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1

ALTERED HCV SPECIFIC T CELL IMMUNITY VERY EARLY IN INTERFERON FREE HCV DAA THERAPY

L. Barrett1, G. Shivasabesan1, C. Wang1, A. Osinusi1,2, A. Kohli1,2, E.G. Symonds3, J. McHutchinson4, S. Kottilil1. 1Laboratory of Immunoepidemiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, 2SAIC-Frederick Inc, Frederick, 3CCMD, CC, National Institutes of Health, Bethesda, MD, 4Gilead Sciences, Inc., Foster City, CA, USA

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Background: Immune mechanisms of achieving sustained virologic response among HCV infected subjects are unclear. Studies of spontaneous clearance suggest a role for adaptive immunity in effective viral clearance, however, such studies in standard of care therapy trials are confounded by exogenous interferon administration. The objective of this study was to assess changes in HCV specific immune responses associated with suppression of viral replication during interferon free anti-HCV direct acting antiviral (DAA) therapy.

Methods: Sixty HCV GT-1 subjects were treated with the potent NS5B inhibitor GS-7977 and ribavirin for 24 weeks. Comprehensive peripheral blood mononuclear cell (PBMC) and liver infiltrating lymphocyte (LIL) T cell, B cell, and NK cell immunophenotyping was performed for 10 patients and 5 controls at baseline, day 10, and end of treatment (EOT). HCV specific immune responses were quantified by IFN-gamma ELISpot and intracellular flow cytometry (IL-2, TNF-alpha, and IFN-gamma) after stimulation with overlapping peptides spanning the entire HCV genome.

Results: Most patients had rapid decline of HCV VL within 7 days of therapy (mean 3.87 log_{10} IU/mL decline), and all patients completing therapy had no detectable virus at EOT. Baseline CD57+ (9.6%±2.3%) and PD-1+ (0.82%±0.27%) T cell counts were higher in chronically HCV-infected individuals than uninfected individuals (7.59%±1.1%, p=0.046; 0.29%±0.1%, p=0.05). Exhausted CD57+PD-1+CD8+ T cells were also higher compared to uninfected (2.38%±0.8% vs 1.6%±0.2%, p=0.049) at baseline. Fewer CD8+ T cells expressed the exhausted phenotype at day 10 (2.38%±1.7% vs 1.67%±0.5%; p=0.05) and EOT. Tim-3+ (9.5%±2.8% vs 9.9%±3.3%, p=0.0001), and PD-1+ (63.7%±2.0% vs 81.1%±1.8%, p=0.0003) cells were more frequent in the liver than the periphery at EOT. Strikingly, there was an increase in peripheral HCV-specific T cell IFN-gamma responses at day 10 and EOT compared to baseline (3.69 spots/10^6 vs 7.4 spots/10^6 PBMC and 13.3 spots/10^6 PBMC; p=0.005), coincident with HCV VL decline.

Conclusions: Inhibition of HCV replication by a DAA regimen is associated with rapidly increased HCV specific immunity and improved peripheral immunophenotype. This is the first study demonstrating HCV specific immune modulation with interferon-free DAA therapy, suggesting immune enhancement is a consequence of viral inhibition not requiring exogenous immune stimulation.

2

A FRAME-SHIFT VARIANT IN THE NOVEL IFNL4 GENE IS ASSOCIATED WITH IMPAIRED HCV CLEARANCE

T. O’Brien1, R.M. Pfeiffer1, M.H. Kuniholm2, B. Muchmore1, P. Akal1, W. Tang1, S. Chen1, A. Wang1, J. Astemborski1, H.L. Bonkovsky3, B.R. Edlin4, C.D. Howell5, T.R. Morgan6, G.B. Sharp7, H.D. Strickler8, D.L. Thomas9, 10, L. Prokunina-Olsson1. 1Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, 2Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, 3IMS, Silver Spring, 4Department of Epidemiology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD, 5Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, 6VA Long Beach Healthcare System, Long Beach, CA, 7Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, 8Division of Infectious Diseases, Johns Hopkins University, Baltimore, MD, USA

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Background and Aims: We recently discovered that dinucleotide frame-shift variant ss469415590 (TT/AG) generates the novel interferon lambda 4 protein (IFN-λ-4, alias IFNAN) in individuals who carry the IFNL4 ss469415590-AG allele (Nature Genetics, in press). IFNL4 is found near IFNL3 (formerly IL28B) on chromosome 19; GWAS marker rs12979860 lies within intron 1 of IFNL4. Linkage disequilibrium between the favorable rs12979860-C variant and IFNL4 ss469415590-AG is strong in Europeans (r²=0.92), but more modest in Africans (r²=0.71). Here we report associations of IFNL4 ss469415590 genotype with spontaneous HCV clearance and early response to treatment of chronic hepatitis C.

Methods: Subjects are African American and European American participants in studies of spontaneous HCV clearance (UHS, ALIVE and WIHS) or response to treatment with pegylated-interferon/ribavirin (Virahep-C and HALT-C). Genotyping for IFNL4 ss469415590-AG was performed with custom Taqman assays. Statistical analyses were stratified by racial ancestry.

Results: Compared to individuals with two copies of the gene that produces IFNL4-4 (ss469415590-AG/AG genotype), those who did not produce IFN-λ-4 (ss469415590-TT/TT genotype) were much more likely to clear HCV spontaneously (range of odds ratios [ORs], 3.5 to 4.7; p-values, <10^{-4} for all comparisons) or in response to treatment (range of ORs for response at week 20 or 24, 3.8 to 10.6; p-values, 0.02 to 10^{-16}). As predicted by the linkage disequilibrium data, ss469415590 and rs12979860 provided similar information for European Americans (data not shown). Among African American individuals, however, associations with HCV clearance were generally stronger for ss469415590 genotype than for rs12979860 genotype (Figure 1).

Conclusions: IFNL4 ss469415590 genotype appears to explain previous associations between genetic variants in the interferon lambda region and impaired HCV clearance. For individuals
ORAL PRESENTATIONS

of African ancestry, IFNL4 ss469415590 genotype predicts HCV clearance better than rs12979860 genotype.

Figure 1. Associations with IFNL4 variants ss469415590 and rs12979860 among African American participants. Odds ratios for: (a) spontaneous HCV clearance (UHS, ALIVE, WHIS); (b) response to treatment with pegylated interferon-alfa/ribavirin at week 24 (Virahep-C) or week 20 (HALT-C Trial). Reference genotypes are ss469415590-D/G and rs12979860-TT, respectively.

3 SAFETY AND EFFICACY OF INTERFERON-FREE REGIMENS OF ABT-450/r, ABT-267, ABT-333 +/- RIBAVIRIN IN PATIENTS WITH CHRONIC HCV GT1 INFECTION: RESULTS FROM THE AVIATOR STUDY


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Background: We assessed safety and efficacy of regimens of ABT-450/r (HCV protease inhibitor dosed with ritonavir 100 mg, identified as a lead compound by Abbott and Enanta) with ABT-267 (NS5A inhibitor) and/or ABT-333 (non-nucleoside NS5B inhibitor) +/- ribavirin (RBV). Overall ITT SVR12 rate for 12-week treatment with 3 DAAs+RBV was 97.5% (77/79) in treatment-naive patients and 93% (42/45) in null responders.

Methods: Non-cirrhotic, GT1 treatment-naive patients and prior peginterferon/RBV null responders received ABT-450/r (100/100–200/100 mg QD) with 1–2 other DAAs (ABT-267 25 mg QD, ABT-333 400 mg BID) +/- RBV for 8, 12, or 24 weeks. We present safety and SVR4 data for patients in 12 and 24-week treatment arms of 3 DAAs+RBV.

Results: Among 247 subjects treated in the 12- and 24-week 3-DAAs+RBV arms, 81.0% had IL28B non-CC genotype, 66.0% had GT1a infection, 54.8% were male and 10.5% were Black, with mean age of 51 years. Baseline HCVRNA was 6.6 log_{10} IU/mL and 37.4% had ≥F2 fibrosis. SVR4 rates were comparable in the 12 and 24 week arms (98.7% and 96.2% in treatment naive and 93.3% and 97.7% in prior null responders, respectively). SVR4 rates (combined 12 and 24 week arms) were comparable in patients with IL28B CC vs. non-CC, GT1a vs. GT1b, baseline HCVRNA <7 log_{10} vs. ≥7 log_{10} IU/mL, and F0–F1 vs. ≥F2 fibrosis (Figure). Among these 247 subjects, 4 patients (1.6%) discontinued due to study drug-related AEs. Seven patients (2.8%) experienced SAEs; 1 (arthralgia) was possibly study drug-related. The moderate-to-severe study drug-related AEs with ≥10% incidence in any arm were asthenia and fatigue. 6 subjects (2.8%) and 1 subject (0.6%) experienced G3–4 laboratory abnormalities in total bilirubin and ALT, respectively; all resolved with continued dosing.

Conclusions: Comparable responses were seen with 12 and 24 weeks of treatment, supporting selection of a 12-week duration of therapy in these populations. SVR rates >90% were achieved in naive and prior null responder patients with a 3-DAAs+RBV regimen, across HCV subtype, IL28B genotype, baseline HCV-RNA or severity of fibrosis.

4 THE EXTRACELLULAR MATRIX PROTEIN LAMININ ALPHA 5 REGULATES THE BEHAVIOUR OF HEPATIC PROGENITOR CELLS IN REGENERATING MOUSE LIVER

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Background and Aims: During chronic liver injury, regeneration occurs through hepatic progenitor cells (HPCs). Understanding the regulation of HPCs may offer therapeutic opportunities to enhance liver regeneration. HPCs are associated with increased laminins in the extracellular matrix. Laminins are heterotrimeric proteins, composed of an alpha, beta and gamma chain. There are 5 alpha chains with different distributions and functions, but the relative contributions of these in HPC-mediated liver regeneration are not known. Our aims were to describe the laminin alpha chains associated with the HPC response and to define the functional effects of specific laminin chains on HPCs.

Methods: The laminin alpha chains were examined by PCR and immunofluorescence in two mouse models of HPC activation. The
functional effects of matrix on cell behaviour were studied in vitro using recombinant laminins and a line of spontaneously immortalised mouse HPCs, and in vivo using transgenic Cre-lox mouse strains that allows conditional knock-out of specific laminin or integrin subunits in HPCs.

Results: The laminin alpha 5 (Lama5) chain is significantly upregulated in both models, with up to a 16-fold increase in gene expression during regeneration (p<0.001). The Lama5 forms a basement membrane which surrounds the progenitor cells. The HPCs express the cell surface receptor alpha-6 beta-1 integrin, a binding partner of Lama5. Compared to other laminin chains, Lama5 selectively promotes HPC adhesion and spreading. These effects are partially blocked by antibodies against beta-1 integrin. Lama5 also significantly enhances HPC migration, resulting in a 2.8-fold increase in cell migration (p<0.001). Furthermore, only Lama5 enhances HPC survival in serum-free medium, with a 3-fold increase in cell viability (p<0.001). HPCs maintained in culture on plastic synthesise Lama5 chain. Knock-down of endogenous Lama5 production using siRNA results in hepatocytic differentiation, with increased albumin production (p<0.001). Disruption of the laminin-integrin interaction in vivo by conditional gene knock-out alters the regenerative response, confirming the importance of this interaction.

Conclusions: Laminin alpha 5-containing matrix is deposited around HPCs during liver regeneration and supports progenitor cell attachment, migration and maintenance of an undifferentiated phenotype. This work identifies a novel target for enhancing liver regeneration.

5 PHASE 3 RANDOMIZED CONTROLLED TRIAL OF ALL-ORAL TREATMENT WITH SOFOSBUVIR + RIBAVIRIN FOR 12 WEEKS COMPARED TO 24 WEEKS OF PEG + RIBAVIRIN IN TREATMENT-NAÏVE GT2/3 HCV-INFECTED PATIENTS (FUSION)

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Background and Aims: Sofosbuvir (SOF)+ribavirin (RBV) has demonstrated high efficacy in Phase2 studies of treatment-naïve GT2/3 HCV-infected patients. FUSION is a Phase 3, randomized, open-label, international, non-inferiority study comparing 12 weeks of SOF+RBV to 24 weeks of Peg+RBV (NCT01497366).

Methods: Treatment-naïve GT2/3 HCV-infected patients were randomized 1:1 to SOF 400 mg daily +RBV 1000–1200 mg daily for 12 weeks or PEG 180 μg weekly +RBV 800 mg daily for 24 weeks (stratified by GT 2/3, HCV RNA <10^4 IU/mL and cirrhosis). GT2/3 patients were enrolled in an approximate 1:3 ratio. The primary endpoint was SVR12 with a pre-specified non-inferiority margin of 15%. Secondary objectives included safety and tolerability, resistance, and additional efficacy outcomes. All subjects have completed treatment and are in follow-up.

Results: 499 patients were randomized and treated; 66% male, 87% white, 30% BMI≥30, mean age 48 (19–77) years, 43% had IL28B CC genotype, 20% had compensated cirrhosis, and 72% GT infection. Safety is summarized in the Table below including AEs occurring in ≥20% of either treatment arm. None of the discontinuations due to AEs in the SOF+RBV arm was deemed related to SOF. Initiation of treatment for depression was less common with SOF+RBV (2%) versus PEG+RBV (11%). Efficacy outcomes including final SVR12 and resistance results will be presented.

Conclusions: SOF+RBV for 12 weeks was well tolerated in treatment-naïve cirrhotic and non-cirrhotic GT2/3 HCV-infected patients. Fewer AEs, discontinuations due to AEs, and Grade 3/4 laboratory abnormalities were observed during treatment with SOF+RBV as compared to treatment with PEG+RBV.

Table: Safety summary

<table>
<thead>
<tr>
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<th>SOF+RBV (N=256)</th>
<th>PEG+RBV (N=243)</th>
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<tbody>
<tr>
<td>Discontinuations due to AE</td>
<td>3 (1%)</td>
<td>25 (10%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>9 (36%)</td>
<td>135 (56%)</td>
</tr>
<tr>
<td>Headache</td>
<td>64 (25%)</td>
<td>107 (44%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>46 (18%)</td>
<td>69 (28%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>31 (12%)</td>
<td>69 (28%)</td>
</tr>
<tr>
<td>≥ Grade 3 AE</td>
<td>18 (7%)</td>
<td>45 (18%)</td>
</tr>
<tr>
<td>Hemoglobin &lt;9 g/dL</td>
<td>3/254 (1%)</td>
<td>9/242 (4%)</td>
</tr>
<tr>
<td>Neutrophils &lt;750/mm3</td>
<td>0/254</td>
<td>36/242 (15%)</td>
</tr>
<tr>
<td>Platelets &lt;50,000/mm3</td>
<td>0/254</td>
<td>18/242 (7%)</td>
</tr>
</tbody>
</table>

6 ALL ORAL THERAPY WITH SOFOSBUVIR + RIBAVIRIN FOR 12 OR 16 WEEKS IN TREATMENT EXPERIENCED GT2/3 HCV-INFECTED PATIENTS: RESULTS OF THE PHASE 3 FUSION TRIAL

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Background and Aims: Treatment-experienced genotype (GT) 2/3 HCV-infected patients have poor response rates to longer duration retreatment with PEG + RBV and currently no alternative treatment options. We report here the results from FUSION, a Phase 3 study designed to evaluate sofosbuvir (SOF) 400 mg once daily+ribavirin (RBV) 1000–1200 mg in a divided daily dose for 12 or 16 weeks in treatment-experienced GT2/3 HCV-infected patients (NCT01604850).

Methods: Randomized, placebo-controlled, double-blind study of treatment-experienced GT2/3 HCV-infected patients who were randomized 1:1 to SOF+RBV for 12 weeks followed by SOF placebo + RBV placebo for 4 weeks or to 16 weeks SOF+RBV (stratified by cirrhosis and HCV genotype). The primary endpoint was SVR12. Secondary endpoints included safety, tolerability, resistance and additional efficacy outcomes.

Table 1. AEs with SOF+RBV for 12 or 16 weeks (Total N = 201)

<table>
<thead>
<tr>
<th>AEs occurring in &gt;10% subjects</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>89 (44%)</td>
</tr>
<tr>
<td>Headache</td>
<td>54 (27%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>48 (24%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>43 (21%)</td>
</tr>
<tr>
<td>Irritability</td>
<td>26 (13%)</td>
</tr>
<tr>
<td>Cough</td>
<td>23 (11%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>21 (10%)</td>
</tr>
</tbody>
</table>

Results: 201 patients were randomized and treated; 70% were male, 87% were white, the mean age was 54 years (range 24–70), 30% were IL28B CC genotype, 34% had compensated cirrhosis, 63% had GT3 infection, 75% had relapse/breakthrough after prior treatment. All subjects have completed treatment and are in follow-up. No patients experienced on-treatment virologic failure. One subject (<1%) discontinued treatment due to an AE. Eight subjects (4%) reported treatment-emergent SAEs. Pooled safety data are summarized in Table 1. Treatment-emergent laboratory
abnormalities ≥ grade 3 occurring in >1% subjects included glucose >250 mg/dl (5%), lymphocytes <500/mm³ (3%), hemoglobin <9 g/dl (2%), total bilirubin >3 mg/dl (2%).

Conclusions: SOF+RBV for 12 or 16 weeks was well tolerated in treatment-experienced GT2/3 HCV-infected patients. SVR12 data for both treatment arms will be presented.

Parallel Session: TRANSLATIONAL RESEARCH IN HCV

7
T CELLS FACILITATE HEPATITIS C VIRUS TRANSMISSION TO POLARISED LIVER AND BRAIN CELL LINES, REVEALING A NEW ROLE FOR VIRAL QUASISPECIES


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Background and Aims: Hepatitis C virus (HCV) infection leads to progressive liver disease and is associated with extrahepatic manifestations including lymphocyte and neuronal abnormalities. Despite high patient viraemia, we are currently unable to grow patient-derived virus robustly in the laboratory and the field is limited to the study of the few characterised infectious clones capable of replicating in cell culture (HCVcc). These predominantly infect hepatocytes, yet infection of blood brain barrier endothelial cells is also possible. Infection of permissive cell lines is particularly challenging when cells are cultured under polarising conditions, which mimic hepatocyte and brain endothelial properties in vivo. This work proposes an alternative pathway for HCV infection that circumvents this challenge and allows robust transmission to polarised cells.

Methods: We use CD4+ T cells isolated from peripheral blood and liver and HCVcc clones to demonstrate transmission to permissive Huh-7 cells, polarised HepG2-CD81 cells and polarised blood brain barrier endothelial cells hCMEC/D3. We explore the role of viral quasispecies using pseudotypes expressing envelope glycoproteins from genotype 1 viruses isolated from the same individual.

Results: We demonstrate that i) CD4+ T cells facilitate the infection of polarised HepG2-CD81 and hCMEC/D3 cells, achieving levels of infection similar to the gold-standard permissive hepatomas Huh-7 and ii) T cell CD81 plays a key role in this process, which is further augmented by the presence of viral quasispecies.

Conclusions: T cells can interact with HCVcc directly to achieve optimal transmission to polarised cell lines. HCV quasispecies enhance this process via T cell CD81.

8
SYNDECANS MEDIATE APOLIPOPROTEIN E–HEPATOCYTE CELL SURFACE INTERACTIONS DURING HEPATITIS C VIRUS INFECTION

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Background: In hepatitis C virus (HCV) infected patients, virions are associated with VLDL-type lipoproteins forming an infectious lipo-viro-particle (LVP). ApoE, which is an integral component of VLDL, has been shown to play a crucial role in HCV life cycle. Using a trans-complementation assay and a RNAi screen we aimed to identify the cellular targets of virion-associated apoE on the hepatocyte cell surface.

Methods: The functional role of apoE and its cellular targets was investigated in a trans-complementation assay. Endogenous apoE was first silenced in Huh7.5.1 cells with a specific siRNA. ApoE expression was then restored using recombinant adenoviral vectors. The functional effect of apoE wild-type (wt) and mutant constructs was then investigated using HCVcc model system. We focused on the functional analysis of the heparan sulfate proteoglycan (HSPG) high affinity domain (HSPG-BD), which overlaps with the well-defined apoE receptor binding domain.

Results: We demonstrated that apoEwt adeno viral transduction restored HCVcc infectious particle production in apoE(-) cells. In contrast, apoE deleted from the HSPG-BD leads to capsid-free HCVcc production suggesting a role of this domain in HCV assembly. Moreover, double alanine substitutions in the apoE HSPG-BD, apoE K143A, K146A or apoE R142A, R145A lead to regular HCVcc production but failed to restore HCVcc infectivity suggesting a role of apoE HSPG-BD in HCV entry. To confirm the role of the apoE-HSPG-BD in HCV entry, we designed an apoE-derived peptide overlapping the HSPG-BD. This peptide inhibits HCVcc binding in a dose-dependent manner. Since syndecans are members of the HSPG family, we next investigated their role in HCV binding. HCVcc entry was markedly inhibited by incubation of Huh7.5.1 cells with a sheddase activator suggesting that HSPG syndecans are functionally relevant for HCV infection. Moreover, using a RNAi screen targeting the individual members of the syndecan family, we identified the syndecans specifically involved in HCV entry.

Conclusion: HCV binding is mediated by apoE-HSPG interactions and requires cell surface expression of defined members of the syndecan family. These results advance our understanding of the very first steps of virus-host interactions and are relevant for the development of antivirals and vaccines targeting HCV entry.

9
ASSOCIATION OF A GENETIC VARIANT OF THE TOLL-LIKE RECEPTOR 9 (TLR9) WITH SPONTANEOUS VIRAL CLEARANCE AND WITH INTERFERON RESPONSE IN CHRONIC HEPATITIS C VIRUS INFECTION

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Background: The Toll-like receptor 9 (TLR9) plays a fundamental role in pathogen recognition and activation of innate immunity during hepatitis C virus (HCV) infection. The transmembrane protein recognizes pathogen-associated molecular patterns and initiates the signaling pathway to induce the production of cytokines which are necessary for the innate immunity and subsequent adaptive immunity. In our study we investigated whether single nucleotide polymorphisms (SNP) in the TLR9 gene may affect on infection progression and on the prediction of sustained virologic response (SVR).

Methods: The study cohort of 491 patients included 331 well characterized patients infected with chronic HCV type 1 from the INDIV-1 study and 160 (33%) patients who spontaneously cleared the infection. The mean age was 43±11 years and 49% were male. All chronic patients were treated with dual combination
10 CYCLOPHILIN INHIBITORS POTENTIATE INTERFERON SIGNALING THROUGH DIMINISHED PKR PHOSPHORYLATION IN HCV-INFECTED CELLS.

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Background and Aims: Cyclophilin (CyP) inhibitors are expected to present a new class of anti-HCV agents. CyPs are cellular cofactors that promote HCV replication, and the anti-HCV effect of CyP inhibitors seen in HCV-infected patients is believed to arise from direct anti-replication function of these drugs. However, recent clinical studies with a CyP inhibitor, SCY-635, revealed that interferon (IFN) a and IFN stimulated genes (ISGs) in patient’s serum was upregulated following administration with SCY-635. These results raised the possibility that CyP inhibitors modulate the IFN signaling in addition to the direct suppression of HCV replication. We examined the effect of CyP inhibitors on the IFN signaling pathway using an HCV-infected cell culture system.

Method: We used persistently HCV-infected Huh7 cells (HCV-Huh7), which were infected with IFN1 and maintained for approximately 20 days. These cells were treated with SCY-635 or siRNA for CyPA for 2 days, and then stimulated with IFN. Cell lysates were collected to detect proteins including ISGs, total and phosphorylated PKR, STAT1, STAT2, and HCV proteins by Western blotting.

Results: The induction of ISGs upon IFNa treatment was suppressed in HCV-Huh7. However, IFNa-mediated ISGs induction was restored by SCY-635. This was not likely to be the result of the suppression of HCV replication since another anti-HCV compound, a protease inhibitor, had little effect on the levels of ISGs. Furthermore, phosphorylation of PKR upon IFNa treatment, (one of the modifications involved in this ISGs upregulation), was remarkably reduced by SCY-635. Knock down of endogenous CyPA with siRNA also decreased phosphorylated PKR, suggesting that the effect of SCY-635 was mediated by CyPA. Other phosphorylation changes triggered by IFNa, including those of STAT1 and STAT2, were not altered by SCY-635.

Conclusions: The CyP inhibitor SCY-635 reduced IFNa-induced phosphorylation of PKR in HCV-infected cells. It has been reported that ISGs are upregulated by JAK/STAT pathway but negatively regulated by phosphorylated PKR. The present study suggests that CyP inhibitors release the negative regulation by PKR to permit ISGs induction in HCV-infected cells. This mechanism may contribute to the anti-HCV activity of CyP inhibitors, in addition to the direct suppression of HCV replication.

11 IN VIVO RIBAVIRIN EFFECTS ON INTERFERON STIMULATED GENES TRANSCRIPTIONAL REGULATION INVOLVES CHROMATIN REMODELING AND HISTONE METHYLATION MEDIATED BY THE G9a METHYL-TRANSFERASE

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Background and Aims: Uncovering RBV mechanism of action remains a tantalising objective faraway of being achieved. Since recent evidence suggests its role in potentiating IFN intracellular pathway, we decided to investigate Interferon Stimulated Genes (ISGs) expression in vivo, in liver biopsies of patients undergoing RBV monotherapy and in the in vitro models of differentiated HepaRG (dHepaRG) cells and primary human hepatocytes (PHH).

Methods: RBV- and/or IFN-treated dHepaRG and PHH, and liver specimens from 24 individuals belonging to a cohort of genotype-1 HCV-infected patients who underwent paired liver biopsies before and after 12 months of RBV monotherapy, were analyzed for ISGs expression by Taqman® Low Density Arrays. Gene expression was correlated to intrahepatic viral RNA content and alanine aminotransferase (ALT) variations. Promoter occupancy and epigenetic histone marks at selected target genes were assessed by chromatin immunoprecipitation (ChIP).

Results: Patients’ expression profiling revealed that in vivo RBV treatment reduces the mRNA levels of a large number of ISGs, particularly those found up-regulated in non-responders to PEG-IFN/RBV, providing a first mechanistic hint to explain RBV capacity to foster sustained virological responses in HCV patients. The ISGs down-regulation correlated with liver biochemical response, with a sharper inhibition observed in patients showing ALT normalization following RBV treatment. In PHH and dHepaRG, exposure to RBV alone results in down-regulation of a large set of ISGs that are greatly transcriptionally enhanced under RBV and IFN co-treatment. To figure out how RBV might modulate ISGs transcription, cells were treated with inhibitors of chromatin modifying enzymes. The functional blockade of G9a, responsible for the repressive histone H3 lysine-9 methylation (H3K9me2/3), abolished RBV-triggered ISGs down-regulation and severely impaired RBV ability to cooperate with IFN in co-treatment experiments, while showed no effects on RBV-induced gene activation. ChIP analysis for promoter occupancy of three representative ISGs (CXCL10/IP-10, IFI27 and RSAD2/Viperin) revealed that RBV favored G9a binding and H3K9 tri-methylation both in vitro and in vivo.

Conclusions: Our results indicate that the positive RBV effect on IFN responses could be related to its ability to epigenetically reset intrahepatic ISGs pre-activation to restore a chromatin environment favourable to be newly activated by IFN.
12 HCV KINETICS AND QUASISPECIES EVOLUTION WITHIN THE FIRST HOURS OF TELAPREVIR-BASED TRIPLE THERAPY IN PREVIOUSLY TREATED HCV-PATIENTS

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S6 Journal of Hepatology

Methods: HCV-RNA decay was assessed (detection-limit=15 IU/ml) under telaprevir-treatment (baseline-1h-2h-3h-4h-5h-6h-8h-12h-24h-28h-48h-1w-2w-4w-8w-12w) and modeled according to Neumann et al., Science 1998. NS3-protease sequences were obtained at different time-points (baseline-8h-24h-48h) during telaprevir-treatment by population-sequencing. For 6 patients, viral quasispecies evolution at the same time-points was assessed by ultra-deep 454-pyrosequencing (UDPS).

Results: Twelve HCV-1 infected patients (HCV-1b=9; HCV-1a=3) received telaprevir+pegIFN-2a/ribo after previous failure to pegIFN/ribo-treatment (null-responders=7; partial-responders=1; relapers=4). The median (IQR) follow-up time was 6 (4–8) weeks. Viral-load decay within the first 48h was similar in previous non-responders (median[IQR]: RNA-decay=2.6[2.6–2.9]logIU/ml) and relapers (median[IQR]: RNA-decay=2.6[2.2–3.1]logIU/ml; p = 0.788), and independent from 1a/b HCV-genotype (2.6[2.3–3.3]logIU/ml in HCV-1a vs. 2.7[2.5–2.8]logIU/ml in HCV-1b; p = 0.921). HCV-RNA decay tended to be biphasic, with an initial drop at 8h since first-dose (median[IQR]: RNA-decay=1.2[0.7–2.1]logIU/ml). The mathematical model set a therapy-effect delay-time (τe) of 7.2h. Within the model, median[IQR]: RNA-decay was 2.3[2.1–2.5]logIU/ml, with the exception of a single patient carrying the T54S RAV at baseline, who showed a slower decay (1.6 logIU/ml).

Introduction: Uncovering the rapid dynamics of HCV under pegylated-interferon/ribavirin (pegIFN/ribo) treatment has provided critical information on the mechanism of HCV clearance and viral eradication. With the use of protease-inhibitors in patients often insensitive (or partially-sensitive) to interferon-administration, the study of early viral kinetics may disclose, and predict, viral dynamics underlying drug-resistance may disclose, and predict, viral dynamics underlying drug-resistance development.

Conclusions: The addition of both GS-5885 and GS-9451 to a regimen of PEG + RBV for 24 weeks resulted in high SVR4 rates in treatment-experienced, genotype 1 HCV-infected patients. GS-5885 at a dose of 90 mg QD is being explored in current investigational regimens in combination with sofosbuvir.

13 COMBINATION OF THE NSSA INHIBITOR, GS-5885, THE NS3 PROTEASE INHIBITOR, GS-9451, AND PEGYLATED INTERFERON PLUS RIBAVIRIN IN TREATMENT EXPERIENCED PATIENTS WITH GENOTYPE 1 HEPATITIS C INFECTION

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Table: SVR4 rates, overall and subgroup analysis

<table>
<thead>
<tr>
<th>Prior Treatment History</th>
<th>Sub-genotype</th>
<th>IL-28B Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>Relapers</td>
<td>Breakthrough</td>
</tr>
<tr>
<td>74%</td>
<td>(117/158)</td>
<td>(66/71)</td>
</tr>
<tr>
<td>93%</td>
<td>(66/71)</td>
<td>(4/10)</td>
</tr>
<tr>
<td>40%</td>
<td>(93/134)</td>
<td>(69/134)</td>
</tr>
<tr>
<td>69%</td>
<td>(69/100)</td>
<td>(24/24)</td>
</tr>
<tr>
<td>97%</td>
<td>(97/100)</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>(100/100)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: The addition of both GS-5885 and GS-9451 to a regimen of PEG + RBV for 24 weeks resulted in high SVR4 rates in treatment-experienced, genotype 1 HCV-infected patients. GS-5885 at a dose of 90 mg QD is being explored in current investigational regimens in combination with sofosbuvir.

14 ALL-ORAL SOFOSBUVIR-BASED 12-WEEK REGIMENS FOR THE TREATMENT OF CHRONIC HCV INFECTION: THE ELECTRON STUDY

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Background: Sofosbuvir (SOF), a potent uridine nucleotide analog in Phase 3 development, administered with ribavirin (RBV) to patients with HCV genotype 1 demonstrated 84% SVR12 in treatment-naïve patients and 10% SVR12 in prior null responders. We hypothesized that the addition of another DAA with a different mechanism of action and non-overlapping resistance profile would improve rates of SVR. We administered SOF in combination with GS-5885 (NSSA inhibitor) plus RBV and GS-9669 (non-nucleotide NS5B inhibitor) plus RBV.
Methods: Non-cirrhotic patients with HCV genotype 1 were enrolled to receive 12-week regimens:
1. 25 treatment-naive patients received SOF+GS-5885+RBV,
2. 9 prior null responders received SOF+GS-5885+RBV,
3. 25 treatment-naive patients received SOF+GS-9669+RBV, and
4. 10 prior null responders are to receive SOF+GS-9669+RBV (8 have been enrolled so far).

Results: Available efficacy data are tabulated.

<table>
<thead>
<tr>
<th>Treatment-naive</th>
<th>Prior null responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVR</td>
<td>0.798 ± 0.031</td>
</tr>
<tr>
<td>EOTR</td>
<td>0.797 ± 0.031</td>
</tr>
<tr>
<td>SVR4</td>
<td>0.795 ± 0.031</td>
</tr>
</tbody>
</table>

At the time of abstract submission, no virologic breakthrough had been observed in any treatment group. Preliminary safety review revealed one discontinuation due to the only SAE observed thus far (unrelated to treatment). This treatment-naive patient who received SOF+GS-5885+RBV for only 8 weeks has achieved SVR12.

Conclusions: All-oral regimens containing sofosbuvir provided rapid and consistent antiviral suppression in treatment-naive patients and prior null responders. High response rates in these arms employing a second agent with a different mechanism of action and promising safety profiles support the continued exploration of sofosbuvir in 2- or 3-drug oral regimens in patients with HCV.

Parallel Session: NON-INVASIVE ASSESSMENT IN LIVER DISEASE

15 FIRST INTENTION-TO-DIAGNOSE COMPARISON OF ARFI AND FIBROSCAN IN CHRONIC LIVER DISEASE
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Background and Aims: Fibroscan and ARFI are accurate devices for the non-invasive diagnosis of liver fibrosis. By excluding patients with failure of measurement, previous studies produced only per-protocol analyses. We aimed to compare Fibroscan and ARFI in an intention-to-diagnose (ITD) basis, according to STARD statements.

Methods: 219 consecutive patients with chronic liver disease and biopsy were included. Liver stiffness measurements were performed by Fibroscan (right lobe) and ARFI (right lobe: ARFI-D, left lobe: ARFI-G). ARFI-DG corresponded to the median value of all valid measurements obtained in both lobes. Reference for fibrosis was Metavir F staging. Diagnostic accuracy was evaluated using AUROC and Obuchowski index (adjusted AUROC). For ITD analysis, failures of elastographic measurements were replaced by the median value measured in the opposite group of the biopsy diagnosis.

Results: Fibrosis stage prevalence: F0 2%, F1 3%, F2 25%, F3 25%, F4 9%. Rate of measurement failure: ARFI-D or ARFI-G: 5% versus Fibroscan: 5.9% (p = 0.0022). AUROCs and Obuchowski indexes are presented in the table. In per-protocol analysis, AUROCs of Fibroscan were significantly higher than those of ARFI-D for each diagnostic target (p < 0.022), and those of ARFI-G or ARFI-DG for F4 (p < 0.028). In ITD analysis, Fibroscan AUROCs decreased showing no significant difference with ARFI-D, ARFI-G, and ARFI-DG for each diagnostic target. Comparison of Obuchowski indexes showed the same results.

Conclusions: ARFI and Fibroscan have close and high accuracy for liver fibrosis diagnosis. Due to a higher failure rate, accuracy of Fibroscan decreases in the ITD analysis but remains not significantly different from ARFI accuracy.

Table: AUROCs and Obuchowski indexes of Fibroscan and ARFI

<table>
<thead>
<tr>
<th>AUROC</th>
<th>Obuchowski</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARFI-D</td>
<td>0.730 ± 0.023</td>
</tr>
<tr>
<td>ARFI-G</td>
<td>0.792 ± 0.028</td>
</tr>
<tr>
<td>ARFI-DG</td>
<td>0.795 ± 0.032</td>
</tr>
<tr>
<td>Fibroscan</td>
<td>0.798 ± 0.031</td>
</tr>
</tbody>
</table>

16 PERFORMANCE OF ELASTPQ SHEAR WAVE ELASTOGRAPHY TECHNIQUE FOR ASSESSING FIBROSIS IN CHRONIC VIRAL HEPATITIS
G. Ferraioli1, C. Tinelli2, R. Lissandrini3, M. Zicchetti1, B. Dal Bello1, C. Filice1.
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Background and Aims: ELASTPQ® real-time shear wave elastography (RTSWE) is a non-invasive technique that assesses liver fibrosis by measuring liver stiffness. This study was carried out to evaluate the performance of RTSWE in patients with chronic viral hepatitis in comparison with transient elastography (TE) by using liver histology as the reference standard.

Methods: Consecutive patients with chronic viral hepatitis scheduled for liver biopsy (LB) (Group1), and healthy volunteers (Group2) were studied. In Group1 RTSWE by using the iU22 ultrasound system (Philips Medical Systems, Bothell, WA, USA) with a convex broadband probe and ElastPQ® technique, transient elastography (TE) by using FibroScan® (Echosens, Paris, France) and ultrasound-assisted LB were consecutively performed. In Group2 RTSWE and TE were carried out. Fibrosis was staged according to the METAVIR scoring system. Receiver operating characteristic curve analyses were performed to calculate area under the curve (AUC) for F2, F3, and F4.

Results: 88 subjects (68 males and 20 females) and 33 subjects (17 males and 16 females) were studied in Group1 and Group2, respectively. In Group1 median values of measurements were 4.6 (IQR, 4.5–5.4) kPa and 5.45 (IQR, 4.3–5.7) kPa for F0–F1 stage; 5.9 (IQR, 5.3–9.7) kPa and 9.1 (IQR, 8.4–11.6) kPa for F3 stage, 12.0 (IQR, 10.3–19.6) kPa and 17.1 (IQR, 14.1–22.0) kPa for F4 stage with RTSWE and TE, respectively. In Group2 median values of measurements were 3.3 (IQR, 3.7–4.1) kPa and 3.8 (IQR, 4.5–5.0) kPa with RTSWE and TE, respectively (p = 0.0001 compared with subjects of Group1). AUROCs were 0.88 (95% CI, 0.72–0.96) for RTSWE and 0.77 (95% CI, 0.66–0.85) for TE (p = 0.003); 0.79 (95% CI, 0.79–0.85) for RTSWE and 0.93 (95% CI, 0.85–0.98) for TE (p = 0.16); 0.96 (95% CI, 0.88–0.99) for RTSWE and 0.94 (95% CI, 0.86–0.98) for TE (p = 0.7) for F2, F3, and F4, respectively.

Conclusion: In staging liver fibrosis RTSWE compares favorably with TE. Healthy volunteers show significant lower values with respect to patients with not significant fibrosis.
17 THE FEASIBILITY AND VALUE OF SHEAR-WAVES ULTRASOUND BASED ELASTOGRAPHIC METHODS FOR LIVER FIBROSIS EVALUATION (TRANSIENT ELASTOGRAPHY – TE, ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY – ARFI, SUPERSONIC SHEAR IMAGING – SSI)


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Background and Aim: Ultrasound based elastographic methods are increasingly used in clinical practice for liver fibrosis evaluation. The aims of our study were to assess the feasibility (‘intend to diagnose’) of the 3 shear waves elastographic methods (TE, ARFI and SSI) and to evaluate if SSI is a reliable tool for liver fibrosis evaluation, considering TE as the reference method.

Methods: Our prospective study included 332 consecutive subjects, with or without hepatopathies, in which liver stiffness (LS) was evaluated by means of TE, ARFI and SSI. Reliable measurements were defined as: median value of 10 (TE, ARFI) or 5 (SSI) LS measurements with a success rate >60% and an interquartile range interval <30%, values expressed in kilopascals (kPa) (TE, SSI) or meters/second (m/s) (ARFI). To discriminate between various stages of fibrosis by TE we used the LS cut-offs proposed in the most recently published meta-analysis: 6 kPa for predicting fibrosis F≥3 (92.1% vs. 71.3%, p = 0.0001) and 92.1% vs. 71.3% (p = 0.0001), respectively. The rate of reliable LS measurements was similar for TE and SSI: 72.2% vs. 71.3%, (p = 0.86). For both TE and SSI, older age and higher BMI were associated with impossibility to obtain reliable LS measurements. For SSI, another factor associated with impossibility to obtain reliable LS measurements was the presence of chronic hepatopathies. For ARFI elastography, the age, gender, BMI, ALT level, the presence of chronic hepatopathies and the presence of cirrhosis did not influence the rate of reliable LS measurements.

The distribution of liver fibrosis in this cohort of patients, using TE previously specified cut-off values were: F0: n = 74 (40.2%), F1: n = 24 (13.1%), F2: n = 37 (20.1%), F3: n = 26 (14.1%), F4: n = 23 (12.5%). The value of SSI for predicting various stages of liver fibrosis is presented in the table.

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>SSI Cut-off (kPa)</th>
<th>AUROC</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>&gt;7.1</td>
<td>0.818</td>
<td>72.7</td>
<td>78.4</td>
<td>83.3</td>
<td>65.9</td>
<td>75</td>
</tr>
<tr>
<td>F1</td>
<td>&gt;7.8</td>
<td>0.851</td>
<td>75.6</td>
<td>83.7</td>
<td>80.2</td>
<td>79.6</td>
<td>79.8</td>
</tr>
<tr>
<td>F2</td>
<td>&gt;8.6</td>
<td>0.889</td>
<td>87.8</td>
<td>79.3</td>
<td>60.5</td>
<td>94.6</td>
<td>81.5</td>
</tr>
<tr>
<td>F3</td>
<td>&gt;11.5</td>
<td>0.910</td>
<td>82.6</td>
<td>91.3</td>
<td>57.5</td>
<td>97.3</td>
<td>90.2</td>
</tr>
</tbody>
</table>

Conclusions: The most feasible shear-waves ultrasound elastographic method is ARFI. Higher BMI and older age were associated with impossibility to obtain reliable LS measurements for both TE and SSI. SSI is a reliable method for non-invasive evaluation of liver fibrosis. The accuracy of SSI increases with the severity of fibrosis.

Reference(s)
COST-EFFECTIVENESS OF NON-INVASIVE METHODS FOR THE DIAGNOSIS OF LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B IN THE UK


Background: Although liver biopsy is the reference standard for staging of fibrosis, non-invasive markers (NITs) are increasingly being used as alternatives due to easier applicability. In order to make the best use of limited healthcare resources, the cost-effectiveness of alternative testing strategies should be established in order to inform decisions about which should be adopted for routine use.

Objectives: To assess the cost-effectiveness of alternative test strategies for staging fibrosis in patients with chronic Hepatitis B (CHB).

Methods: Twenty-four test strategies comprising of a range of NIT’s and liver biopsy assessed either alone or in combination were evaluated. Two further strategies, ‘treat none’ or ‘treat all patients’ without diagnostic testing were also assessed. A model was developed to estimate the long term costs and health outcomes associated with each strategy. A systematic review and meta-analysis was undertaken to obtain data on the sensitivity and specificity of the NIT’s in patients with CHB. Costs were calculated for a UK National Health Service perspective and included: the tests costs; the long term costs of managing patients (taking into account whether they had been correctly diagnosed or not) and; the costs of re-testing. Health outcomes were measured using quality adjusted life years (QALYs) which take into account length and quality of life. The analysis was conducted separately for patients with HBeAg(+) and HBeAg(-).

Results: The incremental cost per QALY was determined for all test strategies. Assuming a cost-effectiveness threshold of £50,000 per QALY, the most cost-effective strategy for HBeAg(+) patients is one that combines two NIT’s: FIB-4 serum marker and Fibrotest; providing a QALY gain of 2.38 at an additional cost of £28,940, compared to a strategy where no diagnostic test or antiviral treatment is administered. Compared to a test strategy of liver biopsy alone, the combination of the two NIT’s provides an incremental QALY gain of 0.51 for £12,677 less. For HBeAg(-) patients, the most cost-effective strategy is a combination of a FIB-4 serum marker and Fibroscan.

Conclusion: The use of a combination of NIT’s is the most cost-effective approach in forming treatment decisions in a patient population with CHB.

PERFORMANCE OF LIVER STIFFNESS COMPARED WITH LIVER BIOPSY TO PREDICT SURVIVAL AND DECOMPENSA TIONS OF CIRRHOSIS AMONG HIV/HCV-COINFECTED PATIENTS


Background and Aims: HIV/HCV-coinfected patients are at increased risk of death. Survival in HCV infection depends on fibrosis stage. Liver stiffness measurement (LSM) allows a non-invasive diagnosis fibrosis and also correlates with portal pressure. Thus, LSM could replace liver biopsy (LB) in the assessment of the risk of death and liver events in HIV/HCV coinfection. Because of these, we aimed to compare the prognostic performance of LB with that of LSM to predict survival and liver decompensations among HIV/HCV-coinfected patients.

Methods: 297 HIV/HCV-coinfected patients, with LB and LSM separated by ≤12 months, were included in this cohort. Baseline date was half the period of time between LB and LSM. LB was staged following the Scheuer’s score. LSM was obtained by hepatic transient elastometry. Mortality from any cause and incidence of the first liver decompensation were estimated. The integrated discrimination improvement (IDI) was computed to compare the ability of models to predict outcomes.

Results: Median (IQR) age was 42 (39–45) years. 229 (77%) patients were men. Median (IQR) follow-up was 5 (4.2–5.4) years. 275 (93%) were on antiretroviral therapy at baseline. Median (IQR) CD4 cell count was 514 (352–693) cells/µL, and 233 (79%) individuals showed undetectable plasma HIV RNA at baseline. Overall mortality rate was 1.56 (95% CI: 1.02–2.40) per 100 person-years. The adjusted hazard ratio [AHR (95% confidence interval, 95% CI)] of baseline fibrosis (per fibrosis stage) was 1.52 (1.08–2.15, p = 0.017) and of LSM (per 5 kPa increase) was 1.28 (1.12–1.46, p < 0.001). Assessment of IDI indicated that LSM-including models performed 3.9% better than the LB-based models (p = 0.072). The liver decompensation rate was 1.59 (95% CI: 1.03–2.43) decompensations per 100 person-years. For the prediction of liver decompensations, the AHR (95% CI) of baseline fibrosis by LB (per stage of fibrosis) was 1.67 (1.15–2.43, p = 0.007) and of LSM (per 5 kPa increase) 1.37 (1.21–1.54, p < 0.001). Evaluation of IDI showed that LSM-based models performed 8.4% better than the LB-based models (p = 0.045).

Conclusions: LSM-based prediction achieves a similar yield than LB-based models to predict overall mortality in HIV/HCV-coinfected patients and the former could predict better liver decompensations. LSM may replace LB as prognostic tool in this setting.

21

10-YEARS PROGNOSTIC VALUE OF FIBROTEST AND STEATOTEST FOR LIVER-RELATED AND CARDIOVASCULAR DEATH IN PATIENTS WITH TYPE-2 DIABETES AND/OR HYPERLIPEIDEMIA

H. Perazzo, M. Munteanu, Y. Ngo, F. Huei, M. Couteau, F. Huet, M. Couteau, F. Huet

Background: Cardiovascular and liver-related deaths have been described in nonalcoholic fatty liver disease (NAFLD) that is usually associated with risk factors, as type-2 diabetes and dyslipidemia. Aims: To evaluate the 10-year prognostic value of non-invasive markers (FibroTest-SteatoTest) for the overall survival (OS) and survival without liver-(LRD) and cardiovascular-related death (CVD).

Methods: A cohort of patients with diabetes and/or dyslipidemia without known liver disease was prospectively followed [1999–2012]. Mortality data, according to the International Classification of Diseases, was collected in the French National Registry. Liver diseases were presumed by validated biomarkers: Advanced liver fibrosis (AF-METAVIR F2, FibroTest>0.48) and advanced steatosis (>32%-AS, SteatoTest>0.69). Three sub-populations were analyzed according to the risk factors: diabetics (D), hyperlipidemia (H) and diabetics with hyperlipidemia (DH).
**ORAL PRESENTATIONS**

**Results:** 2322 patients [52% males, age=52yrs, BMI 26 (15–64) Kg/m2, 61%H; 11%D and 28%DH] were included. The prevalence of presumed AF were H 2.5%, D 6.5% and DH 9% (P < 0.0001). As was presumed in H 11%, D 23% and DH 39% (P < 0.0001). During a median follow-up of 12yrs, 183 (7.8%) patients died [35 CVD; 6 LRD (4 hepatocellular-carcinoma and 2 cholangio-carcinoma)]. Both OS and survival without-CVD were significantly higher in H than in D or DH (%Kaplan–Meier, 95% CI): OS 96 (95–97) vs 6 LRD (4 hepatocellular-carcinoma and 2 cholangio-carcinoma)].

**ORAL PRESENTATIONS**

**Parallel Session: TRANSPLANTATION AND ACUTE LIVER FAILURE**

**23**

**A MULTICENTER STUDY OF PROTEASE InHIBITOR-TRIple THERAPY IN HCV-INFECTED LIVER TRANSPLANT RECIPIENTS: REPORT FROM THE CRUSH-C GROUP**

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**Background and Aims:** Triple therapy [TT; peginterferon (P), ribavirin (R) and protease inhibitor (PI)] offers improved sustained virologic response (SVR) rates in non-liver transplant (LT) patients with HCV genotype 1 (G1).

**Methods:** Cohort study of HCV-infected G1 LT recipients receiving TT from 5 U.S. centers. Extended rapid virologic response (eRVR) and SVR-4 rates (by intent to treat), and adverse events (AEs) were analyzed. Telaprevir was used in 90% with P+R lead-in (LI) in 96%. Forty-three with extended LI (≥90 days) were excluded from efficacy analyses. High rates of eRVR are achievable with TT exceeding 90% with P/R dose reductions in 27%/78%. Hospitalizations under grant agreement no. Health-F2–2009–241762, for the project-FLIP.

**Conclusion:** This study shows that in compensated cirrhotic patients a SS and MELD predictive model represents an accurate predictor of clinical decompensation, with an accuracy at least equivalent to that of HVPG. If confirmed by further studies, SS and MELD could substitute HVPG in the overall management of compensated cirrhotic patients.

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**Background and Aims:** The research received funding from the European Union Seventh Framework-Programme (FP7/2007–2013) under grant agreement no. Health-F2–2009–241762, for the project-FLIP.

**22**

**SPLEEN AND LIVER STIFFNESS MEASUREMENT CAN PREDICT CLINICAL COMPLICATIONS IN COMPENSATED CIRRHOTIC PATIENTS: A PROSPECTIVE STUDY**

A. Colecchia1, D. Mandolesi1, A. Colli2, G. Casaazza1, R. Schiumberini1, L. Marzi1, L. Bacchi-Reggiani1, G. Bonato1, A.R. Di Biasi2, M. Taddia1, L. Montrone1, E. Scialoli1, G. Mazzella1, D. Festi1, University of Bologna, Bologna, 2Ospedale A. Manzoni, lecco, 3University of Milano, Milano, 4University of Modena, Modena, Italy

E-mail: davide.festi@unibo.it

**Background and Aims:** The prognosis of compensated cirrhotic patients is strongly associated with the development of portal hypertension (PHT). Currently hepatic venous pressure gradient (HVPG) measurement represents the best predictor of clinical decompensation. Nevertheless, HVPG is an invasive procedure and not widely diffused. The evaluation of liver stiffness (LS) and more recently of spleen stiffness (SS) by Fibroscan has been shown to be correlated to HVPG degree.

**Methods:** Eighty patients with compensated cirrhosis underwent at enrollment to LS, SS, HVPG measurements and upper gastrointestinal endoscopy, and then followed-up for 2 years or until the occurrence of the first clinical decompensation [variceal bleeding, ascites, encephalopathy, hepatorenal syndrome, sepsis, liver transplantation and death].

**Conclusion:** This study shows that in compensated cirrhotic patients a SS and MELD predictive model represents an accurate predictor of clinical decompensation, with an accuracy at least equivalent to that of HVPG. If confirmed by further studies, SS and MELD could substitute HVPG in the overall management of compensated cirrhotic patients.
Preliminary results show SVR-4 is achieved in 41.2%, but the majority of responders remain on treatment. SVR-4 rates were 70% among those with eRVR, and this may be an important predictor of response. These results must be balanced with high rates of AEs, including a mortality risk (2%). Improving tolerability and identifying predictors of SVR are critical to optimizing the risks-benefits of TT.

**Table 1: Virologic response by intent to treat**

<table>
<thead>
<tr>
<th>Week 4</th>
<th>LOD</th>
<th>Week 8</th>
<th>LOD</th>
<th>Week 12</th>
<th>eRVR</th>
<th>LOD EOT</th>
<th>SVR4</th>
<th>SVR among eRVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>64 (69.6%)</td>
<td>61 (75.2%)</td>
<td>68 (79.0%)</td>
<td>54 (61.4%)</td>
<td>13 (43.3%)</td>
<td>71 (42.1%)</td>
<td>7 (50%)</td>
<td></td>
</tr>
<tr>
<td>Total Patients</td>
<td>92</td>
<td>78</td>
<td>86</td>
<td>88</td>
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**24 HIGH MFI PREFORMED CLASS II DONOR SPECIFIC ANTIBODIES ACCELERATE FIBROSIS AND INCREASE MORTALITY AFTER LIVER TRANSPLANT IN HCV-INFECTED PATIENTS**

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**Aim:** To determine the impact of HLA class I & II donor specific alloantibodies (DSA) on fibrosis progression and death in HCV-infected patients after liver transplant (LT).

**Methods:** Since 1985, our biorepository prospectively collects serum samples from donors & recipients in conjunction with a clinical & laboratory research database. 567 consecutive HCV-viremic primary LT recipients without another organ with a clinical & laboratory research database. 567 consecutive protocol serum samples from donors & recipients in conjunction.

**Results:** Patients median age 51, 74% male, 73% Caucasian who were treated with tacrolimus 60% of the time. Patients with preformed class I or class II DSA (MFI>5000) had an increased risk of rejection in the first 6 months (Class I, P=0.04; Class II, P=0.001), an increased risk of HCV PROGRESSION (Class I, P=0.012; Class II, P=0.006), and increased mortality (Class I, P=0.009; Class II, P=0.016) post-LT. Separate stepwise multivariable analyses of preformed class I (HR=1.45; P=0.04) and preformed class II (HR=1.74; P=0.005) DSA found both to be independent predictors of HCV PROGRESSION after controlling for rejection, donor age and race, recipient race, MELD, CMV, and induction. Separate stepwise multivariable analyses of preformed class I (HR=1.63; P=0.07) and preformed class II (HR=1.87; P=0.03) DSA vs. patients without DSA (MFI<1000) found preformed class II DSA an independent risk factor for death (Table).

**In conclusion,** preformed class II DSA increases rejection, accelerates fibrosis, and increases mortality in HCV-infected patients after LT.

**25 LONG TERM – 8 YEAR FOLLOW UP OF A RANDOMIZED TRIAL OF TACROLIMUS MONOTHERAPY VERSUS TRIPLE THERAPY AFTER LIVER TRANSPLANTATION FOR HCV CIRRHOSIS**

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**Background and Aims:** We published early results of a randomised trial of tacrolimus monotherapy versus triple therapy in 103 first transplants for HCV cirrhosis to evaluate the influence of immunosuppression on severity of HCV recurrence. We evaluated differences after 8 years of follow up, including differences in collagen proportionate area (CPA) and in clinical decompensation.

**Methods:** From January 2000 to June 2007, in 3 liver transplant centres (Royal Free Hospital, Edinburgh Royal Infirmary and St Vincent’s Hospital) consecutive transplant recipients older than 18 years receiving cadaveric liver grafts were randomized if they had HCV related cirrhosis.

103 HCV transplanted patients were randomized to tacrolimus monotherapy (MT, n=54) or triple therapy (TT) with tacrolimus (0.1mg/kg/d; divided dose), azathioprine (1mg/kg) and steroids (20mg/day) (n=49), tailing off to zero by 3–6 months. Both groups had serial transjugular biopsies with hepatic venous pressure gradient (HVPG) measurement. Time to reach stage (Ishak) 4 was the predetermined end-point. CPA was measured in all biopsies. All factors documented in the literature as being associated with HCV recurrence were evaluated. Clinical decompensation was defined as whichever occurred first of either, ascites/hydrothorax, variceal bleeding, encephalopathy.

**Results:** No significant pre, peri or post-operative differences between groups. During 95 months median follow up: 10 MT/7 TT died and 5 MT/4 TT were re-transplanted. Stage 4 fibrosis was reached in 24 patients at a median of 30 months: 19MT/10TT with slower fibrosis progression in TT (p=0.009 mantel-cox, p=0.004 breslow). 14 MT versus 3 TT patients reached HVPG≥10mmHg (p=0.002 mantel-cox, p=0.001 breslow); 9 patients in MT versus 2 in TT decompensated. Multivariately, allocated therapy (p=0.009, OR 6.2, 95%CI 1.08–35.5) and histological de novo hepatitis (p=0.0023, OR 0.158, 95%CI 0.032–0.774) were independently associated with decompensation. CPA measured at 1 year post-LT was 4.7% (median) in MT patients vs 3.1% in TT. CPA at last biopsy was 12% in MT vs 8% in TT patients (p=0.004).

**Conclusion:** Long term maintenance immunosuppression with tacrolimus, azathioprine and shorter term prednisolone in HCV cirrhosis recipients resulted in a slower onset of histological severe fibrosis confirmed by Ishak stage and CPA, portal hypertension and less decompensation compared to tacrolimus alone.
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EVEROLIMUS-BASED IMMUNOSUPPRESSION PROVIDES SUPERIOR RENAL FUNCTION AND COMPARABLE EFFICACY VERSUS STANDARD TACROLIMUS IN DE NOVO LIVER TRANSPLANT RECIPIENTS: 24-MONTH RESULTS OF A RANDOMISED CONTROLLED TRIAL
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Background and Aims: The aim of current immunosuppressive strategies is to allow decreased use of calcineurin inhibitors in order to preserve renal function while maintaining the efficacy following transplantation. The H2304 study (NCT00622869) evaluated the efficacy and safety of concentration-controlled everolimus (EVR) to eliminate or to reduce tacrolimus (TAC) vs. standard TAC (TAC-C) in de novo liver transplant recipients (LTxR).

Methods: In this 24-month (M), multicentre, open-label study, 719 de novo LTxR were randomised (1:1:1) on day 30±5 to receive EVR (C0 3–8 ng/ml) with reduced TAC (C0 3–5 ng/ml; EVR+rTAC, N=245) or EVR (C0 6–10 ng/ml) with TAC withdrawal (TAC-WD; N=231) at M4 or TAC-C (C0 6–10 ng/ml; N=243), all with steroids. Composite efficacy failure rate (treated biopsy proven acute rejection rate [tBPAR], graft loss, or death), its components, renal function (estimated glomerular filtration rate [eGFR] by MDRD4), and safety were assessed. Comparison of only EVR+rTAC vs. TAC-C is presented as enrolment into the TAC-WD group (ITT).

Results: At M24, composite efficacy failure rate was comparable between the EVR+rTAC group and the TAC-C group (10.3% vs. 12.5%, p=0.452). Patient and graft survival was also comparable in the two treatment groups. Incidence of BPAR was significantly lower with EVR+rTAC (6.1%) compared with TAC-C (13.3%; p=0.01). Severity of BPAR events (RAI score) was lower in the EVR+rTAC group, with no cases of moderate or severe BPAR vs. 10 in TAC-C group. EVR+rTAC maintained superior renal function vs. TAC-C (ITT population: adjusted mean difference in eGFR change from baseline to M24: 6.66 mL/min/1.73m2 [97.5% CI: 1.9, 11.42]; p=0.0018). For patients who remained on the assigned treatment, the difference in eGFR change was 8.69 mL/min/1.73m2 (97.5% CI: 4.01, 13.36; p<0.0001). Serious adverse events (AEs) were reported in 56.3% patients in EVR+rTAC vs. 54.1% in TAC-C group and 29.8% patients discontinued study drug due to AEs in EVR+rTAC vs. 21.5% in TAC-C group.

Conclusions: Immunosuppression with early EVR-facilitated TAC reduction provides comparable overall efficacy and safety vs. standard TAC with superior renal function sustained for 24 months in LTxR.

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PORTOPULMONARY HYPERTENSION TREATMENT IN ORTHOTOPIC LIVER TRANSPLANTATION ERA
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Introduction: Portopulmonary hypertension (POPH) complicates cirrhosis in about 1–3% of patients. The prognostic role of POPH is not enough established, but it is demonstrated an increased risk of fatal cardiac complications during Orthotopic Liver Transplantation (OLT) for mean pulmonary arterial pressure (PAPm) >35 mmHg and pulmonary vascular resistance (PVR) >3 WU.

Aim: To establish the prognostic significance of POPH and the efficacy of specific therapies in reducing PAPm-PVR values under accepted risk thresholds for OLT.

Material and Methods: We enrolled patients with cirrhosis and POPH, diagnosed by Right Heart Catheterization (RHC) and treated with Sildenafil, Bosentan, Epoprostenol (in monotherapy or in association). RHC was repeated every 4 months, in order to assess response to therapy, defined by PAPm <35 mmHg and/or PVR <3 WU. Patients were followed-up until OLT or death.

Results: Twenty-three patients were included: 13/23 (57%) died, 10 of whom (77%) because of hepatic causes and 3 (23%) because of right heart failure; median follow-up was 944 days (range 111–3050). MELD score at POPH diagnosis discriminates well survivors and non-survivors (16±4 vs 11±3, p=0.005), unlike the presence of signs/symptoms of right heart failure at diagnosis and baseline RHC parameters. Twenty patients were treated and 9 of whom (45%) responded: mean PAPm and PVR values at diagnosis in this subgroup were 45±8 mmHg and 10.4±2.2 WU, after treatment 28±6 mmHg and 3.5±0.9 WU, respectively. These patients had a significantly higher survival rate than others (89% vs 18% p=0.005). MELD score and hemodynamic parameters did not significantly predict response to therapy. The best response rate was found with Bosentan monotherapy (5/7 patients, 71%) and mean response time with this treatment was 162±97 days.

Conclusions: Prognosis of POPH patients is strongly affected by MELD score at diagnosis, and they die mainly because of liver-related causes. Bosentan monotherapy seems to be effective in most patients and to work in about six months, irrespective from baseline hemodynamic parameters. Therefore, in each POPH patient candidate for OLT a therapy should be started whenever possible.

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A PROTEOMIC APPROACH TO IDENTIFY DYSREGULATED LIPID TRANSPORTER PROTEINS WHICH COULD PREDICT THE SEVERITY AND OUTCOME OF PATIENTS WITH ACUTE CHRONIC LIVER FAILURE
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Acute on chronic liver failure (ACLF) is a serious ailment with high mortality and with limited treatment options. It is not known
whether altered levels of various lipid metabolism and transport related proteins could predict the severity of ACLF.

**Aim:** To identify candidate circulating biomarkers using proteomics, to assess the severity and prognosis in ACLF patients.

**Methods:** After depleting major plasma proteins, low abundant protein fraction was trypsin digested, iTRAQ labeled and fractionated using (SCK,RP) HPLC subjected to high resolution mass spectrometry for quantitative expression profiling. Validation of protein expression profiling were performed in; Gr. 1 ACLF (n=40), Gr. 2 compensated cirrhosis (n=20) and Gr. 3 healthy controls (n=20).

**Results:** 305 differentially regulated proteins were identified of which 120 were more than 2 fold differentially regulated. Proteins involved in transport and metabolism of lipids were significantly reduced in Gr. 1 (Pon1, ApoA1, ApoA2, and ApoC3; >2 folds). Pon 1 was significantly reduced in Gr. 1 (25ug/ml), vs. Gr. 2 (45ug/ml). Levels of efflux transporters (Apo A1, Apo A2, Apo C1, Apo C3, Apo B, Apo E) were significantly reduced in Gr. 1 vs. Gr. 2, Gr. 3 (p<0.05). Levels of fPon1 and Apo B were significantly reduced in non survivors compared to survivor in Gr. 1 (p<0.05). Ratios of Pon1/ApoA1, Apo A2, ApoC1 were severely deranged in non survivors in Gr. 1 (p<0.05). The level of Pon 1 and the ratio of Pon 1/A1, A2, C1 correlated inversely with the MELD, SOFA and CTP scores (p<0.05). Moreover, level of Pon1 and the ratio of Pon1/Apo A1, A2 and C1 showed a direct correlation with survival in ACLF patients (p<0.03, r2>0.3).

**Conclusions:** In the ACLF patients, circulating Pon 1 level and the ratio of Pon1/Apo A1, A2, C1 were significantly reduced in the non-survivors compared to survivors. These lipid transporter proteins could serve as biomarkers for assessing the outcome of patients with ACLF.

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**Efficacy of Adipose Tissue-Mesenchymal Stem Cells Transplantation in Rats with Acetaminophen Liver Injury**

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**Background and Aims:** Acetaminophen intoxication is a leading cause of acute liver failure. Liver transplantation for acute liver failure is limited by the availability of donor organs. In this study, we aimed at identifying if the transplantation of adipose tissue-mesenchymal stem cells (ASCs) may exert therapeutic effects on acetaminophen-induced liver injury.

**Methods:** ASCs were isolated from human subcutaneous tissue and were transfected with a green fluorescent protein (GFP). Sprague-Dawley rats were administrated 300mg/kg of acetaminophen intraperitoneally and were transplanted with ASCs or vehicle. After 24 hours from acetaminophen administration, rats were sacrificed. Hepatic levels of isoprostanes, 8-hydroxyguanosine (8-OHG), nitrites/nitrates and reduced glutathione (GSH) were determined as markers of oxidative stress; JNK phosphorylation and hepatic levels of TNF-α, MCP-1, IL-1α and ICAM-1 were also assessed.

**Results:** Transplantation of ASCs decreased AST, ALT and prothrombin time to the levels observed in control rats. Transplanted animals had normal plasma ammonia and did not display clinical encephalopathy. Liver sections of intoxicated rats treated with vehicle showed lobular necrosis and diffuse vacuolar degeneration; in rats transplanted with ASCs liver injury was almost absent. Transplantation of ASCs decreased liver isoprostanes, 8-OHG and nitrite-nitrates to the levels of control rats, while preserving GSH. Consistently, hepatic levels of TNF-α, MCP-1, IL-1α, ICAM-1 and phospho-JNK were markedly increased in rats treated with vehicle and were restored to the levels of controls in animals transplanted with ASCs.

**Conclusions:** In this study, we demonstrated that ASCs transplantation is effective in treating acetaminophen liver injury by a potent antioxidant and anti-inflammatory activity.

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**Arterial Blood Lactate as a Prognostic Marker in Acute Liver Failure: Validation in 1000 Patients**


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**Background:** Arterial blood lactate (ABL) measurements are simply and rapidly determined using point-of-care (POC) testing and may be a useful early prognostic biomarker in patients with acute liver failure (ALF). However, reports of its use are limited and controversial. In a very large cohort of ALF patients, we examined prognostic value of individual and sequential ABL measurements, comparing with standard laboratory measures and in different ALF etiologies.

**Patients and Methods:** 1031 consecutive patients with ALF were studied, 589 (57%) with acetaminophen (APAP) and 442 (43%) with non-APAP ALF. Median age was 36 years (IQR (26–45), INR 3.6 (2.3–6.1); 64% developed encephalopathy ≥ grade 3. 226 (22%) died, 575 (56%) survived with medical care alone and 230 (22%) underwent liver transplantation (LT). ABL was measured on first 3 days of admission using a POC analyser. Test performance was assessed using Receiver Operating Characteristic (ROC) techniques.

**Results:** ABL was higher in patients who died (5.9 mMol/l (IQR 3.2–10.5) or underwent LT (3.9 (2.4–6.8)) than in medical survivors (2.3 (1.5–3.5), p<0.0001) and differences remained highly significant on days 2 and 3 (both p<0.0001). Discrimination between medical survivors and those who died (LT patients excluded) increased over admission; Day 1 Area under the ROC curve (AUROC) for ABL was 0.742 (95%CI 0.69–0.79), day 2 0.789 (0.75–0.83), day 3 0.822 (0.78–0.86) (p<0.01 for comparison of D1 vs. D3, Hanley & McNeil test). Day 1 ABL did not differ when patients with APAP and non-APAP etiologies were compared but discrimination between survivors and non-survivors was better in APAP; AUROC 0.836 (0.79–0.88) vs. non-APAP 0.773 (0.72–0.83).

In APAP patients AUROC was greater than INR (0.673 (0.61–0.73), Creatinine (0.645 (0.59–0.70), bilirubin (0.524 (0.46–0.59) (p<0.0001). Using a 3.5mMol/l ABL threshold in APAP patients, day 1 sensitivity was 78% and specificity 73%. Specificity increased over time and on day 3 was 93%.

**Conclusions:** POC-determined ABL discriminates very well between survivors and non-survivors of ALF. In APAP-related disease it was the best single laboratory predictor of outcome. Persistent elevation of blood lactate is closely associated with a poor outcome and is a highly specific predictor of death.
Parallel Session: LIVER REGENERATION

31 NOVEL METHOD TO ISOLATE AND EXPAND EPCAM+CD24+CD133+ ADULT HEPATIC PROGENITOR CELLS WITH LIVER REPOPULATION CAPACITY

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Background and Aims: In chronic liver injury hepatic progenitor cells (HPCs) can differentiate into cholangiocytes and hepatocytes. HPCs are a potential therapeutic target but HPC isolation and expansion has been challenging. We aim to isolate HPCs which are rapidly expandable and phenotypically stable which can be engrafted as an alternative to liver transplant.

Methods: C57BL/6 mice were supplemented with Choline Deficient Ethionine Supplemented Diets to establish progenitor cells response. HPCs were isolated and purified from liver non parenchymal cells either by an innovative method involving a highly restrictive serum free condition or sorted for EpCAM+CD24+/CD133+/CD45−/CD31−/Ter119− population. HPCs were cultured at clonal density and expanded using a novel diluted trypsin assisted re-plating method. In vitro expanded HPCs were transplanted into recipient mice with liver injury via intrasplenic injection. Livers of recipient mice were analysed for engraftment and functionality of transplanted cells in vivo.

Results: HPCs isolated by both methods are capable of forming clonal colonies. These highly pure HPCs populations express progenitor markers Sox9, Osteopontin, and panCK. Isolated HPCs can be expanded and passaged whilst maintaining their phenotypic stability. We have generated two primary lines from these cells which are able to differentiate along the hepatocyte lineage and are capable of synthesising albumin in vitro; differentiation is concurrent with HPC phenotypic loss. These cells can be separated into three distinct populations (EPCAM+CD24+, EPCAM-CD24+, EPCAM-CD24−), which demonstrate divergent characteristics by transcriptome array and can be used as an in vitro model of cellular hierarchy. Only the EPCAM+CD24+ population is capable of contributing to the other HPC populations. Transplanted HPCs engraft the damaged liver in large numbers and stably contribute to a sizable proportion of the adult parenchyma.

Conclusion: We have developed a novel method to purify and expand HPCs in vitro. These HPCs maintain their phenotypic stability after expansion and are able to contribute to hepatic lineages in vitro, in vivo and are a potential alternative to whole organ transplantation.

32 DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO HEPATIC CELLS AND DEVELOPMENT OF A LIVER TISSUE ON A CHIP

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Background: The therapeutic potential of human pluripotent stem (hPS) cells is threatened, among various problems, by the difficulty to homogeneously direct cell differentiation into specific lineages.

Aims: a. To efficiently derive mature hepatic cells from hPS cells; b. To integrate the specific lineages into a microfluidic platform to obtain a functional liver tissue on a chip.

Methods: Human embryonic stem cells (cell line HES2, National Stem Cell Bank, Madison WI) and induced hPS cells (cell line ADH#1, IECMS, Kyoto University) were grown on mouse embryonic fibroblasts (Chemicon, Temecula, CA) and were differentiated on matrigel. Then we developed a robust multi-stage microfluidic-based technology to efficiently derive mature cells from stem cells. Obtained cells have been fully characterized both with hepatic markers expression (alpha-fetoprotein, cytokeratins 18, 19, albumin, CYP3A) and functional tests (glycogen storage, indocyanine green uptake, albumin secretion).

Results: We efficiently differentiated both human embryonic and induced pluripotent stem cells. Two hepatic lineages were obtained: hepatocyte-like and cholangiocyte-like cells showing high CYP3A expression, indocyanine uptake, glycogen storage and albumin secretion over a 14-day period. This technology, based on frequency-controlled medium delivery in the microfluidic channels, allowed to accurately control hPS cells expansion and fate toward early endoderm commitment, hepatic development and functional maturation on a chip. Compared to conventional cell culture, microfluidic platform allowed shortening of the time required for differentiation, while leading to higher hepatic markers expression and enhanced functional activity. The proportion between hepatocyte- and cholangiocyte-like cells was 3:1. In particular, we obtained 75% of cells with glycogen storage capacity, whereas the number of CYP3A-positive cells resulted in a 59% of the total, with a 20% increase compared to the standard hepatocytes differentiation. Albumin production was about 40% higher than the one observed in standard conditions.

Conclusions: The engineerization of pluripotent cell differentiation into hepatic lineages will allow us to further understand the mechanisms involved in tissue development. Moreover, mature hepatic cells fully integrated on a chip could be directly used for temporal-defined toxicological assays and drug screening.

33 TARGETING THE Hippo PATHWAY TO IMPROVE THE REGENERATIVE CAPACITY OF THE LIVER

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Background: Partial hepatectomy (PH) of 70% of healthy liver provokes quiescent, differentiated adult hepatocytes to re-enter cell cycle and proliferate to replace the lost liver mass. Clinically, patients present with livers that have faulty regenerative potential with limited treatment options. We are exploring a gene therapy approach using in vivo siRNAs to target the Hippo pathway to improve the regenerative capacity of compromised livers. Mammalian Hippo proteins, MST1 & MST2 are protein kinases that target the transcriptional co-activator YAP1 retaining an inactive form in the cytoplasm. Inactivation of MST1& MST2 liberates YAP where it translocates to the nucleus and stimulates cell proliferation. We hypothesize that KD of MST1 & MST2 will push hepatocytes into cell cycle through activation of YAP1.

Methods: Mouse livers were genetically manipulated the using siRNA:liposome technology to knockdown MST1 and MST2 and hepatocyte proliferation was monitored by EdU incorporation. We are exploring a gene therapy approach using in vivo siRNAs to target the Hippo pathway to improve the regenerative capacity of compromised livers. Mammalian Hippo proteins, MST1 & MST2 are protein kinases that target the transcriptional co-activator YAP1 retaining an inactive form in the cytoplasm. Inactivation of MST1& MST2 liberates YAP where it translocates to the nucleus and stimulates cell proliferation. We hypothesize that KD of MST1 & MST2 will push hepatocytes into cell cycle through activation of YAP1.

Results: We have identified siRNA sequences that lead to 89% and 92% knockdown of MST1 and MST2 in mouse liver hepatoma cell line in vitro. In addition, our data shows that siRNA:liposome complexes injected i.v. in vivo resulted in >80% knocked down of...
expression in the liver using FV1 as a control gene target. Using siRNAs targeting MST1 & MST2 coupled with liposomes reduced expression to 72 and 64%, respectively in liver after 72 hours. Efficiency of the KD was confirmed by RT-qPCR and immunoblot. Knockdown of MST1 and MST2 resulted in an increase of YAP nuclear translocation and subsequent hepatocyte proliferation measured by incorporation of EdU and Ki67 immunostaining. Moreover, after MST1 and MST2 knockdown there was a 3-fold increase in the YAP target gene, BIRC5/surviving in the liver.

**Conclusion:** In conclusion, knockdown of MST1 & MST2 using siRNAs coupled with liposomes provoked hepatocyte proliferation in wild-type mice. And finally to determine if targeting MST1 & MST2 is clinically relevant, we will demonstrate that there is impairment of these protein/signalling pathways in diseased or include healthy mice. And finally to determine if targeting MST1 & MST2 using siRNAs coupled with liposomes provoked hepatocyte proliferation in wild-type mice. And finally to determine if targeting MST1 & MST2 is clinically relevant, we will demonstrate that there is impairment of these protein/signalling pathways in diseased or include healthy mice.

34 TGR5 PROTECTS THE LIVER FROM BILE ACID OVERLOAD DURING LIVER REGENERATION IN MICE

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**Background and Aims:** Many regulatory pathways are involved in liver regeneration after partial hepatectomy (PH), to initiate growth, protect liver cells and sustain functions of the remnant liver. Bile acids (BA) rise in the blood early after PH and stimulate both hepatocyte proliferation and protection, through their binding to the nuclear receptor FXR. However, the impact of the membrane bound BA receptor TGR5 (G Protein-Coupled BA Receptor 1, GPBAR1) after PH in mice remains to be studied.

**Methods:** Liver histology (H&E, neutrophil staining), hepatocyte proliferation (phosphorylated histone 3), BA concentrations (HPLC-tandem MS) in plasma, bile, urine and liver, bile flow and composition, and cytokine production (Lumexin) were studied in wild type (WT) and TGR5-KO mice, before and after PH. Bile duct ligation (BDL), cholic acid (CA) or cholestyramine (CT)-enriched diet, and clodronate liposomes (Kupffer cell depletion) treatment were also performed.

**Results:** Basal BA composition (plasma, liver, bile, urine, stools) was more hydrophobic in TGR5-KO than in WT mice. After PH, hepatocyte necrosis (“bile infarcts”), prolonged cholestasis (plasma and liver BA overload), and exacerbated inflammatory response (cytokine overproduction, hepatic neutrophil infiltration) were observed in TGR5-KO mice, as well as a delayed regeneration (liver mass restoration, mitotic index). Hepatocyte adaptive response to post-PH BA overload (CYP7a1 and Ntcp mRNA downregulation, Ostb mRNA upregulation) was similar in WT and TGR5-KO mice. However, kidney (BA elimination in urine) and biliary (enhancement of bile flow and bicarbonate secretion) adaptive responses to PH were impaired in TGR5-KO as compared with WT mice. After BDL or upon cholic acid (CA)-enriched diet, TGR5-KO mice exhibited more severe liver injury with neutrophil infiltration, and impaired BA elimination in urine as compared with WT controls. The post-PH TGR5-KO mice phenotype was completely rescued by CT treatment, and improved by Kupffer cell depletion.

**Conclusion:** TGR5 is crucial for liver protection against BA overload after PH, primarily through the control of bile hydrophobicity and cytokine secretion. In the absence of TGR5, intrahepatic stasis of abnormally hydrophobic bile and excessive inflammation, in association with impaired bile flow adaptation and deficient urinary BA efflux, lead to BA overload-induced liver injury and delayed regeneration.

35 LOSS OF CASPASE-8 IN HEPATOCYTES ACCELERATES THE ONSET OF LIVER REGENERATION IN MICE THROUGH PREMATURE NF-κB ACTIVATION

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**Background and Aims:** The cytokine TNF-α (TNF) plays a critical role early in liver regeneration following partial hepatectomy (PH). TNF stimulates at least three different pathways leading to NF-κB activation, apoptosis signaling via caspase-8 (Casp8) and activation of cjun N-terminal kinases (JNK). The present study aims to better define the role of Casp8 during liver regeneration.

**Methods:** We performed partial hepatectomy in mice lacking Casp8 specifically in hepatocytes (Casp8CreERT2) and determined their liver regeneration capacity by measuring liver mass restoration and kinetics of cell cycle markers cyclin D (G1-phase), E (G1/S-phase) and A (S-phase). The immediate response to liver resection was evaluated by analyzing NF-κB and JNK dependent signaling pathways.

**Results:** Casp8CreERT2 mice showed an accelerated onset of DNA synthesis after PH, signs of delayed hepatocyte mitosis, but overall normal liver mass restoration. Analysis of immediate TNF dependent signaling pathways revealed premature activation of NF-κB and JNK/cjun related signals. In order to define the role of NF-κB in this setting we blocked NF-κB activation in these mice by concomitant inactivation of the NF-κB Essential Modulator (NEMO) in hepatocytes (NEMO*+/cre). Lack of NEMO largely reverted aberrant DNA synthesis in Casp8CreERT2 mice but resulted in incomplete termination of the regeneration process and hepatomegaly.

**Conclusion:** Casp8 comprises a non-apoptotic function during liver regeneration by balancing NF-κB and JNK activation. While loss of Casp8 triggers NF-κB activation and thus improves liver regeneration, combined loss of Casp8 and NEMO impairs a controlled regenerative response and drives hepatomegaly.

36 NCAM AND PSA-NCAM MODULATE HEPATIC PROGENITOR CELL BEHAVIOR DURING LIVER DEVELOPMENT AND REGENERATION

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**Background and Aims:** NCAM is a hepatic progenitor cell (HPC) and liver cancer marker but its function is unknown. We therefore investigated its role in liver development and HPC mediated liver regeneration. NCAM mediates interactions between cells and surrounding matrix. Polysialic acid (polySia) is an unusual post-translational modification of NCAM.

**Methods:** The post-translational modification of NCAM with polySia (PSA-NCAM), and the role of the polySia synthesising enzymes SThSial and StbSialV were examined in HPCs in vivo using the CDE and DDC diet models of liver injury and regeneration, in vitro using models of proliferation, differentiation and migration and by use of mouse models with gene defects in the polysialyltransferases (STbSia 2+−4+), and StbSia 2+−4−. Results: During liver development polySia is required for the correct formation of bile ducts and portal tracts as both StbSia 2+−4−, and StbSia 2+−4− mice had abnormal portal tracts and bile ducts.
In adult liver, bile duct cells, HPCs and their surrounding cells including activated stellate cells were NCAM positive. Following liver damage and HPC activation, NCAM+ve cells expanded from the HPC niche, and gene expression of Ncam and St8sia4 significantly increased. In vitro studies using HPC cell lines and hepatic mesenchymal cell lines (both NCAM+ve) revealed that polySia was mainly produced by HPCs through the enzyme St8siaV. The polySia cleaving enzyme Endo-N weakened the cell–cell and cell-matrix bonds which accelerated HGF induced cell migration. Differentiation of HPC to hepatocytes resulted in cleavage of NCAM via the NCAM cleaving enzymes ADAMs 8 and 10. Conclusions: PolySia production is required for normal liver development. In the normal liver NCAM supports intercellular contacts of HPCs as well as binding of HPCs to the surrounding matrix. During regeneration, the polySia addition to NCAM weakens cell–cell and cell-matrix interactions thus allowing HPC to migrate. ADAMs 8 and 10 mediated NCAM cleavage from HPCs occurs during HPC-hepatocyte differentiation.

**Background and Aims:** Liver progenitor cells (LPC) proliferate in response to liver injury when the regenerative capacity of hepatocytes is impaired. However, their role in tissue repair and their contribution to newly generated hepatocyte is still controversial. The aim of this study is to assess the contribution of LPC to liver regeneration by tracing Hepatocyte Nuclear Factor (Hnf)α+ biliary cells.

**Methods:** HNF1α expression was evaluated in human livers. The fate of Hnf1α+ biliary cells was assessed in Hnf1αCreER/Rosa26R mice. Mice were treated with tamoxifen to induce persistent Co-expression of HNF1α and EpCAM was observed in ductular reaction cells from human cirrhotic liver biopsies. In mouse intact liver, HNF1α expression was restricted to the biliary compartment. As expected, LPC expansion was mild in the PH mouse model and absent in acute liver injury. By the contrary, DDC and CDE-treated mice showed an enhanced adhesion and proliferation of HPCs on laminin in an extracellular laminin regulates the homeostatic balance between proliferation and differentiation in the HPC population. Galectin 3 (Gal-3) is a 30kDa glycoprotein belonging to the β-galactoside-binding lectin family that is upregulated in injured liver in mice and humans. Gal-3 binds to integrins and regulates adhesion to laminin. It was the object of this study to examine the role of Gal-3 in liver regeneration and HPC activation. HPC activation was studied following hepatocellular injury induced by administration of a choline deficient, ethionine supplemented (CDE) diet and biliary injury induced by 3,5-diethoxycarbonyl-1,4-dihydridrocollidine (DCC) supplemented diet in wild type and Gal-3−/− mice. HPC expansion was significantly reduced in Gal-3−/− mice with reduced ductular reaction following CDE and reduced ductular proliferation, cholangitis and periductal fibrosis following DCC diet. Gal-3−/− mice failed to form a HPC niche, with reduced laminin formation. In vivo HPCs secrete Gal-3 which enhanced adhesion and proliferation of HPCs on laminin in an undifferentiated form; effects that were attenuated in Gal3−/− HPCs and following treatment with the Gal-3 inhibitor lactose. Gal-3−/− HPCs differentiated prematurely and regulated cell cycle genes leading to cell cycle arrest. We conclude that Gal-3 is required for the undifferentiated expansion of HPCs in their niche in injured liver.

**Conclusions:** Hnf1α biliary cells can give rise to ductular reaction cells with LPC characteristics as well as to periportal hepatocytes. However, under these experimental conditions the biliary compartment contributes minimally to the regeneration of the liver parenchyma. These results support the hypothesis that although LPC participate in liver regeneration and can generate mature hepatocytes, hepatocytes are the main source of regenerating cells.
GENOME-WIDE ASSOCIATION STUDY IDENTIFIES NEW VARIANTS ASSOCIATED WITH THE RISK OF CHRONIC HEPATITIS B


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Background and Aims: Hepatitis B virus (HBV) infection is the predominant risk factor for chronic hepatitis B (CHB), liver cirrhosis, and hepatocellular carcinoma. Recently, several genome-wide association studies (GWAS) of CHB identified human leukocyte antigen (HLA) loci, including HLA-DP and HLA-DQ in Asian populations, as being associated with the risk of CHB. To confirm and identify the host genetic factors related to CHB infection, we performed another GWAS using a higher-density chip in Korean CHB carriers.

Methods: We analyzed 1,400 samples from Korean population (400 CHB cases and 1,000 population controls) using a higher-density GWAS chip (1,140,419 single nucleotide polymorphisms (SNPs)). In subsequent replication analysis, we further analyzed in an independent study of a Korean CHB cohort consisting of 2,909 Korean samples (971 cases and 1,938 controls). Logistic regression methods were used for statistical analysis adjusting age and sex as covariates.

Results: This study identified two new risk-associated loci for CHB on the HLA region of chromosome 6, e.g., rs652888 on euchromatic histone-lysine-methyltransferase 2 (EHMT2, \( P = 7.07 \times 10^{-13} \)) and rs1419881 on transcription factor 19 (TF19, \( P = 1.26 \times 10^{-18} \)). Conditional analysis with nearby HLA CHB loci that were previously known, confirmed the independent genetic effects of these two loci on CHB. In an independent Korean CHB cohort, validation analysis also showed strong associations with CHB. Combined analysis of a fixed-effects model demonstrated strong significant associations of these two SNPs (OR=1.38, \( P = 7.07 \times 10^{-13} \) for rs652888 of EHMT2; OR=0.73 (\( P = 1.26 \times 10^{-18} \)) for rs1419881 of TCF9).

Conclusions: Genome-wide association study and subsequent validation study identified new variants associated with the risk of chronic hepatitis B. These findings may advance the understanding of genetic susceptibility to CHB.
Institute of Gastroenterology and Hepatology, Songklanagarind
HBsAg < 2000 IU/mL
HBsAg clearance 12/234 (5) 5/77 (6) 0.7725 10/126(8) 10/223 (4) 0.2305

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CHRONIC HEPATITIS B ACCORDING TO ASIAN AND CAUCASIAN
OF PEGINTERFERON alfa-2a (40KD) IN 1233 PATIENTS WITH
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S18 Journal of Hepatology
vol. 58 | S1–S24
2013

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Introduction: S-Collate is a multinational, prospective, observational cohort study investigating on-treatment predictors of hepatitis B surface antigen (HBsAg) clearance in peginterferon alfa-2a (Peg-IFNa-2a) (40KD)-treated chronic hepatitis B (CHB) patients. 1802 patients were treated with Peg-IFNa-2a. This analysis reports outcomes 6 months post-treatment in CHB patients according to Asian/Oriental or Caucasian race.

Methods: Patients treated with Peg-IFNa-2a for a maximum of 1 year were included in the analysis (N = 1233). Response rates were calculated for patients with available measurements 6 months post-treatment.

Results: Of 679 Asian patients, the majority were HBsAg-positive (N = 455, 67%). Of 554 Caucasians, the majority were HBsAg-negative (N = 417, 75%). In patients with known mode of infection (N = 444), perinatal transmission was more prevalent in Asians (75–84%) than Caucasians (25–33%). Mean age was similar between Asians and Caucasians; 30–31 years for HBsAg-positive patients and 37–39 years for HBsAg-negative patients. Response rates 6 months post-treatment were similar between Asians and Caucasians in both HBsAg-positive and HBsAg-negative patients for most endpoints (Table). In Asian patients, who are considered difficult to treat, 65% and 57% of HBsAg-negative patients achieved HBV DNA <2000 IU/mL and HBsAg < 2000 IU/mL + ALT normalization, respectively; these rates were significantly higher than in HBsAg-negative Caucasians (Table). Early on-treatment HBsAg level/decline was a predictor of response, irrespective of race and HBsAg status. For example, in HBsAg-positive patients, HBsAg ≤20,000 IU/mL at week 24 resulted in higher rates of HBsAg loss + HBV DNA <2000 IU/mL than HBsAg >20,000 IU/mL (Asian: 24% [29/123] vs 5% [1/19]; Caucasian: 60% [3/5] vs 14% [2/14]). However, low availability of on-treatment measurements, resulting in small patient numbers, was a limiting factor. Rates of adverse and serious adverse events were comparable across groups, with 5% of Asians and 4% of Caucasians withdrawing treatment due to adverse events.

Conclusions: In a ‘real-life’ setting, response rates to Peg-IFNa-2a were overall similar between Asians and Caucasians; however, significantly more HBsAg-negative Asian patients achieved HBV DNA <2000 IU/mL ± ALT normalization 6 months post-treatment. Peg-IFNa-2a was well-tolerated, regardless of race.

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PREDICTING POST PARTUM HEPATITIS B VIRUS FLARES USING INNATE IMMUNE BIOMARKERS
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Aim: To examine innate immune function in chronically infected HBV pregnant women during and after pregnancy and correlate it with post-partum hepatic flares, +/- antiviral medications used to prevent vertical transmission.

Background: Maternal tolerance to fetal antigens, gives way, post partum to immune system reconstitution and consequently in HBV infection to immune mediated hepatic flares, which can be life threatening to the mother. The characteristics of these immune changes and their impact and association with a variety of innate immunological and virological parameters is not well established.

Methods: Plasma and PBMCs were collected from patients at two sites at 5 different time points; first half of pregnancy, 3rd Trimester, 6 weeks post partum, 3 months post partum and 1 year post partum. TLR expression was measured on monocytes, NK cells and NK T cells by flow cytometry. PBMCs were also stimulated with TLR ligands LPS (TLR4), Pam3Cys (TLR2), CpG2006 (TLR9), PolyC (TLR3), R848 (TLR7/8) and assayed for cytokines by ELISA. NK cells (CD56 bright and 10A expression) were also examined to see if they are activated and able to kill hepatocytes using a TRAIL mediated mechanism. HBV viral load, quantitative HBeAg and HBsAg were also done at each timepoint.

Results: 127 women were recruited 23% were HBeAg positive and 28% had a hepatitis flare (>2 ULN). Our results of 101 patients demonstrate elevated TLR2 expression in both monocytes (p < 0.02) and NK cells (p < 0.01) with increased specific TLR2 (P < 0.04) cytokine production, occurring in the third trimester in patients who developed post partum flares, with and without an increase in
viral load. Activated NK cells overexpressed TRAIL and conditioned media from these patients caused caspase associated apoptosis of both hepatic cell lines and primary hepatocytes. **Conclusions:** These data demonstrate the importance of innate immune responses in relation to pregnancy-associated HBV flares. Cytokines may be driven by various TLRs and in turn activate TRAIL mediated mechanisms of apoptosis of hepatocytes leading to fibrosis. These results can also help elucidate the underlying mechanisms of HBV flares and serve as a model for dynamic alterations in ALT elevations and liver inflammation of HBV-associated flares in general.

**43 LONG TERM TENOFOVIR DISOPROXIL FUMARATE (TDF) THERAPY AND THE RISK OF HEPATOCELLULAR CARCINOMA**

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**Background:** Efficacy trials have shown that antiviral therapy improves short to intermediate term outcomes in patients with chronic hepatitis B, such as HBV DNA suppression, HBe seroconversion and regression of fibrosis and cirrhosis. The effect of antiviral therapy on incidence of HCC has not been well established.

**Aim:** To examine HCC incidence using a prediction model.

**Methods:** The incidence of HCC in patients treated with TDF was obtained from the 6-year follow-up data of the registration trials for HBeAg-positive (GS-US-174–0103) and HBeAg-negative (GS-US-174–0102) patients. The predicted risk of HCC in individual patients was estimated using a model validated in cirrhotics and non-cirrhotics (the REACH-B model: Yang et al., Lancet Oncology, 2011). Standardized incidence ratios [SIR] were calculated between the observed and predicted numbers of HCC in the study cohort.

**Results:** In the two studies, 641 patients received TDF for 6 years (375 subjects in study 102 and 266 in 103). During this time, 13 cases of HCC were reported. Nine were HBeAg– at baseline; among them 3 were cirrhotic. Four were HBeAg+ at baseline; among them 3 were cirrhotic. From the 13 HCC cases, 4 were genotype (gt) C, 5 gt-D, 1 gt-B, 1 gt-E, 1 gt-F and 1 unable to genotype. In the figure, the arrow indicates the 10th HCC case which occurred at 3.3 years, at which time the REACH-B model predicted 11.2 cases. Beyond that time point, there was a progressive divergence between the predicted and observed number of HCC cases. The SIR was 0.94 (95% confidence interval [CI]=0.47–1.88) at 2.4 years, 0.89 (95%CI=0.48–1.66) at 3.3 years and 0.55 (95%CI=0.32–0.94) at the latest follow-up (median 5.52 years).

**Conclusion:** Based on the REACH-B risk calculator, after long term therapy with TDF, the incidence of HCC decreased compared to the predicted risk.

**44 PERFORMANCE OF HEPATOCELLULAR CARCINOMA RISK SCORES IN CHRONIC HEPATITIS B PATIENTS RECEIVING ENTECAVIR TREATMENT**


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**Background and Aims:** Several risk scores have been developed to predict hepatocellular carcinoma (HCC) in untreated chronic hepatitis B patients. Their performance in patients receiving antiviral therapy and the clinical significance of on-treatment changes in the risk scores are unknown. This study tested the accuracy of these scores before and during entecavir treatment in predicting HCC development.

**Methods:** All patients who had received entecavir treatment for at least 12 months were prospectively followed. Three reported risk scores (CU-HCC, GAG-HCC and REACH-B) were calculated at baseline and 2 years after entecavir treatment. The primary outcome was HCC development at 5 years.

**Results:** Among 1531 entecavir-treated patients (age 51±12 years, 71.7% males, 21.7% clinical cirrhosis), 47 (2.9%) patients developed HCC after a mean follow-up of 42±13 months. Old age, cirrhosis and failed complete hepatitis B virus DNA suppression were independent factors associated with HCC development by Cox regression analysis. The area under the receiver operating characteristics curves of the baseline CU-HCC, GAG-HCC and REACH-B scores to predict HCC at 5 years was 0.81 (95% CI 0.75–0.87), 0.76 (0.70–0.83) and 0.70 (0.60–0.80), respectively. The corresponding sensitivities and specificities were 93.6% and 47.3% for CU-HCC, 55.3% and 80.2% for GAG-HCC, and 95.2% and 15.9% for REACH-B scores. The on-treatment scores had similar accuracies in predicting HCC at 5 years. None of the patients with baseline CU-HCC score below the cutoff of 5 points developed HCC. In contrast, HCC occurred in 12.2% of patients with CU-HCC score persistently above 5 (P<0.001 compared to those below 5) and 3.9% of those with score decreasing from above to below 5 after entecavir treatment (P=0.007).

**Conclusions:** The CU-HCC score has high sensitivity in predicting HCC in chronic hepatitis B patients receiving entecavir treatment. Complete viral suppression is important in HCC prevention. An improvement in CU-HCC score during entecavir treatment reduces but does not eliminate the risk of HCC.

**Acknowledgements:** This study was partially supported by the Research Fund for the Control of Infectious Diseases of the Hong Kong SAR Government (Ref 11100372) and the Direct Grant of The Chinese University of Hong Kong (Ref 2041703).
**ORAL PRESENTATIONS**

### 45 IMMUNE EVASION OF HEPATITIS DELTA FROM CD8+ T CELL IMMUNE RESPONSE

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**Background and Aims:** The immunopathology of hepatitis delta virus (HDV) infection remains unclear. However, CD8+ T cell response seems to play a key role in the infection's outcome, as it does in many other viral infections. A high genetic diversity was observed in the genotypes. In the presence of cytotoxic T lymphocyte (CTL) immune response, those viruses with mutations within MHC class I restricted epitopes are able to evade effector T cell recognition, establishing chronic infection.

In the light of these observations, the aims of the study were: analysis of the genotype(s); characterization of the variability of the large hepatitis delta antigen (L-HDAg); identification of the immune escape of HDV from CD8+ T cell response and, finally, a thorough comprehension of the immunopathogenesis of HDV and the role of selected mutants under immune pressure.

**Methods:** 130 chronically infected HDV patients from 6 different collaborating centers were enrolled in this study. The whole large hepatitis delta antigen (L-HDAg) of these patients was amplified, sequenced and genotyped. HLA typing of the subjects and computational prediction of the potential epitopes within the L-HDAg were performed. The mutation rates in the predicted and previously identified epitopes were compared in the presence and the absence of the given HLA allele.

**Results:** All sequences were branched under genotype one. HLA typing of PCR positive patients showed normal allele distribution, compared with the frequency of HLA class I alleles in the general European population. Preliminary results indicate a higher frequency of mutations in an identified HLA-A02 epitope and in a predicted HLA-A24 epitope in HLA-A02 and HLA-A24 positive patients, respectively, than in negative ones. Currently we are analyzing the sequential samples of some chosen patients to determine the adaptation of the virus to the host over time.

**Conclusions:** The results suggest that CD8+ T cell response-associated selection pressure plays an important role in the infection's progression to the chronic phase and in the evolution of circulating HDV isolates. Genetic diversity, resulting in rapid mutations over a short period of time, has hampered attempts to develop potential therapeutic vaccines for use in the chronic phase of HDV infection.

### 46 LONG-TERM FOLLOW-UP AFTER PEG-IFNa2a-BASED THERAPY OF CHRONIC HEPATITIS DELTA

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**Background:** Interferon alpha is the only effective treatment option for hepatitis delta. Recent trials investigating the efficacy of pegylated interferon alfa (PEG-IFNa) showed HDV-RNA negativity rates of 25–30% 24 weeks after therapy. However, the clinical and virological long-term outcome of HDV-infected patients treated with PEG-IFNa is unknown.

**Methods:** We performed a retrospective-prospective follow-up of patients treated in the HIDIT-1 trial (NEJM 2011;364:322–31). Patients had been treated for 48 weeks with either PEG-alfa-2a plus adefovir dipivoxil (ADV) (Group-I), PEG-IFN-alfa-2a alone (Group-II), or adefovir dipivoxil alone (Group-III). Long-term follow-up data of patients who completed 24 weeks of post-treatment observation in the HIDIT-1 trial were available for 18 (75%), 19 (76%), and 21 (81%) of patients in groups I, II and III with a median time of follow-up of 3.9 (0.5–5.3), 4.2 (0.4–5.4), and 4.2 (0.4–5.5) years.

**Results:** Clinical endpoints (death, liver transplantation, hepatic decompensation, HCC) during post-treatment follow-up were observed in 2 (11%), 1 (5%), and 3 (14%) patients, respectively with an overall annual event rate of 2.4%. Patients in group-III received significantly more often (re-) treatment with PEG-IFNa (n=10; 48%) than in patients in group I and II (17% and 26%; p=0.04). Patients initially treated in the PEG-IFNa arms had significantly more often low HBV-DNA levels at the last follow-up visit compared to patients treated with ADV alone (94% and 58% vs. 43%; p=0.012). HBsAg observed in 2 (11%), 1 (5%), and 3 (14%) patients, respectively with an overall annual event rate of 2.4%. Patients in group-III received significantly more often (re-) treatment with PEG-IFNa (n=10; 48%) than in patients in group I and II (17% and 26%; p=0.04). Patients initially treated in the PEG-IFNa arms had significantly more often low HBV-DNA levels at the last follow-up visit compared to patients treated with ADV alone (94% and 58% vs. 43%; p=0.012). HBsAg tested negative in 6 patients until the end-of-follow-up (4 group-I, 2 group-II). 16 patients who were HDV-RNA negative 6 months after PEG-IFNa treatment entered the follow-up study (8 Group-I and 8 Group-II). Out of these, 8 individuals (3 group-I and 5 group-II) tested HDV-RNA positive at least once during further follow-up with 7 patients being HDV-RNA positive at the most recent visit.

**Conclusions:** The annual post-treatment rate of clinical events in hepatitis delta patients eligible for PEG-IFNa therapy is about 2–3%. A close monitoring after therapy is recommended even in patients being HDV-RNA negative 6 months after PEG-IFNa-based treatment as late relapses may occur. We are currently investigating if these cases represent real late HDV-RNA relapses or de novo infections.
47 TESTING FOR HEPATITIS B VIRUS (HBV) ALONE DOES NOT INCREASE VACCINE COVERAGE IN NON-INFRINGEMENTS
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Background and Aims: Despite low prevalence of HBV infection in France, 2500 new infections occur each year – the majority of which were preventable with vaccination. HBV testing could serve as an occasion to screen and vaccinate non-immunized individuals. The objective of this study was to describe current practices of vaccination for non-immunized subjects at-risk for HBV-infection after HBV testing.

Methods: Participants were recruited from two multi-center HBV-testing campaigns in Paris, France (Phase 1: 2011 and Phase 2: 2012). Non-immunized subjects were identified and determined if indicated for HBV-vaccination (based on French guidelines). Subjects were then contacted via telephone 3–9 months after testing and asked questions on their decision to vaccinate or not.

Results: Of 4924 subjects with results on all HBV markers [ELISA: Hepatitis B surface (HBs) antigen, anti-HBs antibodies, anti-Hepatitis B core antibodies]. 1898 (38.0%) had not been immunized. Among them, 1645 (86.7%) were indicated for HBV-vaccination. A total of 840 subjects were successfully contacted, of whom 97 (11.5%) had been vaccinated against HBV after screening. Sub-group analysis indicated a stark contrast in vaccination coverage across centers, ranging from 0–56%. Reasons explaining vaccine refusal were more associated with physician-patient motivation rather than direct opposition to vaccination, regardless of study phase (Table 1).

Table 1 Reasons for non-vaccination, n (%) 2011 (N=423) 2012 (N=320) Total (N=743)

<table>
<thead>
<tr>
<th>Reason</th>
<th>2011 (N=423)</th>
<th>2012 (N=320)</th>
<th>Total (N=743)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did not return for results</td>
<td>65 (15.4)</td>
<td>34 (10.6)</td>
<td>99 (13.3)</td>
</tr>
<tr>
<td>Vaccination not proposed by physician</td>
<td>134 (31.7)</td>
<td>23 (7.2)</td>
<td>157 (21.4)</td>
</tr>
<tr>
<td>No indication for vaccination according to physician</td>
<td>33 (11)</td>
<td>11 (3.4)</td>
<td>44 (5.9)</td>
</tr>
<tr>
<td>Patient did not want vaccination</td>
<td>78 (18.4)</td>
<td>144 (45.0)</td>
<td>222 (29.8)</td>
</tr>
<tr>
<td>Perceived to have no risk-factors</td>
<td>9 (2.1)</td>
<td>80 (25.0)</td>
<td>89 (22.0)</td>
</tr>
<tr>
<td>Perceived to already have vaccination</td>
<td>9 (2.1)</td>
<td>11 (3.4)</td>
<td>20 (2.7)</td>
</tr>
<tr>
<td>Unfavorable opinion of vaccinations in general</td>
<td>20 (4.7)</td>
<td>22 (6.9)</td>
<td>42 (5.7)</td>
</tr>
<tr>
<td>Unfavorable opinion of HBV-vaccination</td>
<td>41 (9.7)</td>
<td>40 (12.5)</td>
<td>81 (10.9)</td>
</tr>
<tr>
<td>Have not yet received vaccination, but still considering it</td>
<td>138 (32.6)</td>
<td>115 (35.9)</td>
<td>253 (34.1)</td>
</tr>
</tbody>
</table>

Conclusions: HBV-vaccination after HBV screening was very low in this study, which was largely due to physician-patient motivation towards vaccination. Increased vaccination coverage might be achieved by emphasizing its need at the organizational level.
Conclusions: Patients with compensated cirrhosis (F0–F2) can expect a mean lifetime cost of €1,030, or €33,590 in patients with mild fibrosis (F0) at mean age 47. As disease progresses, the costs increase, with €21,500 (F1 at mean age 45), €35,350 (F2 at mean age 51), and €49,820 (F3 at mean age 56). However, because 50% (F4) as compared to no treatment in HCV mono-infected adult patients, the incremental cost of treating patients with compensated cirrhosis (F0–F2) is €32,520, increasing to €102,340 for patients with advanced liver disease (F3–F4). Following French treatment guidelines (treatment in ≥F2), triple therapy with telaprevir or boceprevir incurred immediate costs of about €37,000, with patients with F4 disease attaining a mean cost of €73,370 (cirrhosis at mean age 59). Following French treatment guidelines with triple therapy (telaprevir or boceprevir containing regimen), the cost of treating patients with compensated cirrhosis (F0–F2) was €33,590 (F0 at mean age 47); €36,280 (F1 at mean age 45), €49,820 (F2 at mean age 51); and €56,370 (cirrhosis at mean age 59). Following French treatment guidelines with triple therapy (telaprevir or boceprevir containing regimen), the cost of treating patients with compensated cirrhosis (F0–F2) was €33,590 (F0 at mean age 47); €36,280 (F1 at mean age 45), €49,820 (F2 at mean age 51); and €56,370 (cirrhosis at mean age 59).

50 IS ADDING HCV SCREENING TO THE ANTENATAL NATIONAL SCREENING PROGRAM IN AMSTERDAM, THE NETHERLANDS COST-EFFECTIVE?

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Introduction: Hepatitis C virus infection (HCV) can lead to severe liver disease. Recently new improved treatment options have been introduced. Pregnant women are already routinely screened for several infectious diseases, however not yet for HCV infection. Here we examine whether adding HCV screening to routine screening is cost-effective.

Methods: To estimate the cost-effectiveness of implementing HCV screening of all pregnant women and HCV screening of first generation non-Western pregnant women compared to no screening, a Markov model was developed. This model was parameterized with prevalence data from pregnant women retrospectively tested for HCV in Amsterdam, the Netherlands, and literature sources. In addition, we estimated the effect of possible treatment improvement in the future.

Results: Screening all pregnant women resulted in the incremental cost per women screened of €41,0008 life years gained, and thus an incremental cost-effectiveness ratio (ICER) of €52,473 which is above the cost-effectiveness threshold of €50,000. For screening first generation non-Western migrants, the ICER was €47,113. Best case analysis for both scenarios showed ICERs of respectively €19,505 and €17,533. We estimated that if costs per treatment will decline to €3750, screening all pregnant women will be cost-effective.

Conclusions: In the current situation, adding HCV screening to the already existing screening program for pregnant women is not cost-effective for women in general. However, adding HCV screening for first generation non-Western women shows a moderate cost-effective outcome. Yet, best-case analyses shows potentials for an ICER below €20,000 per life-year gained. Treatment options will improve further in the next coming years enhancing cost-effectiveness even more.

51 HCV TREATMENT RATE IN SELECT EUROPEAN COUNTRIES IN 2004–2010

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Background and Aims: With the launch of the new direct acting antiviral (DAA) therapies targeting hepatitis C virus (HCV) infection, there is an expectation that more HCV patients will be treated. However, little data is available on the historical treatment rate prior to the launch of DAs. The objective of this study was to determine the annual number of HCV treated patients by country and the corresponding treatment rate in 2004–2010. The study was limited to 22 European countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, and United Kingdom) where reliable data was available.

Methods: The number of pegylated interferon units was gathered by country and year. The units were adjusted for uses in non-HCV indications and for under-reporting. The average number of units per patient per year was calculated by gathering genotype distribution in each country, the duration of treatment for each genotype, and the average Peg/Riba therapy persistence. The annual number of treated patients was estimated using the adjusted units.
sold and the average number of units per patient per year. The treatment rate was estimated by also considering the HCV viremic population size.

Results: Two countries, Russia and Italy, accounted for more than half of the HCV infected population in the countries studied. France consistently had the highest treatment rate in Europe followed by Germany and Sweden. Eastern European countries had the lowest treatment rate in the region. The number of treated patients in Russia increased eight fold between 2004 and 2010. However, the overall HCV prevalence remained over 2 million resulting in low overall treatment rate.

Conclusions: There was significant variability in treatment rate (0.1%-6.1%) in Europe between 2004–2010. Increases in treatment rate and prevention is required in Eastern European countries to offset new infections and to reduce the total number of HCV infections over time.

52 INTRAHEPATIC CHOLESTASIS OF PREGNANCY AND PREEXISTING OR FUTURE HEPATOBILIARY DISEASES: A POPULATION-BASED COHORT STUDY OF 125,281 SWEDISH WOMEN SHOWS STRONG ASSOCIATIONS WITH HEPATITIS C

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Background and Aims: Intrahepatic cholestasis of pregnancy (ICP) is the most common liver disease in pregnancy and is associated with impaired fetal outcome. We aimed to estimate the risk of the mother for later diagnosis of hepatobiliary diseases and the odds for women with prevalent hepatobiliary diseases to develop ICP.

Methods: We analyzed data of women with first and consecutive births between 1973 and 2009 registered in the Swedish Medical Birth Register. By linkage with the Swedish Patient Register, we identified 11,936 women with ICP who were matched to 113,942 women without this diagnosis. Diagnosis of preexisting or later hepatobiliary disease was obtained from the Patient Register. Main outcome measures were hazard ratios (HRs) for later hepatobiliary disease in women with ICP at <1 year, 1–5 years, >5 years after delivery and odds ratios (ORs) for developing ICP in preexisting hepatobiliary disease. Risk estimates were calculated through Cox regression and logistic regression analysis.

Results: Women with ICP were more often diagnosed with later hepatobiliary disease (HR 2.63; 95%CI 2.72–2.77; increment at 1% per year), hepatitis C or chronic hepatitis (HR 4.16; 3.14–5.51 and 5.96; 3.43–10.33, respectively), fibrosis/cirrhosis (HR 5.11; 3.29–7.96), gallstone disease or cholangitis (HR 2.72; 2.55–2.91, and 4.22; 3.13–5.693, respectively) as compared to women without ICP (p < 0.0001 for all HRs). Later ICP was more common in women with prepregnancy hepatitis C (OR 5.76; 1.30–25.45; p = 0.021), chronic hepatitis (OR 8.66; 1.05–71.48; p = 0.045), and gallstone disease, (OR 3.29; 2.02–5.36; p < 0.0001).

Conclusions: Women with ICP have substantially increased risk for later hepatobiliary disease. We found beyond gallstone-related morbidity a strong positive association between ICP and hepatitis C both before and after ICP diagnosis. Thus, we advocate testing for hepatitis C in women with ICP, in particular, since this potentially life-threatening infection can be treated successfully in the majority of patients.
injecting network on the effective introduction of the new highly efficacious HCV treatment in people who inject drugs (PWID).

**Methods:** A discrete time, stochastic, individual based transmission model was developed, using a set of networks to describe contact patterns. The structure of the networks was informed by empirical data collected from a street based cohort of 258 PWID. Using this model, we tested the effect of five community treatment strategies on the HCV incidence and prevalence compared to no treatment. Treatment models included treating (a) highly connected PWID, (b) random PWID and (c) random PWIDs and their HCV positive contacts (“bring a friend”).

![Figure 1](image)

**Figure 1.** Boxplots of outcomes for 100 iterations of the network. The upper panel is the outcome assuming a treatment efficacy of 60% and the lower panel is the outcome assuming a treatment efficacy of 80%. The plots are shaded in three colours representing different coverage of treatment per year. From left to right, blue is the lowest coverage, of 15 per 1000 PWID per year, purple is 25 PWID per year and pink is 50 PWID per year.

**Results:** Results for the strategies vary within different treatment coverage and within both high (80%) and low (60%) efficacy treatments. Within each set of parameters, all treatments perform better than no treatment, and all other strategies perform better than random selection at reducing prevalence over a twenty year period (Figure 1). The benefit of the “bring a friend” strategy over the random strategy is most marked at higher efficacy treatment with coverage of 25 and 50 per thousand. The “bring a friend” strategy was significantly better than the random strategy for high efficacy treatment with high coverage (P < 0.05) as predicted by this model, and is not inferior to other strategies in any of the efficacy/coverage combinations.

**Discussion:** Our results suggest that the structure of PWID networks plays a role in HCV transmission impacting on the effectiveness of treatment strategies. With the advent of new HCV treatments there is the opportunity to exploit PWID networks to target HCV treatment to ensure it is of greatest benefit to both the individual undergoing treatment and also the community more generally as we strive to reduce the overall prevalence of HCV amongst PWID.
Selective Activation of Intrahepatic Immunity with TLR8 Agonist: A Potent Therapeutic Strategy to Boost Antiviral Immunity in Human Liver

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Background and Aims: The liver is considered as an organ that induces suboptimal or tolerogenic immune responses, hence certain hepatotropic viruses, such as HBV or HCV, can establish their persistence. Nonetheless, efficient immunity can be elicited resulting in viral clearance. These facts initiated our study to define novel immune-based strategies to control persistent viral infections by characterizing the functional profiles of immune cells resident within human liver upon Toll-like receptor (TLR) activation.

Methods: Intrasinusoidal immune cells (lymphocytes and monocytes) were collected from perfusion of healthy (n=20) and pathological livers (n=8). Functional profiles were quantified both at mRNA and protein levels, via multiplex gene expression, cytokine array and flow-cytometry technologies at the steady state and upon stimulation with TLR agonists or with anti-CD3/CD28-coupled beads (i.e., TCR-mediated activation).

Results: High throughput analysis of the ability of stimulated intrasinusoidal immune cells to produce antiviral cytokines identified a unique role for TLR8, which triggered a vast production of IFN-gamma from intrasinusoidal cells. Despite the observation that pathologic liver-derived immune cells were activated to a lesser extent by TLR8 agonist compared to their healthy counterparts, this TLR8-mediated activation was much stronger than that elicited by addition of IL-7, a cytokine secreted by hepatocytes upon infection.

Conclusions: We demonstrated that TLR8 agonist induced a selective IFN-gamma production mediated by NK and NKT cells resident in liver sinusoids. These subsets of lymphocytes appeared to be highly sensitive to cytokine-mediated activation. These data open a new therapeutic possibility to boost an antiviral immunity within the liver microenvironment using a selective TLR agonist activation.

Suppression of Hepatitis B Virus (HBV) Transcription and Replication by Small Molecules that Target the Epigenetic Control of Nuclear cccDNA Mini-chromosome

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Background: The HBV cccDNA is organized into mini-chromosomes in the nucleus of infected cells by histone and non-histone proteins. By using a cccDNA-specific chromatin immunoprecipitation (ChIP)-based assay, we showed that HBV replication is regulated, both in a cell replication system and in the liver of HBV chronically infected patients, by the acetylation status of cccDNA-bound H3/H4 histones. We have also shown that interferon-α (IFNα) inhibits HBV transcription and replication in vitro and in vivo by favoring the long-term recruitment to the nuclear cccDNA mini-chromosome of the class III HDAC hSirt1 and of the PRC2 repressive complex, including the transcriptional co-repressors HDAC1 and Ezh2.

Aim: We sought to assess the feasibility to inhibit HBV transcription and replication by targeting the epigenetic control of nuclear cccDNA mini-chromosome with small compounds active on different classes of chromatin modifying enzymes.

Methods: Capsid-associated HBV-DNA (TaqMan real-time PCR), cccDNA (TaqMan real-time PCR) and pgRNA levels (quantitative real-time PCR with specific primers), were assessed in HepG2 cells transfected with full length HBV genomes left untreated or treated with (a) class I and class III histone deacetylase inhibitors (HDACI); (b) p300 and PCAF histone acetyltransferases (HAT) inhibitors; (c) hSirt1 activators and (d) JMJD3 histone demethylase inhibitors.

Results: The combined inhibition of p300 and PCAF HATs (compound EML-264) resulted in an evident reduction of HBV replication that mirrored the decrease of pgRNA transcription. The hSirt1/2 activators MC2562 and MC2791, albeit with different efficiency, both inhibited HBV replication and cccDNA transcription. Potentiation of Ezh2 activity through the inhibition of JMJD3 histone demethylase with compound MC3119 resulted in a >50% reduction of pgRNA transcription. Notably, inhibition of hSirt1/2 (MC2344) or Ezh2 (MC2887) strongly induced cell death, thus hampering the evaluation of their effects on viral replication.

Conclusions: Altogether these results represent a proof of concept that activation of hSirt1 and Ezh2 by small molecules can induce an “active epigenetic suppression” of HBV cccDNA minichromosome similar to that observed with IFNα.
SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI) IS A PIVOTAL MEDIATOR OF ANTI-INFLAMMATORY RESPONSES IN ACUTE LIVER FAILURE
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Background: Acetaminophen-induced ALF (AALF) is characterised by circulating monocyte dysfunction and a predisposition to sepsis. Recent data also implicate hepatic macrophages (h-mϕ) in the resolution of inflammation/tissue repair in AALF. Following tissue injury, secretory leukocyte protease inhibitor (SLPI) promotes anti-inflammatory responses inhibiting TLR-4/NF-κB pathway.

Methods: SLPI levels were determined in 7 AALF and 8 control liver tissue using ELISA and immunohistochemistry (CD68/SLPI). Monocytes (mo) were immunophenotyped in 37 AALF patients and 15 healthy controls (HC). Using phosphoflow, TLR-4 induced NF-κBp65 in ex-vivo mo in 10 AALF patients and 10 healthy controls (HC) was determined. SLPI (pg/ml) was measured in 55 AALF patients at admission. Effects of recombinant human (rh)-SLPI (0.1–0.5 mcg/ml) on the phenotype/function of CD14+ isolated monocytes was determined in 3 cell culture experiments. SLPI neutralization effects were determined incubating CD14+ monocytes and monocyte-derived mo/mϕ, mo derived from HC, in (i) AALF liver homogenates (n=5) (ii) AALF patient sera (n=20) in the presence and absence of α-SLPI (5mcg/ml).

Results: Compared to HC, intrahepatic (116 vs 442pg/ml; p < 0.004), systemic (71200 vs 43310; p < 0.001) and regional (HV-PV [72130 vs 60410; p=0.03]) SLPI levels were significantly elevated in AALF, peaking on admission versus day 3 (67570 vs 58590; p=0.03). SLPI expression is detected in biliary epithelial cells and necrotic areas. Circulating monocytes in AALF show an anti-inflammatory phenotype (CD14+CD16+CD163+HLA-DR-) and a significant reduction in LPS-induced NF-κBp65 expression compared to HC (ratio: 0.8 vs 1.5; p=0.001). Rh-SLPI induced a CD14+CD16+CD163+ phenotype decreasing LPS-induced TNF-α (941 vs 555; p<0.001) whilst preserving IL-10 secretion (191 vs 128; p=ns). Compared to HC, culture in AALF liver homogenates and ALF sera expands the number CD14+CD16+CD163+ mo/mϕ with decreased LPS induced TNF-α (58 vs 2887; p<0.001), IL-6 secretion (30 vs 2500; p<0.001). Exposure of mo/mϕ to α-SLPI reversed the decrease in LPS-induced TNF-α (70 vs 419; p<0.01) secretion whilst preserving IL-10 (58 vs 87; p=ns).

Conclusions: Our data indicate that SLPI is a pivotal mediator of anti-inflammatory responses in AALF through modulation of mo/mϕ function and could be instrumental to monocyte dysfunction and susceptibility to sepsis. Our data also implicate SLPI as a key hepatic microenvironmental trigger promoting resolution of inflammation/tissue repair.

HRas SIGNAL TRANSDUCTION MEDIATES HEPATITIS C VIRUS CELL ENTRY BY TRIGGERING THE ASSEMBLY OF THE HOST TETRASPANIN RECEPTOR COMPLEX
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Background and Aims: Hepatitis C virus (HCV) entry is the first step of infection and requires the cooperative interaction of
several host cell factors, including CD81 and claudin1. We recently identified a role of epidermal growth factor receptor (EGFR) in HCV entry by regulating CD81-claudin1 co-receptor interaction, suggesting that EGFR-signaling plays a role in virus entry. To identify the molecular mechanisms of EGFR-signaling pathway(s) required for HCV entry, we aimed to uncover the molecular link between EGFR-signaling and the regulation of CD81-claudin1 co-receptor association.

Methods: We combined RNAi screening in Huh7.5.1 cells, small molecule inhibition into primary human hepatocytes (PHH), phosphoryar analysis in PHH and liver biopsies and advanced imaging studies including fluorescence recovery after photobleaching (FRAP) and fluorescence resonance energy transfer (FRET).

Results: RNAi screening comprising cellular signaling pathways demonstrated that silencing the GTPase HRas markedly inhibited HCV entry. Moreover, a trans-dominant active HRas mutant increased HCV entry and rescued the defect following EGFR inhibition, suggesting that HRas is a molecular switch regulating EGFR-mediated HCV entry. Indeed, FRET analyses demonstrated that similar to EGFR, silencing HRas expression reduced CD81-claudin1 association. Moreover, differential proteome analyses (SLAC) identified HRas specific association with a complex containing CD81. FRAP assays demonstrated that EGFR/HRas functions modulate lateral diffusion and membrane trafficking of CD81, allowing to form the host co-receptor complex. Furthermore, proteomic analyses identified novel signaling effectors within tetraspanin-enriched microdomains as candidates mediating this essential step of the HCV life cycle.

Conclusion: Taken together, our results support a model where HRas/CD81 complexes provide a functional link between the EGFR/HRas pathway and CD81-claudin1 association that are prerequisites of HCV entry and indicate a crucial role of EGFR/HRas signaling for CD81 trafficking. These data increase our understanding of the molecular mechanisms underlying EGFR-mediated HCV entry and open new perspectives for the development of antivirals targeting signaling pathways.

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LYMPHOTOXIN beta RECEPTOR ACTIVATION LEADS TO DEGRADATION OF HBV cccDNA FROM INFECTED HEPATOCYTES
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Background and Aims: Despite availability of efficient therapies against hepatitis B virus (HBV), long-term persistence of cccDNA requires life-long treatments that may cause side effects. The discovery of compounds directly targeting the cccDNA is thus one of the major challenges to cure chronic HBV infections. Here we aimed at testing the effect of lymphotixin beta receptor (LTBR) agonization on HBV infection and replication.

Method: Cell culture models including HBV-infected HepAR cells and primary human hepatocytes as well as HBV-transgenic mice were used to test the effect of antibodies agonizing human LTBR (BS1) or mouse LTBR.

Results: We observed a strong and dose-dependent anti-HBV effect by activation of the LTBR both in vitro and in vivo. All HBV replication markers were decreased upon treatments including cccDNA in cells where HBV infection was already established. The non-cytopathic degradation of cccDNA was found to be dependent on NF-κB activation through RelA phosphorylation but was independent of non-canonical NF-κB signaling or production of type I interferon. LTBR-activation resulted in overexpression of APOBEC3B resulting in hypermutations of cccDNA and subsequent specific degradation of cccDNA. As a consequence, and in contrast to nucleos(t)ide analogues currently used for the treatment of chronic HBV infection, no rebound of HBV replication was observed after stop of treatment.

Conclusions: We here describe an effective, long lasting and non-cytopathic mechanism of effective HBV-cccDNA depletion – forming the basis for a novel alternative therapeutic approach to cure chronic HBV infection.

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SVR12 RATES AND SAFETY OF TRIPLE THERAPY INCLUDING TELAPREVIR OR BOCEPREVIR IN 221 CIRRHOTIC NON RESPONDERS TREATED IN THE FRENCH EARLY ACCESS PROGRAM (ANRS CO20-CUPIC)

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Background: Triple therapy was associated with higher SVR rates in phase III trials. However, few patients with cirrhosis were included in these trials. We report the SVR12 rates and safety profile of telaprevir (TVR) and boceprevir (BOC) with peg-IFN/ribavirin (RBV) in cirrhotic experienced patients treated in the French Early Access Program.

Methods: 674 genotype 1 patients with compensated cirrhosis (Child A) were prospectively included and received 12W TVR/PEG-IFN-2a/RBV+36W PEG-IFN/RBV, or 4W PEG-IFN-2a/RBV+12W TVR/PEG-IFN-2a/RBV+32W PEG-IFN/RBV, or 4W PEG-IFN-2b/RBV+44W BOC/PEG-IFN/RBV. The analysis is restricted to 221 patients reaching W60 of follow-up.

Results: Efficacy data are shown in the table. SAEs were observed in 57.0% (TVR) and 40.4% (BOC). Death, infection and hepatic decompensation were reported in 2.8%, 6.5% and 0.9% for TVR and 0.9%, 4.4% and 1.8% for BOC, respectively. Anemia <8 g/dL or blood transfusion were reported in 17.8% (TVR) and 8.7% (BOC).

Conclusions: In this large “real life” cohort of cirrhotic patients, SVR12 rate was comparable with the results in the sub group of patients with severe fibrosis or cirrhosis of phase III studies. Data on the entire population included in CUPIC (674 patients) will be presented at the EASL meeting.

Table: Virological efficacy

<table>
<thead>
<tr>
<th>Undetectable HCV RNA (ITT) n (%)</th>
<th>BOC n = 114</th>
<th>TVR n = 107</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 12</td>
<td>52 (46)</td>
<td>79 (74)</td>
</tr>
<tr>
<td>Week 24</td>
<td>58 (51)</td>
<td>65 (61)</td>
</tr>
<tr>
<td>Week 48 (EOT)</td>
<td>44 (39)</td>
<td>45 (42)</td>
</tr>
<tr>
<td>SVR12 (Total)</td>
<td>44 (39)</td>
<td>49 (46)</td>
</tr>
<tr>
<td>SVR12 in relapers</td>
<td>24/46 (52)</td>
<td>32/45 (71)</td>
</tr>
<tr>
<td>SVR12 in partial responders</td>
<td>20/64 (31)</td>
<td>17/58 (29)</td>
</tr>
<tr>
<td>SVR12 in null responders</td>
<td>0/2 (0)</td>
<td>0/3 (0)</td>
</tr>
</tbody>
</table>
**61** TREATMENT WITH SOFOSBUVIR + RIBAVIRIN FOR 12 WEEKS ACHIEVES SVR12 OF 78% IN GT2/3 INTERFERON-INELIGIBLE, -INTOLERANT, OR -UNWILLING PATIENTS: RESULTS OF THE PHASE 3 POSITRON TRIAL


**Methods:** GT 2/3 HCV-infected patients deemed IFN-ineligible, -intolerant, or -unwilling were randomized and treated; 54% were male, 91% white, 16% had compensated cirrhosis, 51% GT2, 45% were -unwilling) were randomized and treated; 54% were male, 91% white, 16% had compensated cirrhosis, 51% GT2, 45% were IFN-ineligible, IFN-intolerant, or unwilling HCV-infected patients have no currently approved treatment options. We report here the results from POSITRON, a Phase 3, randomized, placebo-controlled study comparing 12 weeks of SOF + ribavirin (RBV) to placebo (NCT01542788).

**Results:** 278 patients (44% IFN-ineligible, 9% -intolerant, 47% -unwilling) were randomized and treated; 54% were male, 91% white, 16% had compensated cirrhosis, 51% GT2, 45% were IFN-status was observed in GT3 patients only. No S282T in NS5B was detected among the cirrhotics. The difference in SVR12 by cirrhosis status was observed to placebo (0%, p < 0.003).

**Conclusions:** SOF+RBV for 12 weeks achieved a high SVR12 rate without evidence of resistance and was well tolerated in cirrhotic and non-cirrhotic GT2/3 HCV-infected patients who do not currently have any treatment options.

**Table: Safety summary**

<table>
<thead>
<tr>
<th></th>
<th>SOF+RBV (N = 207)</th>
<th>Placebo (N = 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any treatment emergent AE</td>
<td>185 (89%)</td>
<td>55 (78%)</td>
</tr>
<tr>
<td>Discontinues due to AEs</td>
<td>4 (2%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>91 (44%)</td>
<td>17 (24%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>46 (22%)</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>Headache</td>
<td>43 (21%)</td>
<td>14 (20%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>39 (19%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Any TE egrade 3 labs</td>
<td>17/206 (8%)</td>
<td>17 (24%)</td>
</tr>
<tr>
<td>Hemoglobin &lt;9 g/dL</td>
<td>2/206 (1%)</td>
<td>0</td>
</tr>
</tbody>
</table>

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**62 PHASE II HCVac STUDY OF TG4040 IMMUNOTHERAPEUTIC IN COMBINATION WITH PEGIFNα2a AND RIBAVIRIN IN GENOTYPE 1 CHC TREATMENT NAÏVE PATIENTS: SVR24 FINAL RESULTS**


**Methods:** This randomized open label study evaluated two schedules of TG4040 in combination with PegIFNα2a (P) and ribavirin (R) vs P/R alone (ArmA); ArmB with 6 TG4040 injections initiated 4wks after P/R and pre-vaccination ArmC with 13 TG4040 injections initiated 12wks before P/R. The primary endpoint was cEVR. Peripheral blood mononuclear cells and serum were respectively assayed for T-cell responses against vaccine antigens (IFN-g ELISPOT) and for MVA-specific antibodies.

**Results:** 153 patients were enrolled; 31 in ArmA, 63 in ArmB, and 59 in ArmC (1:2:2). Overall population was well balanced between arms; 55% of patients were male, 97% Caucasian, 78% had 1b genotype and 25% had IL28B C-C genotype. The study met its primary endpoint in TG4040 pre-vaccination arm. This effect was sustained over time reaching 62% of SVR24.

**Table: Safety summary**

<table>
<thead>
<tr>
<th></th>
<th>Control Arm A</th>
<th>Experimental Arm B</th>
<th>Experimental Arm C</th>
</tr>
</thead>
<tbody>
<tr>
<td>cEVR – Primary endpoint</td>
<td>30% (9/30)**</td>
<td>46% (28/61)</td>
<td>64% (34/53)**</td>
</tr>
<tr>
<td>ETR (48 weeks of P/R)*</td>
<td>68% (21/31)</td>
<td>56% (35/63)</td>
<td>67% (37/55)</td>
</tr>
<tr>
<td>SVR12*</td>
<td>42% (13/31)</td>
<td>48% (30/63)</td>
<td>56% (31/55)</td>
</tr>
<tr>
<td>SVR24*</td>
<td>48% (15/31)</td>
<td>51% (32/63)</td>
<td>62% (34/55)</td>
</tr>
</tbody>
</table>

**Conclusion:** TG4040 immunotherapeutic is a recombinant poxvirus (MVA strain) encoding NS3, NS4 and NS5B. It aims at triggering HCV-specific T-cell immune responses capable of controlling viral replication.
CORRELATION OF EARLY DETECTION OF HCV NS3-RESISTANCE AND VIROLOGICAL FAILURE IN PATIENTS TREATED WITH TRIPLE THERAPY INCLUDING TELAPREVIR OR BOCEPREVIR

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E-mail: ceccherini@med.uniroma2.it

Background: The role of resistance testing in chronic HCV infected patients treated with triple therapy including the protease inhibitors (PIs) boceprevir or telaprevir in clinical practice is still unclear. Aim of this study was to analyze the resistance profiles to boceprevir/telaprevir treatment, investigating the impact of baseline and early NS3-protease resistance-associated variants (RAVs).

Methods: Seventy patients (HCV-1a/1b/1g=23/46/1; previous non-responders/relapers/naïve/unknown = 43/19/6/2; advanced-fibrosis/cirrhosis = 25/24) treated with pegIFN-2a/ribavirin+ boceprevir (N=23) or telaprevir (N=47) were analyzed. HCV-RNA (detection-limit=12/15 IU/ml) and NS3-protease sequences were evaluated at baseline, at early time-points (48h–1 week–2 weeks–4 weeks) and then every 4 weeks. HCV NS3 quasi-species evolution was also assessed in 10 patients by Ultra-deep 454-Pyrosequencing (UDPS). Results: In this interim analysis, patients received boceprevir for a median (IQR) time of 25 (13–48) weeks or telaprevir for 12 (4–20) weeks. Overall, viral-failure was observed in 13/70 (18.6%) boceprevir/telaprevir-treated patients. Viral-failure was strongly associated with previous non-response to pegIFN/ribavirin (12/13 patients, 92.3%), and more frequent in HCV-1a subtype (7/23, 30.4%) vs HCV-1b (5/46, 10.9%). The single HCV-1g patient failed.

Of baseline and early NS3-protease resistance-associated variants (RAVs).

Conclusions: In this (still limited) number of cases, early detection of RAVs, either at baseline or at 48h, was always associated with PI-failure in previous non-responders patients. If confirmed in a larger cohort, this may suggest the clinical relevance of early resistance test in predicting viral failure in selected patients.

GS-5885 + GS-9451 + PEGINTERFERON AND RIBAVIRIN (PR) FOR SIX OR TWELVE WEEKS ACHIEVES HIGH SVR12 RATES IN TREATMENT-NAÏVE GENOTYPE 1 IL28B CC PATIENTS

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Background and Aims: The IL28B CC polymorphism (rs12979860) is associated with improved virologic responses to interferon-based HCV therapy. However, no prospective trials have reported outcomes with less than 12 wks in this interferon responsive population.

Methods: We conducted a prospective, randomized, multi-center, open-label study to compare the antiviral efficacy, safety, and tolerability of an interferon-sparing regimen of GS-5885 30 mg QD (NS5a inhibitor) + GS-9451 200 mg QD (PI) + PR for 6 or 12 wks (Arm 1) vs. PR for 24 wks (Arm 2). A total 244 genotype 1, non-cirrhotic, treatment-naïve subjects with IL28B CC genotype were randomized 1:1. Subjects in Arm 1 with HCV-RNA < LLoQ at Wk 2 (vRVR) were re-randomized to receive 6 or 12 wks of treatment. Subjects in Arm 2 with HCV RNA < LLoQ at Wk 4 (RVR) received 24 wks of PR.

Results: Virologic response for both study arms are shown in Table 1.

Early on-treatment virologic response occurred in >90% of patients on 4 drugs vs <50% with PR alone. SVR12 rates with 6 or 12 wks of quadruple therapy were comparable to 24 wks of PR. Baseline VL and BMI were significant predictors of relapse with 6 wks of treatment. There was no difference in GT 1a and 1b responses. Treatment-related adverse events were rare and consistent with PR. Mean changes in hematologic labs were similar in both Arms. Early total bilirubin elevations were observed more frequently in Arm 1. Fewer study treatment dose modifications or discontinuations for safety were seen with 6 wks of treatment.

Conclusions: High SVR12 rates (82–100%) were seen in treatment-naïve, non-cirrhotic, IL28B CC patients with early virologic response treated with two direct-acting antivirals plus PR and are comparable to PR for 24 weeks.
SAFETY OF TRIPLE THERAPY WITH TELAPREVIR OR BOCEPREVIR IN HEPATITIS C PATIENTS WITH ADVANCED LIVER DISEASE – PREDICTIVE FACTORS FOR SEPSIS

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E-mail: karoline.rutter@meduniwien.ac.at

Introduction: Cure rates of chronic hepatitis C patients with advanced fibrosis or cirrhosis by peginterferon/ribavirin (PegIFN/RBV) are unsatisfactory, but can be increased by the addition of protease inhibitors. Besides the CUPIC study, few data on tolerability and safety in patients with advanced liver disease receiving triple therapy are available.

Methods: 110 CT-1 patients (F3: n = 31; F4: n = 79, m/f: 79/31; mean age: 55.2±8.0, platelet count median [range] 130[35–332]g/L, IL-28B: CC: 10, TC: 72, TT: 13; na: 15; non-responders: 69, relapsers: 27, treatment naïve: 14) received triple therapy: 180μg PegIFN alfa2a/2b/RBV with either 800mg/TID boceprevir (BOC: Victrelis®, MSD) after a 4 week lead in; n = 61) or 750mg/TID telaprevir (TPV; Incivo®, Janssen; for 12 weeks; n = 49). Total treatment duration was 48 weeks.

Results: Severe adverse events (SAE) were observed in 22 (20%) patients on treatment (severe infections: 11, rash [including one patient with DRESS syndrome]; 4, severe anaemia; 5, myocardial infarction; 1, psychosis; 1). In 14 of them treatment was discontinued. 3 patients died due to sepsis. Platelet count <100,000 G/L had no impact on the overall SAE rates (16.7% vs 17.7%) but was associated with the occurrence of severe infections under treatment (4/30; 13.4% vs 7/80; 8.7%, p < 0.05). More injection related SAEs were observed in patients with serum albumin <35 g/dl (55.6% versus 5.4%, p < 0.05) or fibrosis grade 4 (F4: 12.6%; F3: 3.2%, p < 0.05). Pretreatment hepatovenous pressure gradient (HVPG) measurement was available in 27 cirrhotic patients. Infection related SAEs occurred in 6 of 18 patients with HVPG ≥10 mmHg but in none of the 9 with HVPG <10 mmHg (p < 0.05). Early treatment discontinuation was necessary in 51 (46.8%) patients (breakthrough: 5, futility: 26, AE: 20). 24 patients had undetectable HCV-RNA at end of treatment (EOT). 16 patients are on follow up, 5 relapsed and 3 had a SVR (interim ITT analysis: 6%). 35 patients are still on treatment.

Conclusion: Triple therapy in patients with advanced liver disease is associated with a poor outcome and a high rate of severe adverse events including three cases of death. Low platelet count, low serum albumin and HVPG ≥10 mmHg were predictive markers for severe septic events. These patients may benefit from antibiotic prophylaxis on triple therapy.
Methods: Thirty-five patients (n=35) naïve CHC-G1 were recruited. Group A (n=18), p-IFN 135mcg once weekly, Telaprevir 750mg two tablets-TID four days and three tablets BID post dialysis for three days; along with RBV 400 mg daily for 12 weeks followed by p-IFN 135mcg plus RBV 400 mg till 24 weeks. Group B (n=17) p-IFN 135mcg once weekly as Group A with RBV 200mg for 12 weeks followed by p-IFN 135mcg with RBV 400mg till 48 weeks. Viral load to follow RGT.

Results: See the table.

Conclusion: This study demonstrates higher SVR comparing traditional SOC on hemodialysis CHC-G1 patients. Extended 48 weeks had no benefits. Multi-center trials to follow.

68 ANTIVIRAL EFFICACY OF THE NS3 PROTEASE INHIBITOR, GS-9451, NON-NUCLEOSIDE NS5B INHIBITOR, TEGOBUVIR, AND PEGYLATED INTERFERON PLUS RIBAVIRIN IN TREATMENT-NAIVE GENOTYPE 1 HEPATITIS C INFECTED PATIENTS


Table (abstract 67)

<table>
<thead>
<tr>
<th>Sub-genotype</th>
<th>IL28 Status</th>
<th>Baseline HCVRNA (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>1b</td>
<td>Non-CC</td>
</tr>
<tr>
<td>G1a</td>
<td>10/18 (53%)</td>
<td>12/18 (67%)</td>
</tr>
<tr>
<td>G1b</td>
<td>10/10 (50%)</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>G1c</td>
<td>10/8 (67%)</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>19/36 (53%)</td>
<td>19/36 (53%)</td>
</tr>
</tbody>
</table>

E-mail: kkypreos@med.upatras.gr

Background and Aims: The antiviral efficacy and safety of combining tegobuvir and GS-9451 with PEG/RBV for a shortened treatment duration in early viral responders was assessed in treatment naïve chronic HCV genotype 1 infected patients.

Methods: 239 non-cirrhotic genotype 1 HCV patients were randomized 2:1:1 to receive:

ARM 1: tegobuvir (30 mg BID) + GS-9451 (200 mg QD) + PEG (180 µg/week) + RBV (1000–1200 mg/day).

ARM 2: GS-9451 + PEG/RBV or

ARM 3: PEG/RBV.

A treatment duration of 16 weeks was explored in Arm 1 for patients who achieved an undetectable viral RNA at week 2. Patients receiving the 4- or 3-drug therapy (Arm 1 or Arm 2, respectively) who achieved an undetectable viral RNA at weeks 4–12 stopped treatment at week 24; the remaining patients stopped treatment at week 24.

Results:

Table: SVR 12 rates, overall and subgroup ITT analysis

<table>
<thead>
<tr>
<th>Sub-genotype</th>
<th>IL28 Status</th>
<th>Baseline HCVRNA (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
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</tr>
<tr>
<td>G1b</td>
<td>10/10 (50%)</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>G1c</td>
<td>10/8 (67%)</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>19/36 (53%)</td>
<td>19/36 (53%)</td>
</tr>
</tbody>
</table>

Conclusions: The addition of GS-9451 to a PEG/RBV regimen results in improved SVR12 rates in treatment-naïve, genotype 1 HCV patients relative to PEG/RBV.

Parallel Session: NON-ALCOHOLIC FATTY LIVER DISEASE: FROM PATHOLOGY TO THERAPY

69 NOVEL CAUSATIVE RELATIONSHIP BETWEEN LOW HDL AND DIET-INDUCED NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: During the biogenesis of HDL, lipid free or minimally lipidated apoA-I interacts functionally with the lipid transporter ABCA1 to form immature discoidal HDL which are then converted into mature spherical particles by the action of lecithin:cholesterol acyl transferase (LCAT). Here we investigated the mechanistic relationship between low and dysfunctional HDL and diet-induced NAFLD development using mouse models.

Methods: We employed male apoA-I-deficient (apoA-I−/−) mice that lack classical apoA-I containing HDL and male deficient (LCAT−/−) mice that have immature discoidal HDL.

Results: Mice were fed the standard western-type diet for 24 weeks and then histological and biochemical analyses were performed. ApoA-I−/− mice showed increased diet-induced hepatic triglyceride deposition and disturbed hepatic histology while they exhibited reduced glucose tolerance and insulin sensitivity. Quantification of FASN-1, DGAT-1, and PPARα mRNA expression suggested that the increased hepatic triglyceride content of the apoA-I−/− mice was not due to de novo synthesis of triglycerides. Similarly, metabolic profiling did not reveal differences in the energy expenditure between the two mouse groups. However, apoA-I−/− mice exhibited enhanced intestinal absorption of dietary triglycerides, accelerated clearance of postprandial triglycerides, and a reduced rate of hepatic very low density lipoprotein triglyceride secretion. In agreement with these findings, adenovirus-mediated gene transfer of apoA-I in apoA-I−/− mice fed western-type diet for 12 weeks resulted in a significant reduction in hepatic triglyceride content and an improvement of hepatic histology and architecture. Similar to apoA-I−/− mice, LCAT−/− mice were characterized by increased diet-induced hepatic triglyceride deposition and impaired hepatic histology and architecture. Adenovirus-mediated gene transfer of LCAT in LCAT−/− mice that were fed western-type diet for 12 weeks resulted in a significant reduction in hepatic triglyceride content and a great improvement of hepatic histology and architecture.

Results: Taken together, our data establish that the HDL metabolic pathway is a central contributor to the deposition of dietary triglycerides to the liver and the development of NAFLD. Our data further support that the coexistence of reduced HDL levels and NAFLD in an individual with metabolic syndrome may not be a mere coincidence, rather it underlays a strong causative relationship between these two conditions.
70 INTRAHEPATIC CHANGES IN BILE ACID COMPOSITION PROTECTS BSEP (ABCB11) KO MICE FROM HEPATIC INFLAMMATION IN METHIONINE CHOLINE DEFICIENT DIET INDUCED NASH

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**Background:** Bile acids (BAs) activate G-coupled and nuclear receptors controlling hepatic glucose and lipid metabolism as well as inflammation. Non-alcoholic-steatohepatitis (NASH) is characterized by increased hepatic lipid storage and inflammation. BAs are transported into bile via the bile salt export pump (BSEP; ABCB11). BSEP transgenic mice display less fat accumulation but severe inflammation. We therefore hypothesized that absence of BSEP may sensitize to hepatic steatosis while at the same time reducing inflammation in a mouse model of NASH.

**Methods:** Wildtype (WT) and BSEP knockout (KO) mice were fed a methionine-choline-deficient (MCD) diet for 5 weeks to induce NASH. Liver RNA profile analysis was performed by RT-PCR. Serum biochemistry, hepatic TG, BA content/composition as well as liver histology were assessed.

**Results:** MCD feeding induced hepatic TG accumulation (1.8-fold) in WT mice and to a lesser extent in BSEP KO mice. In line, mRNA expression of de novo lipogenesis and fatty acid transport markers was repressed to a higher extent in MCD fed BSEP KO mice. In contrast to WT animals, BSEP KO were protected from hepatic inflammation (reflected by Tnfα, F4/80, Macp1 and iNOS expression) induced by MCD feeding. mRNA expression of the BA-importer Ntcp was down-regulated by 60% in WT mice upon MCD but to a greater extent in BSEP KO mice. Conversely, expression of Mrp4 and Ostb was increased (10-fold; 2.8-fold) only in BSEP KO mice. Measurement of hepatic bile acid composition uncovered a distinct increase in hepatic CDCA (3-fold) and CA (3-fold) concentration (both BAs are known FXR agonists) in BSEP KO mice compared to WT controls, whereas amounts of hepatic UDCA and b-muricholic acid were decreased.

**Conclusion:** Absence of BSEP protects from hepatic inflammation and reduces fatty acid storage by modulating hepatic bile acid transport and intrahepatic bile acid composition. These effects are most likely mediated via FXR induction. Thus, pharmacological modulation of bile acid transport/metabolism could constitute a new therapeutic option for modulating inflammation during progression from fatty liver to NASH. Supported by the project FLIP (HEALTH-F2-2009-241762) and SFB LIPOTOX (F3008).

72 MAPPING EXPRESSION QUANTITATIVE TRAITS LOCI (eQTL) FOR PNPLA3 GENE IDENTIFIES ADDITIONAL SNPS ASSOCIATED WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) INDEPENDENT OF rs738409

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**Background:** The PNPLA3 I148M (rs738409) polymorphism is associated with NAFLD. However, questions remain how PNPLA3 is regulated and whether additional polymorphisms contribute to its function or confer susceptibility to advanced NAFLD.

**Aim:** To identify genetic polymorphisms affecting hepatic PNPLA3 expression, and to investigate their effect on hepatic fat accumulation and NAFLD phenotype.

**Methods:** Using genome-wide hepatic eQTL mapping, we assessed for SNPs significantly associated with PNPLA3 mRNA expression. Identified SNPs were genotyped in 27 healthy liver donors, and correlated with hepatic total fat (HTF), triglyceride (HTG) and cholesterol (H-Chol) levels. In addition, we examined the association between these SNPs and histological features of NAFLD in the recent GWAS data generated by the FLIP (Fatty Liver: Inhibition of Progression) consortium.

**Results:** A genome-wide significant \( p < 10^{-5} \) eQTL locus was mapped to the PNPLA3-SAMM50 region. Detailed linkage disequilibrium (LD) analysis revealed two major groups of SNPs, represented by rs139051 \( (p = 7.2 \times 10^{-10}) \) and rs2294918...
(p = 3.5 × 10^{-7}), independently affected PNPLA3 expression (LD R²=0.3). The previously identified rs738409 was not strongly associated with PNPLA3 expression (p = 0.01). LD level between rs738409 and rs139051 or rs2294918 was relatively weak (R² < 0.15), suggesting that these loci independently influenced PNPLA3 function.

The SNP rs139051 was significantly associated with HTF (r² = 0.33, p = 0.002), THG (r² = 0.25, p = 0.008) and H-Chol (r² = 0.14, p = 0.05) in the liver samples. There is also a significant association between rs738409 and increased HTF (r² = 0.21, p = 0.011), HTG (r² = 0.21, p = 0.02) and H-Chol (r² = 0.20, p = 0.02).

In the FLIP GWAS, rs2294918 was significantly associated with NAFLD (p = 1.9 × 10^{-10}), severe steatosis (1.0 × 10^{-9}), steatohepatitis (1.5 × 10^{-7}), and fibrosis (7.4 × 10^{-7}). Neither rs738409 nor rs139051 were represented on the GWAS chip. No SNPs were in strong LD with rs139051. However rs2896019, a SNP in strong LD (R² = 0.68) with rs738409, had the strongest genome-wide association with NAFLD (p = 4.1 × 10^{-10}), severe steatosis (7.7 × 10^{-22}), steatohepatitis (2.3 × 10^{-24}), and fibrosis (1.2 × 10^{-15}).

**Conclusions:** We identified SNPs, independent of I48M, affecting hepatic PNPLA3 expression and associated with hepatic lipid accumulation and advanced NAFLD. These data suggest a combination of qualitative (rs738409 as a missense, protein function-altering allele) and quantitative (rs139051 and rs2294918 as strong eQTLs) regulation of PNPLA3 function and play a role in the pathogenesis of NAFLD.

### 73 KRÜPPEL LIKE FACTOR 6 (KLF6) PROTECTS FROM NAFLD PROGRESSION THROUGH REGULATION OF ADIPOSE TISSUE INSULIN RESISTANCE

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**Background and Aims:** We have previously shown that adipose tissue insulin resistance index (Adipo-IR), which reflects impaired insulin action in inhibiting peripheral lipolysis, is associated with higher degree of liver fibrosis and hepatic insulin resistance in patients with NAFLD. The intronic polymorphism in the zinc finger transcription factor Kruppel-like Factor 6 (KLF6), KLF6-IVS1-27C>A, is associated with lower NAFLD progression and lower hepatic insulin resistance (Hep-IR). KLF6 is also involved with adipogenesis that is promoted by insulin. Thus, the aim of this study was to evaluate if KLF6-IVS1-27C>A was associated with lower Adipo-IR and better lipid metabolism in previously genotyped 1218 healthy subjects of European ancestry recruited from 19 centers (RISC consortium).

**Methods:** RISC data collected included measures of body mass index (BMI), fat mass, fasting free fatty acids (FFA), insulin (INS), lipid profile (total cholesterol, HDL, LDL, triglycerides, apolipoprotein A-I, A-II, B, C-III and E), beta hydroxybutyrate (BHB) a marker of hepatic beta oxidation, Adipo-IR (fasting FFAxIns), Hep-IR (EGPxINS in a subgroup of n = 368). Genotype/phenotype relationships were studied by linear trend analysis corrected for age, sex, and recruitment center.

**Results:** In the whole dataset we observed a stepwise increase in Adipo-IR in association with KLF6 genotype (KLF6_AA 10.7 ± 2.6 n = 12; KLF6_GA 16.7 ± 1.1 n = 151; KLF6_GG 17.2 ± 0.4 n = 1055) that was statistically significant by linear regression analyses corrected for age, sex, recruitment centre and fat mass (p = 0.023) as well as a stepwise increase in Hep-IR (KLF6_AA 343 ± 102 n = 5; KLF6_GA 422 ± 44 n = 38; KLF6_GG 496 ± 19 n = 325, p = 0.02). Increased Adipo-IR was associated with Hep-IR and increased concentrations of TG, BHB, LDL, Apo-AII, Apo-B, Apo-CIII and fatty liver index, indicating hepatic fat overload. However, no significant linear trend was observed between KLF6 and increased cholesterol, triglyceride, Apolipoproteins or BHB plasma concentrations.

**Conclusions:** KLF6-IVS-27G, the genotype associated with NAFLD progression is linked to an increase in Adipo-IR. We propose that KLF6 plays a central role also in regulating peripheral lipid metabolism in response to insulin, contributing not only to the development and progression of NAFLD, but also to the pathogenesis of the metabolic syndrome itself.

### 74 APPLICATION, UTILITY AND VALIDATION OF A NEW HISTOLOGICAL SYSTEM FOR CLASSIFICATION OF NAFLD IN PATIENTS WITH METABOLIC SYNDROME

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**Background:** Liver histology remains the gold standard for diagnosing NASH, but histological definitions vary among pathologists and the use of the NAS is controversial. We previously developed an algorithm along with a scoring system (SAF) for the classification of liver injury in morbidly obese patients undergoing bariatric surgery (Hepatology 2012; 56(5):1751–9).

**Aim:** To assess the utility of this algorithm and the reproducibility of the SAF score in patients with hepatic steatosis and metabolic risk factors explored in hepatological centers within the FLIP consortium.

**Methods:** Forty liver biopsies of > 20 mm length were selected from patients with suspected NAFLD and no other causes of liver disease. In a 1st round of reading, 6 expert liver pathologists independently categorized each biopsy for the diagnosis of steatosis or NASH according to their own practice. In a 2nd reading session, the same slides were reclassified by each pathologist using the algorithm and SAF score (S = Steatosis, A = Activity, the sum of ballooning and lobular inflammation, F = Fibrosis according to Kleiner staging system). Agreement and the k score were compared between readings.

**Results:** In the initial evaluation, the strength of concordance for the diagnostic category was moderate (κ = 0.54, 77% agreement). With the new algorithm, concordance became substantial (κ = 0.63, 85% agreement). Concordance was substantial for Steatosis (κ = 0.61) and Activity (κ = 0.75), and moderate for Fibrosis (κ = 0.53). Significant discordance was observed in substaging of fibrosis stage 1 (1a vs 1b vs 1c). Pooling of these substages into a single score F1 greatly increased the concordance of staging from moderate to almost perfect (κ = 0.84).

**Conclusion:** The new SAF algorithm decreases observer variability between expert liver pathologists for the histological classification of NAFLD. The concordance for the components of the SAF score was substantial for steatosis and activity and almost perfect for fibrosis after pooling of the Kleiner 1 substages. This expert validation supports the diagnostic utility of the new SAF score and algorithm in the larger population of patients with suspected NAFLD investigated in liver units.

Funded by FP7 under grant agreement HEALTH-F2-2009-241762.
HEPATOCYTE-DERIVED MICROPARTICLES WITH A SPECIFIC ANTIGENIC COMPOSITION ARE RELEASED IN BLOOD DURING NAFLD DEVELOPMENT: IMPLICATIONS FOR BIOMARKERS

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Background and Aims: Nonalcoholic steatohepatitis (NASH) is a serious public health problem. There is currently a lack of effective treatments and noninvasive diagnostic markers. We have recently demonstrated that hepatocyte-derived microparticles (MPs) are critical signals that contribute to angiogenesis and liver damage in NASH (Presidential Plenary AASLD 2012). Here we tested the hypothesis that circulating hepatocyte-MPs are novel targets for noninvasive monitoring of NASH.

Methods: Male C57BL/6 mice were placed on Choline Deficient L-Amino Acid (CDAA) diet, Choline Supplemented L-Amino Acid (CSAA) or regular Chow diet for 4 and 20 weeks. These time points were chosen as they have been shown to be associated with early stage and established NASH, respectively. Circulating MPs were isolated from platelet-free plasma (PFP), detected by flow cytometry and extensively characterized by electron microscopy. Liver specimens were cytoskeleton or vesiculation proteins, 2.9% protein of the cytoplasmic proteins, 58.8% cytoplasmic proteins, 8.8% nuclear proteins and 5.9% extracellular proteins. Analysis of the molecular function characterization of circulating MPs using a comprehensive proteomic approach by LC-MS/MS analysis. A gene expression microarray was performed using Affymetrix oligo arrays. The antigenic composition of circulating MPs was identified using a comprehensive proteomic approach by LC-MS/MS analysis. A gene ontology analysis of the proteins identified 26.5% plasma membrane proteins, 58.8% cytoplasmic proteins, 8.8% nuclear proteins and 5.9% extracellular proteins. Analysis of the molecular function of these proteins demonstrated that 35.3% were enzymes, 41.2% were cytoskeletal or vesiculation proteins, 2.9% protein of the nucleosome and 5.9% ribonucleoproteins.

Conclusion: Our data identified circulating MPs with a unique antigenic composition as potential novel biomarkers for noninvasive diagnosis of NASH.

THE EFFECT OF WEIGHT LOSS ON NONALCOHOLIC FATTY LIVER DISEASE IN AN OVERWEIGHT AND OBESE POPULATION

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide and is frequently associated with obesity and insulin resistance. Weight loss is recommended for overweight and obese patients with NAFLD. We aimed at studying the effect of weight loss on metabolic and histological parameters of NAFLD in an obese population.

Methods: Patients presenting for a problem of obesity underwent a metabolic and liver assessment. If NAFLD was suspected, liver biopsy was proposed. Patients were invited to participate in a weight reducing program (hypocaloric diet in combination with physical activity or bariatric surgery). Patients were re-evaluated after 12 months of treatment, including liver biopsy. All biopsies were scored according to the NASH CRN.

Results: 120 patients (70.8% female) were prospectively included (mean age 46.4±1.08 years); 49.2% were treated with lifestyle intervention and 50.8% underwent bariatric surgery. In 46.7% of patients a second liver biopsy was performed (58.9% were treated with lifestyle intervention). After 12 months of treatment mean BMI fell from 39.0±0.51kg/m² to 31.5±0.43kg/m² (P<0.001) and visceral fat dropped from 214.7±31.4cm² to 136.8±46.2 cm² (P<0.001). A significant improvement was observed in the metabolic profile with reduction in lipid profile and insulin resistance (HOMAIR). Serum alanine aminotransferase (P<0.001), aspartate aminotransferase (P<0.001), gamma glutamyltransferase (P<0.001), and alkaline phosphatase (P=0.037) significantly decreased. Significant histological improvement was noted in the NASH Activity Score (from 3.96±0.30 to 1.48±0.29, P<0.001) and its individual components; steatosis (from 1.55±0.14 to 0.52±0.11, P<0.001), lobular inflammation (from 1.21±0.12 to 0.45±0.10, P<0.001) and ballooning (from 1.20±0.10 to 0.52±0.11, P<0.001) and this was also significant in both intervention groups separately. However, mean decreases were higher in the surgery group. Fibrosis stage also showed significant improvement in patients who underwent bariatric surgery (from 1.09±0.24 to 0.52±0.25, P=0.046).

Conclusions: Weight loss by lifestyle intervention can achieve a significant, histologically documented, improvement of NAFLD in overweight and obese patients. Bariatric surgery results in even more pronounced improvement, including fibrosis regression. This work is part of the project “Hepatic and adipose tissue and functions in the metabolic syndrome” (HEPADIP) European Commission 6th Framework Program (Contract LSHM-CT-2005-18734).

EMBOLIZATION OF LARGE SPONTANEOUS PORTOSYSTEMIC SHUNTS FOR REFRACTORY HEPATIC ENCEPHALOPATHY: A EUROPEAN MULTI-CENTER SURVEY ON SAFETY & EFFICACY

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Refactory hepatic encephalopathy (HE) remains a major cause of morbidity in cirrhotic patients. Large spontaneous portosystemic
shunts (SPSSs) have been previously suggested to sustain HE in these patients. We aimed to retrospectively assess the feasibility, efficacy and safety of patients treated with embolization of large SPSSs for the treatment of chronic therapy-refractory HE in a European multicentric working group and to identify patients that may benefit from this procedure.

Between July 1998 and January 2012, 37 patients (Child A6-C13, MELD 5–28) with refractory HE were diagnosed with single large SPSSs which were considered eligible for embolization. On a short-term basis (i.e., within 100 days after embolization), 22 out of 37 patients (59.4%) were free of HE (P < 0.001 vs before embolization) of which 18 (48.6% of patients overall) remained HE-free over a mean period of follow-up of 697 ± 157 days (P < 0.001 vs before embolization). Overall, we noted improved autonomy, decreased number of hospitalizations or severity of the worst HE episode after embolization in three quarters of the patients. Logistic regression identified the MELD-score as strongest positive predictive factor of HE recurrence with a cut-off of 11 for patient selection. As to safety, we noted 1 major non-lethal procedure-related complication. There was no significant increase in de novo development or aggravation of preexisting varices, portal hypertensive gastropathy or ascites.

In conclusion, this multicenter European cohort study demonstrated a role for large SPSSs in chronic protracted or recurrent HE and substantiated the effectiveness and safety of embolization of these shunts provided there is sufficient functional liver reserve.

78 AN OPEN LABEL RANDOMISED CONTROLLED TRIAL OF PROBIOTICS FOR PRIMARY PROPHYLAXIS OF HEPATIC ENCEPHALOPATHY IN PATIENTS WITH CIRRHOSIS

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Background: Hepatic encephalopathy (HE) is associated with poor prognosis. Probiotics alter gut flora with non urease producing organism with decrease ammonia production. Present study assessed effects of probiotics for primary prophylaxis of HE.

Methods: In prospective randomised controlled trial, patients with no history of overt HE were divided into:

- group 1 (Probiotics, n = 86, received VSL#3, one capsule TDS) and
- group 2 (control, n = 74).

Minimal HE (MHE) was diagnosed when psychometric hepatic encephalopathy score (PHES) was ≤5 and overt HE with West Haven criteria.

All patients underwent psychometric tests, critical flicker frequency (CFF), glucose hydrogen breath test (GHBT) for small intestinal bacterial overgrowth (SIBO) and lactulose hydrogen breath test (LHBT) for oro-cecal transit time (OCTT). Primary end point was development of overt HE.

Results: 160 patients (age 48.6 ± 11.1 years, M:F 96:64) were included. 25 (15.6%), 51 (31.9%) and 82 (52.5%) patients were in CTP class A, B and C respectively. Mean CTP score was 9.74 ± 2.63 and MELD score was 19.32 ± 5.91. Baseline laboratory parameters, CTP score, MELD score, CFF, PHES and OCTT were comparable. 42 (48.8%) in group 1 and 33 (44.6%) in group 2 had MHE (p = 0.88). 33 (38.4%) and 26 (35.1%) patients in group 1 and 2 had SIBO respectively. Mean follow up of group 1 and group 2 patients was 28.5 ± 11.8 and 27.3 ± 12.8 weeks respectively (p = 0.87). 11 (6.9%) patients were lost during follow up. 6 (7.5%) in group 1 and 7 (10.1%) in group 2 died (p = 0.81). There was significant improvement in arterial ammonia levels, SIBO, OCTT, PHES, CFF and MHE after 3 months of treatment with probiotics. 7 (8.8%) patients in Group 1 and 14 (20.3%) patients in Group 2 developed overt HE (p < 0.05, hazard ratio 2.1, 95% CI, 1.31–6.53). In patients without MHE, absolute risk reduction (ARR) was 7.8% (95% CI, 2.2–11.4%) and number need to treat (NNT) was 31 (95% CI, 14.2–58.6). However, in patients with MHE (ARR) was 23.8% (95% CI, 5.4–42.2%) and NNT was 5.1 (95% CI, 2.4–18.4).

Conclusion: Probiotics are effective in primary prophylaxis of HE.

79 THE OUTCOME OF SHUNT REDUCTION AFTER TIPS BY THE PARALLEL TECHNIQUE: A PROSPECTIVE STUDY

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Background and Aim: Transjugular intrahepatic portosystemic shunt placement (TIPS) became the standard treatment for a subcategory of patients with refractory ascites and variceal bleeding. It has the disadvantage that it provokes chronic hepatic encephalopathy (HE) and in some patients, with limited liver function, TIPS-induced liver failure (LF). Reduction of the diameter of the TIPS stent is feasible by the parallel technique. However the experience is still limited.

Methods: TIPS reduction was performed by the placement of a 10 mm self-expanding stentgraft together with a 5–6–7 mm balloon expandable stent. After a learning group op 17 pts (Maleux G JVIR 2007), 54 pts were included in this prospective study. Baseline characteristics are listed in the table.

Table I. Baseline characteristics

<table>
<thead>
<tr>
<th>Chronic HE (n = 35)</th>
<th>LF (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59 (41–79)</td>
</tr>
<tr>
<td>Gender: males n (%)</td>
<td>26 (74%)</td>
</tr>
<tr>
<td>Alcoholic cirrhosis n (%)</td>
<td>22 (63%)</td>
</tr>
<tr>
<td>Baseline MELD, points</td>
<td>11 (6–21)</td>
</tr>
<tr>
<td>Indication: Bleeding/ascites, n (%)</td>
<td>7 (20%)/24 (69%)</td>
</tr>
<tr>
<td>Interval: TIPS-reduction</td>
<td>3 (1–36) Months</td>
</tr>
<tr>
<td>Pressure gradient: before/after, mmHg</td>
<td>8 (0–19)/12 (4–28)</td>
</tr>
</tbody>
</table>

*Data expressed as median-range.

Results (6 months): Pts with medical therapy resistant chronic HE: improvement of HE = 25/35 (71%), recurrence of initial indication = 8/35 (25%) and survival = 30/35 (86%). Pts with TIPS-induced LF: improvement 9/19 (47%) and survival 9/19 (47%), of these pts 3 received a liver transplantation.

Conclusions: Stent reductions with the parallel technique improved chronic hepatic encephalopathy in 71% of the patients and offered them a 6 months survival of 86%. In patients who developed TIPS-induced liver failure 47% recovered and TIPS reduction can serve in these patients as a bridge to liver transplantation. Factors affecting prognosis of shunt reduction are currently evaluated in a multicenter trial.

80 SAFETY AND TOLERABILITY OF HUMAN MESENCHYMAL STEM CELLS FOR PATIENTS WITH DECOMPENSATED LIVER CIRRHOSIS: A PRELIMINARY STUDY

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Background and Aim: The study is aimed to evaluate the safety and feasibility of infusions of human umbilical cord mesenchymal stem cells (hUCMSCs) in patients with decompensated liver cirrhosis (DLC).

Methods: It is in an open, dose escalation study. Three doses of hUCMSCs are 5 × 10^7 cells, 1 × 10^8 cells and 2 × 10^8 cells, respectively. The cells were administrated with IV infusion. Each
patient received 3 times infusion every the fourth day, with a follow-up for 52 weeks. The criteria for Adverse Event (AE) was mainly in accordance to the NCI-CTCAE 4.0 version. The study got an approval from IRB, and all subjects have signed ICF before study enrollment (ClinicalTrials.gov identifier: NCT01342250).

**Results:** 20 patients were recruited (14 male and six female, mean age 54.2±5.9 years) from Nov 2010 to May 2011. 17 of them were diagnosed as HBV, while one was HCV. All patients were tolerant with the infusion. Two patients died for complications after 6 months of the first infusion. The overall survival rate was 90% at week 52. The most common AEs were fever, neutrophilia, hyperammonemia, hyperbilirubinemia, and prolonged prothrombin time (PT). Only one SAE (prolonged PT) might be related to cells to 2x10^8 cells/per infusion for DLC patients on the dose limiting toxicity (DLT) test. The study suggests that hUCMSCs could improve clinical outcomes at 52 weeks of follow-up for these patients. And we will initiate a series of muti-center, randomized, blinded studies to explore the safety and efficacy further.

**Conclusions:** It is safe and tolerant when the cells dose escalated to 2x10^6 cells/per infusion for DLC patients on the dose limiting toxicity (DLT) test. The study suggests that hUCMSCs could improve clinical outcomes at 52 weeks of follow-up for these patients. And we will initiate a series of muti-center, randomized, blinded studies to explore the safety and efficacy further.

### 81 BACTERIAL INFECTIONS, ENDOTOXEMIA AND SYSTEMIC INFLAMMATION IN CIRRHOSIS ARE ASSOCIATED WITH AN IMBALANCE OF VON WILLEBRAND FACTOR (VWF) AND ITS CLEAVING PROTEASE ADAMTS13

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**Background:** Increased serum concentrations of VWF antigen (VWF:Ag) are associated with portal hypertension and increased mortality in cirrhosis. Homeostatic competence and thrombogenic potential of VWF depends on its multimer size and are primarily determined by the VWF-cleaving protease ADAMTS13. Whether bacterial translocation (BT), bacterial infections and systemic inflammation modulate the regulation of VWF and ADAMTS13 in cirrhosis is largely unknown.

**Methods:** Patients with compensated cirrhosis without bacterial infections (Group I, n=25), patients with decompensated cirrhosis without bacterial infections (Group II, n=31) and patients with decompensated cirrhosis and overt bacterial infection and/or SIRS (Group III, n=24) were prospectively included and analyzed for VWF:Ag, ADAMTS13, Lipoplyasaccharide-binding protein (LBP) and the presence of bacterial DNA. To assess the functional competence of VWF, ristocetin cofactor activity (VWF:RCo) in plasma from cirrhotic patients was measured using platelets from healthy donors.

**Results:** ADAMTS13 antigen and activity were decreased in patients with decompensated cirrhosis with the lowest levels in patients with bacterial infections or SIRS (antigen 1.57 [I], 0.96 [II], 0.48 mg/dl [III], p=0.0001; activity 87% [I], 70% [II], 47% [III], p=0.004). Simultaneously, VWF:Ag increased over the three groups (1.6 [I], 2.7 [II], 3.6 IU/ml [III], p=0.007) indicating an increasing imbalance of VWF:Ag and its cleaving protease. Conclusively, plasma from patients in group III showed a higher ability to aggregate normal platelets in presence of Ristocetin (VWF:RCo 463), than patients in group II (257; p<0.01) and group I (211; p<0.001). Increasing VWF:RCo activity correlated with increasing LBP serum concentration as marker of BT and endotoxemia (p=0.035) as well as with increasing MELD (p<0.0001) and Child Pugh score (p<0.0001) as markers of decreasing liver function. Even in the absence of portal vein thrombosis, linearly increasing plasma VWF:RCo activity were associated with exponentially increasing D-Dimer levels indicating increased fibrinolytic activity and the possible formation of microthrombi.

**Conclusions:** Bacterial translocation, bacterial infections and systemic inflammation in cirrhosis are accompanied by an imbalance of increased VWF levels and decreased ADAMTS13 activity promoting a pro-thrombotic function of VWF. The presence of VWF multimers may hence contribute to thrombotic events, thrombopenia and organ failure in cirrhosis.

### 82 THE TNF-alpha –238 G-ALLELE PREDISPOSES TO SEVERE BACTERIAL INFECTION IN PATIENTS WITH END-STAGE LIVER DISEASE ENLISTED FOR LIVER TRANSPLANTATION

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**Background:** Augmented susceptibility to infections increases mortality in patients with end-stage liver disease (ESLD). Activation of pro-inflammatory cytokine production via TLR4, which is a major defense mechanism against bacterial infections, shows inter-individual differences. We aimed to determine the contribution of genetic variants in TLR4 and pro-inflammatory cytokines to severe infections in patients with ESLD.

**Methods:** We retrospectively assessed incidence of severe bacterial infections (SBI) (spontaneous bacterial peritonitis, pneumonia, urinary infection, erysipelas, bacteremia) requiring hospitalization and i.v. antibiotics administration in a cohort of 243 adult cirrhotic ESLD patients enlisted for orthotopic liver transplantation (OLT) from 1995 to 2010 in Prague. Patients with hepatocellular carcinoma corresponding to Child–Pugh’s classification A, patients with metabolic liver disorders with normal liver function, patients with primary sclerosing cholangitis (PSC), and patients with acute liver failure were excluded. All patients were genotyped for TLR4 +1196C/T, CD14 −159C/T, TNFα −238C/A, TNFα −863C/A, IL-1B −31C/T and IL-1RA variable number of tandem repeats (VNTR) allelic variants. Associations were validated in a second cohort of 237 cirrhotic ESLD patients enlisted for OLT from 1995 to 2011 in Rotterdam.

**Results:** Sixty nine (69/243, 28%) patients with SBI while enlisted for OLT in the Prague cohort. Patients homozygous for TNFα −238 (GG genotype; (n=221)) showed a significantly increased risk of SBI (OR 9.33, P=0.009) compared to patients with the TNFα −238GA genotype, which is supposed to have increased the transcriptional activity of TNFα in the Rotterdam cohort, seventy two (72/237, 30%) ESLD patients suffered from SBI while enlisted for OLT. In this cohort, the association between TNFα −238GG and increased risk for SBI was confirmed (OR 3.76, 0.001).
p = 0.032). The association was independent of clinical variables that were also included in multivariate analysis.

**Conclusion:** Our results indicate that a genetic variant in the TNFA gene that increases its transcriptional activity independently modifies the risk of SBI in patients with ESLD. These findings may help to identify those patients who are predisposed to SBI.

83 THE NEW CONSENSUS DEFINITION OF ACUTE KIDNEY INJURY IS VALID IN PREDICTING MORTALITY IN HOSPITALIZED INFECTED CIRRHOTIC PATIENTS: THE NACSELD EXPERIENCE

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Cirrhotics with infections have a high mortality risk which is associated with the development of acute kidney injury (AKI). Due to inherent issues with interpreting static creatinine as a renal function marker in cirrhosis, a consensus conference proposed that cirrhosis-associated AKI to be defined as “rise in serum creatinine >50% from baseline or >0.3 mg/dl in <48 hours”.

**Aim:** To validate the new AKI definition in the prediction of mortality in a multi-center cohort of infected hospitalized cirrhotics.

**Methods:** 30-day mortality, length-of-stay (LOS) and organ failure rates were compared between patients with/without AKI in NACSELD. AKI episodes were classified as transient (creatinine returned to baseline), progressive (increasing creatinine or dialysis) or persistent (creatinine rises but plateaus without dialysis). The effect of treatment on AKI was also analyzed.

**Results:** 337 patients (56% men, 56 ± 10 yrs, 31% alcohol, MELD 20 ± 8) from 12 centers were enrolled. Of these, 166 (49%) developed AKI based on the consensus criteria during hospitalization. The leading infections were urinary (28%), SBP (21%), spontaneous bacteremia (14%) and pneumonia (11%). The majority of infectious isolates were gram-positive (34%), followed by gram-negative (28%) and no growth (24%). On admission, the patients who developed AKI had a higher Child–Pugh (13 vs. 6.5 days, p < 0.0001) and lower serum albumin (2.6 vs. 2.8 gm/dL, p < 0.00001) than those who never developed AKI.

**Conclusion**:

- **Outcomes:** Patients with AKI had a higher risk of 30-day mortality (34% vs. 7%, p < 0.0001), ICU transfer (46% vs. 20%, p < 0.0001), ventilation (27% vs. 6%, p < 0.0001), hepatic encephalopathy (73% vs. 45%, p < 0.0001), shock (31% vs. 8%, p < 0.00001) and a longer LOS (13 vs. 6.5 days, p < 0.0001) compared to non-AKI patients. AKI rates were statistically similar between specific infections/organisms.

- **AKI Course:** 56% was transient, 28% persistent and 16% resulted in dialysis; death rate was the highest in progressive (80%) compared to persistent (40%) and transient (15%) compared to those who never developed AKI (7%, p < 0.0001). 12% of patients did not receive AKI-specific therapy, 36% received only albumin while 52% received other treatments.

- **Conclusions:** Mortality risk increases 10-fold with progressive AKI compared to those without AKI. The consensus definition of AKI is valid and predicts 30-day mortality, LOS and organ failures in this multi-center study.

84 IMPROVEMENT OF INTESTINAL PERMEABILITY AND REDUCING BACTERIAL TRANSLATION BY BETABLOCKER TREATMENT IS ASSOCIATED WITH A LOWER RISK OF VARICEAL BLEEDING

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**Introduction:** Evaluation of gastrointestinal permeability and bacterial translocation in cirrhotic patients with portal hypertension (PHT) prior and after nonselective betablocker (NSBB) treatment.

**Methods:** 50 cirrhotic patients underwent the following measurements prior and under NSBB treatment:

- i. portal pressure was assessed by the hepatic venous pressure gradient (HVPG),
- ii. gastroduodenal and intestinal permeability was evaluated by the sucrose-lactulose-mannitol (SLM) test, and
- iii. levels of LPS-binding protein (LBP) and interleukin-6 (IL-6) were quantified by ELISA. Bleeding rates and mortality were recorded during follow-up by clinical visits.

**Results:** Patient characteristics: 72% male, 18% ascites, 60% alcoholic etiology. Prior to NSBB treatment, abnormal gastroduodenal and intestinal permeability were found in 72% and 59% of patients, respectively. Patients with severe portal hypertension (HVPG ≥20 mmHg; n = 35) had increased markers of gastroduodenal/intestinal permeability (urine sucrose levels p = 0.049; sucrose/mannitol ratios p = 0.007; intestinal permeability indices p = 0.002), and of bacterial translocation (LBP p = 0.002; IL-6 p = 0.025) than patients with HVPG <20 mmHg. NSBB treatment did not only result in a significant reduction in HVPG, but also in improvement of gastroduodenal/intestinal permeability and a decrease of bacterial translocation (LBP -16% p = 0.018; IL-6 -41% p < 0.0001) levels These improvements were not limited to hemodynamic responders. Patients with abnormal results by the SLM test indicating abnormal gastroduodenal (p = 0.066) or intestinal permeability (p = 0.084) had a clear trend towards a higher incidence of variceal bleeding. Accordingly, patients with high LBP (p = 0.180) and/or IL-6 (p = 0.038) levels were also at increased risk of variceal bleeding during follow-up. However, these findings did not translate into an increased mortality in patients with abnormal gastroduodenal (p = 0.870) or intestinal permeability (p = 0.994), nor in patients with high levels of LBP (p = 0.571) and IL-6 (p = 0.594).

**Conclusions:** Abnormal gastroduodenal and intestinal permeability are common findings in cirrhotic patients and are correlated with the degree of portal hypertension. NSBB treatment ameliorates gastroduodenal/intestinal permeability and reduces bacterial translocation, which may contribute to the reduced risk of variceal bleeding observed under NSBB treatment.
THE TRANSCRIPTION FACTOR c-JUN/AP-1 PROMOTES HBV-RELATED LIVER TUMORIGENESIS IN MICE

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Introduction: Hepatocellular carcinoma (HCC) frequently develops as a consequence of chronic inflammatory liver disease such as chronic hepatitis B virus (HBV) infection, suggesting that chronic inflammation and cancer are mechanistically linked. The transcription factor c-Jun/AP-1 is strongly expressed in response to inflammatory stimuli, promotes hepatocyte survival during acute hepatitis and acts as an oncogene during chemically-induced liver carcinogenesis in mice.

Aims: We therefore addressed the question whether c-Jun is a major molecular link between chronic hepatitis and hepatocarcinogenesis.

Methods: Transgenic mice expressing all three forms of HBV envelope proteins [Tg(HBsAg)] were crossed with knockout mice lacking c-Jun specifically in hepatocytes. Tumorogenesis as well as molecular alterations at distinct stages of tumorigenesis were analyzed.

Results: Hepatic expression of c-Jun was strongly induced at several time points of tumorigenesis in Tg(HBsAg) mice, whereas expression of other AP-1 components remained unchanged. Importantly, formation of premalignant foci and tumors was almost completely abolished in Tg(HBsAg) mice lacking c-Jun. This phenotype correlated with impaired hepatocyte proliferation at different stages of tumorigenesis and increased expression of the cell cycle inhibitor p21. Further, expression of epidermal growth factor receptor was strongly reduced, while hepatocyte survival was not affected. Progression and prognosis of HBV-related HCC closely correlates with expression of osteopontin (Opn), which is an established AP-1 target gene. Opn expression was indeed strongly reduced in Tg(HBsAg) livers and primary mouse hepatocytes lacking c-Jun, suggesting that c-Jun regulates hepatic Opn expression in a cell-autonomous manner.

Conclusions: These findings suggest that c-Jun has several functions in hepatitis-associated tumorigenesis. While regulating hepatocyte survival during acute hepatitis, during HBV-related tumorigenesis c-Jun promotes hepatocyte proliferation and progression of dysplasia. Therefore, targeting c-Jun may be a strategy to treat hepatitis-associated tumorigenesis.

REGULATION OF ALTERNATIVE SPlicing BY SLU7 IS ESSENTIAL FOR HCC CELL SURVIVAL

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Introduction: The splicing machinery performs the controlled removal of introns and the linking of exons to generate mature and diverse mRNAs. Impaired mRNA splicing is associated with a variety of diseases including HCC. We previously reported that aberrant splicing of p73 in HCC cells is associated with amphiregulin (AR)-mediated down-regulation of the splicing factor Slu7. We also demonstrated that Slu7 expression is reduced in HCCs and also in cirrhotic livers. We now further characterize the role of Slu7 in HCC.

Methods: Human HCC cell lines HepG2, PLC/PRF/5, Hep3B, non-transformed HepaRG cells and primary human hepatocytes. siRNA-mediated Slu7 knockdown. Cell cycle was analyzed by FACS and cell death by DNA fragmentation/detection of nucleosomes (ELISA). Gene expression was analyzed by qPCR/Western blotting.

Results: Slu7 silencing induces HCC cell apoptosis, suggesting that the remnant expression of Slu7 in HCC cells is essential for survival. This effect was not observed either in HepaRG cells or primary hepatocytes, suggesting the existence of compensatory mechanisms in normal cells. HCC cells death is in part p53-mediated, and is preceded by G2/M arrest. Slu7 silencing promotes the aberrant splicing of the antiapoptotic gene BCL2L12, inducing the exon3-defective BCL2L12A isoform previously associated with G2/M arrest. Slu7 silencing also increases the expression of exon8-deleted SIRT1a deacetylase isoform. We demonstrate that this isoform contributes to G2/M arrest and apoptosis in HCC cells. Moreover, PLC/PRF/5 cells apoptosis occurred in the absence of DNA fragmentation due to impaired ICAD (inhibitor of caspase-associated DNase) splicing. All these data demonstrate that Slu7 is necessary for the correct splicing of genes implicated in HCC cell survival. Alternatively, we found that Slu7 knockdown in hepatocytes potentiates the proliferative signalling of oncogenic growth factors, such as AR.

Conclusions: Fine tuning of Slu7 expression in liver parenchymal cells is critical for cell fate. Slu7 down-regulation in hepatocytes favours neoplastic transformation, allowing the induction of oncogenic p73 isoforms and increasing responsiveness to growth/oncogenic factors. Nevertheless, Slu7 expression is still essential for HCC cell survival, being required for the correct splicing of genes such as SIRT1a and BCL2L12, suggesting Slu7 as a new target for HCC treatment.

A LIVER-SPECIFIC DELETION OF NIPP1 CAUSES BILE DUCT HYPERPLASIA AND AN INCREASED SENSITIVITY TO DIETHYLNITROSAMINE-INDUCED CARCINOMA

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NIPP1, an established interactor of protein phosphatase-1 (PP1), is an essential regulator of embryonic development and cell proliferation in multicellular eukaryotes. It acts, at least partially, by regulation of Polycomb Group (PcG) proteins, which are involved in the silencing of genes implicated in cell proliferation and differentiation. Deregulation of PcG proteins contributes to the development of cancer. Importantly, NIPP1 associates with a subset of PcG targets and is required for the repression of these genes. A complete NIPP1 knockout in mice is embryonic lethal just before onset of gastrulation. To further explore the function of NIPP1 in vivo, we generated mice where NIPP1 is mediated Slu7 knockdown. Cell cycle was analyzed by FACS and cell death by DNA fragmentation/detection of nucleosomes (ELISA). Gene expression was analyzed by qPCR/Western blotting.

Results: Slu7 silencing induces HCC cell apoptosis, suggesting that the remnant expression of Slu7 in HCC cells is essential for survival. This effect was not observed either in HepaRG cells or primary hepatocytes, suggesting the existence of compensatory mechanisms in normal cells. HCC cells death is in part p53-mediated, and is preceded by G2/M arrest. Slu7 silencing promotes the aberrant splicing of the antiapoptotic gene BCL2L12, inducing the exon3-defective BCL2L12A isoform previously associated with G2/M arrest. Slu7 silencing also increases the expression of exon8-deleted SIRT1a deacetylase isoform. We demonstrate that this isoform contributes to G2/M arrest and apoptosis in HCC cells. Moreover, PLC/PRF/5 cells apoptosis occurred in the absence of DNA fragmentation due to impaired ICAD (inhibitor of caspase-associated DNase) splicing. All these data demonstrate that Slu7 is necessary for the correct splicing of genes implicated in HCC cell survival. Alternatively, we found that Slu7 knockdown in hepatocytes potentiates the proliferative signalling of oncogenic growth factors, such as AR.

Conclusions: Fine tuning of Slu7 expression in liver parenchymal cells is critical for cell fate. Slu7 down-regulation in hepatocytes favours neoplastic transformation, allowing the induction of oncogenic p73 isoforms and increasing responsiveness to growth/oncogenic factors. Nevertheless, Slu7 expression is still essential for HCC cell survival, being required for the correct splicing of genes such as SIRT1a and BCL2L12, suggesting Slu7 as a new target for HCC treatment.
enhanced anisokaryosis and a clear increase in the occurrence of ballooned hepatocytes. In the NIPP1L-KO mice an immunostaining of the liver sections for the cholangiocyte and progenitor cell marker cytokeratine 19 revealed the beginning of an oval cell response. After one year, the liver of knockout mice showed an obvious bile duct hyperplasia which, surprisingly, was associated with the recurrence of NIPP1 expression. Interestingly, the injection of diethyl-nitrosamine (DEN) during 25 weeks resulted in an increased sensitivity towards the development of both hepatocellular and cholangiocarcinoma in the NIPP1L-KO mice. At this stage most hepatocytes and cholangiocytes in the NIPP1L-KO showed a normal expression of NIPP1, hinting at the replacement of damaged liver cells by NIPP1-containing cells, probably originating from progenitor cells that had escaped Cre-recombination. In conclusion, NIPP1 appears to be indispensable for cell proliferation in the mouse liver and seems required for a balanced differentiation of oval cells into cholangiocytes and hepatocytes. Nevertheless, the NIPP1L-KO mice show an increased predisposition towards the development of liver cancer, hinting at long-term effects from NIPP1-depleted cells.

88 THE FUNCTION OF Fos AND Fos-Jun Dimers in Liver Cancer Development

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Background and Aims: Hepatocellular carcinoma (HCC) belongs to the cancer types which are associated with chronic inflammation. The crucial crosstalk between hepatocytes and non-parenchymal cells, especially immune cells, is well established and many important signaling molecules like MAPKs, NF-kB, AP-1 and STAT3 are involved. We have shown that the proto-oncogene c-Jun, a component of the dimeric AP-1 transcription factor, is required for mouse liver tumorigenesis (Eferl et al., Cell, 2003). In addition, we have demonstrated that c-Jun promotes cell survival during tumor initiation by controlling c-Fos/SIRT6-dependent expression of the anti-apoptotic protein Survivin (Min et al., Nat Cell Biol, 2012). The c-Jun partner c-Fos is frequently over-expressed in HCC, however, the in vivo function of c-Fos in liver cancer remains to be defined.

Methods: The well-established chemical carcinogenesis (DEN) protocol was applied to different mouse models. Conditional deletion of c-fos in hepatocytes and non-parenchymal liver cells was achieved using Mx-cre and hepatocyte-specific Alfp-cre. Importantly, we also generated mice carrying novel tetracycline (tet)-switchable hepatocyte-specific c-fos and forced jun-fos alleles.

Results: Mice with genetic inactivation of c-fos in hepatocytes displayed a significant reduction in tumor burden when subjected to chemical carcinogenesis, whereas deletion of c-fos in hepatocytes and non-parenchymal liver cells did not lead to significant alterations in HCC development. Moreover, hepatocyte-specific ectopic expression of c-Fos in adult mice led to spontaneous dysplastic lesions and promoted DEN-induced liver carcinogenesis. Interestingly, when c-Fos dimerization was restricted to a single Jun partner, the resulting c-Jun~c-Fos and JunD~c-Fos expressing mice altered the carcinogenic process which led to dysplastic lesions and promoted the development of dysplasia. The knock out of c-Fos in liver cancer development. c-Fos appears to have an oncogenic function in hepatocytes, through dimerization with c-Jun or JunD, but not JunB. Interestingly, c-Fos shows a tumor suppressive function in non-parenchymal liver cells. Future studies will lead to a better understanding how c-Fos controls development of liver tumors and possibly influences the tumor microenvironment. This will help to identify new prognostic biomarkers and therapeutic targets.

89 A TRANSPON-BASED PRIMARY TUMOR RESECTION MODEL OF INTRAHEPATIC CHOLANGIOCARCINOMA (ICC) WITH EXTRAHEPATIC DISTANT METASTASES

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Background and Aims: Intrahepatic cholangiocarcinoma (ICC) is a tumor with worldwide increasing incidence and dismal prognosis. Although surgical resection (RO) of the primary tumor is the only potential curative treatment, most of the patients die because of frequent tumor recurrence and outgrowth of metastases after surgery. Mouse tumor resection models are urgently needed to reflect the clinical relevant situation and investigate novel therapies in the context of ICC. In this study, we established a corresponding endogenously induced murine tumor model of resectable, single locus ICC formation.

Methods: We investigated tumor development at a defined intrahepatic locus by injection of Sleeping Beauty-based, oncogenic transposon plasmids into the liver lobe followed by subsequent electroporation. The plasmids included the constitutively activate KRasG12V oncogene together with a plasmid for Cre-recombinase and were applied in p53-fl/fl mice to induce the p53-knockout. This setup was chosen since molecular pathogenesis of ICC frequently involves both KRas-activation and p53-aberrations.

Results: Mice developed a single intrahepatic tumor lesion within 3–7 weeks following electroporation. Development of ICC was verified by histological analysis. Molecular analysis after electroporation of defined lineage-specific fluorescent reporter plasmids provided evidence for hepatocytes as origin of ICC formation. At the time of primary tumor growth no formation of metastases in the lungs could be detected. But formation of satellites close to the primary tumor and vascular invasion could be observed as an indicator of early metastasis. After RO-resection of the primary tumor we were able to prolong median survival with the observation of local disease recurrence, peritoneal carcinomatosis and lung metastases. This resection model of the endogenous, intrahepatic tumor induction allows preclinical testings of adjuvant therapies.

In conclusion, we have successfully developed a murine model of endogenously induced ICC with a single, resectable, primary tumor. This model has favorable characteristics for the study of recurrence patterns after resection and the mechanisms of metastasis. It also holds promise for preclinical evaluation of novel multimodal or adjuvant therapies to prevent recurrence and outgrowth of metastases after RO-resection.

90 HOXA13 AND HOTXIP EXPRESSION LEVELS PREDICT PATIENTS’ SURVIVAL AND METASTASIS FORMATION IN HEPATOCELLULAR CARCINOMA

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Background: The epigenetic mechanisms controlled by the polycombs and trithoraxs genes as well as the HOX genes transcriptional factors family have been linked to the genesis and evolution of liver cancer. Previously, we observed that among the HOX genes, HOXA13 is highly deregulated in hepatocellular
cancer (HCC). More recently, a lncRNA located at the 5’ end of the HOXA locus (in physical contiguity with HOXA13), named HOTTIP, has been identified. HOTTIP binds the WDR5/MLL complexes driving gene transcription along the entire HOXA locus. In this study we aimed to evaluate the impact of HOTTIP and HOXA13 deregulation on HCC pathogenesis.

**Methods:** Total RNA extracted from 60-paired biopsies obtained from HCC patients was used to quantify HOTTIP/HOXA13 expression levels via qRT-PCR and subjected to global transcriptome analysis. HOTTIP/HOXA13 expression levels have been correlated with patients’ clinicopathological data. Non HCC-conditions (normal liver donor, liver inflammation, steatosis, cirrhosis) samples have been used as controls.

**Results:** qRT-PCR data confirmed that HOXA13 is highly deregulated in HCC with no major alteration found in non HCC-conditions. Furthermore, we outlined that HOTTIP is also deregulated in HCC but not in non HCC-conditions and that its expression directly correlates with HOXA13 levels. We also found that virus-related (HBC and/or HCV) HCC samples present the highest expression levels of both HOTTIP and HOXA13 among HCC patients. In addition, we found that both HOTTIP and HOXA13 expression levels predict patients’ overall survival as well as metastasis formation. Finally, the global transcriptome analysis revealed that HOTTIP/HOXA13 overexpression in HCC identifies a specific subset of genes mostly involved in mRNA processing. In vitro siRNA experiments against HOXA13/HOTTIP in HCC cell lines further validated the strong interplay between HOTTIP and HOXA13.

**Conclusions:** We demonstrated for the first time that HOTTIP expression directly correlates with HOXA13 levels in HCC. Moreover, we reported that HOTTIP/HOXA13 deregulation as a key feature in HCC. Finally, we outlined HOTTIP/HOXA13 as predictive markers of HCC patients’ outcome and incidence metastasis formation.

**91 IMMUNOTHERAPY WITH VECTOR ENCODING ALPHA-FETOPROTEIN FUSED WITH PROTEASOME TARGETING SIGNAL PROVIDES NOTABLE PROTECTIVE IMMUNITY AGAINST HEPATOCELLULAR CARCINOMA IN MICE**

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**Background and Aims:** Alpha-fetoprotein (AFP) is a marker of hepatocellular carcinoma (HCC). DNA vaccines against AFP were shown to generate strong immune response. Previously we demonstrated that DNA vaccine bearing HIV-1 reverse transcriptase (RT) gene and mouse ornithine decarboxylase (ODC) degradation signal induced a strong Th1 immune response against RT HIV-1 in mice. It was suggested that the DNA vaccine bearing AFP+ODC signal directed for degradation in proteasome, would induce strong CD8+CTL response against tumor cells expressing AFP and might retard or even prevent HCC appearance.

**Materials and Methods:** Vectors expressing murine AFP (pAFP), mAFP+ODC degradation signal without AFP exportation signal (pAAFPODCsignal) and a number of other constructs were designed. Protein expression was examined in transfected HEK 293T cells. Proteasomal degradation was evaluated by the cycloheximide chase, proteasome inhibition assay and immunofluorescence. Th1 immune response was assessed by ELISA. Tumors in C57BL mice were induced by subcutaneous admittance of 2x10⁶ hepatoma cells (Hepa 1–6 cell line), or by injection of diethylnitrosamine (DENA). Three trials were performed. “Therapeutic” trial – 14 days after tumor cell challenge mice were vaccinated intramuscularly with 100 μg of plasmid. “Prevention” trial – mice were vaccinated four times (50 μg, 2 week intervals). Two weeks after the last vaccination animals were challenged with tumor cells. “DENA therapeutic trial” – infant mice received 25μg/g DENA and were immunized with the constructs 4 times with 2 week intervals starting at 3 months of age and at the age of 10 months the animals were sacrificed. At the end of each trial tumors, livers and serum were examined.

**Results:** Protein ΔAFPODCsignal degraded fast and specific in the proteasomes of transfected cells (half-life 1.5–2h). Preventive vaccination with pAAFPODCsignal yielded 5-fold reduction in mean tumor volume compared to the non-immunized group on day 77 after tumor cell challenge. pAAFPODCsignal impaired growth of HCC by 35% in animals with tumors induced by DENA.

**Conclusions:** Immunotherapy with the DNA encoding AFP and proteasome targeting tag induce not only prominent immune response, but most important a significant retardation of tumor growth in vaccinated animals, becoming a promising candidate vaccine for the HCC prevention.

**92 FORCED IL-6 SIGNALING IS SUFFICIENT TO PROMOTE MALIGNANT TRANSFORMATION IN TELOMERASE-IMMORTALIZED HUMAN FETAL HEPATOCYTES FOLLOWING CHALLENGE WITH OXIDATIVE STRESS**

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**Background and Aims:** HCC is the most prevalent cancer associated with chronic inflammation. Telomere stabilization is considered a prerequisite and early event in multi-step hepatocarcinogenesis; however, hTERT-mediated telomerase reactivation alone does not induce transformation. In chronic inflammation, further downstream oncogenic events are driven by genotoxic reactive oxygen species (ROS) in the context of pro-proliferative signaling pathways. In this project, we investigated the antioxidant response and cellular transformation events in untransformed hTERT-immortalized human fetal hepatocytes (FH-hTERT) with forced activation of IL-6 signaling.

**Methods:** To activate the IL-6 signaling pathway, we generated clones with stable expression of a constitutively active gp130 (L-gp130), responsible for downstream signal transduction. Derived single cell-clones were characterized and challenged with H₂O₂ following glutathione depletion by buthioninesulfoximine (BSO). Quantification of ROS was performed by FACS (Carboxy-H2DCFDA) and DNA-double strand breaks were determined by fluorescent staining for gamma-H2AX. DNA damage and antioxidant response were assessed by qRT-PCR. Finally, to monitor malignant transformation, we determined anchorage-independent growth in soft agar as an established in vitro marker.

**Results:** Stable transfection with L-gp130 resulted in enhanced phosphorylation of STAT3 and MAPK1/2 with upregulation of CCND1. These molecular changes did not induce a transformed phenotype in FH-hTERT/L-gp130 cells and, interestingly, even somewhat suppressed proliferation. Treatment with H₂O₂/BSO resulted in 2- to 3-fold higher ROS levels in FH-hTERT/L-gp130 clones compared to control FH-hTERT cells. In contrast, p21 was not induced and suppressed in some clones in comparison to FH-hTERT control cells. Surprisingly, FH-hTERT/L-gp130 clones developed colony growth capabilities in soft agar with a frequency of up to 20 colonies per 5,000 seeded cells only after challenge with H₂O₂/BSO (in comparison, HuH-7 without treatment 91±12, FH-hTERT with and without treatment no colony formation, FH-hTERT/L-gp130 without treatment no colony formation). As possible mechanism,
we observed a decreased expression of antioxidant genes in FH-hTERT/L-gp130, in particular SOD3, DHCR24, and SEPP1.

**Conclusions:** Forced IL-6 signaling is sufficient to promote malignant transformation in hTERT-immortalized human hepatocytes following exposure to ROS.

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**Parallel Session: CHOLESTASIS AND AUTOIMMUNE LIVER DISEASES**

**93 AUTOIMMUNE ACUTE LIVER FAILURE IN CHILDREN: CLINICAL CLUES AND A NEW DIAGNOSTIC SCORE**

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**Background:** Autoimmune hepatitis (AIH) is considered an underdiagnosed cause of acute liver failure (ALF) both in adults and children. If treatment is initiated promptly, patients with AI-ALF may survive without transplantation (OLT). There are no reported series of children with autoimmune acute liver failure (AI-ALF) may survive without transplantation (OLT). There are no reported series of children with AI-ALF.

**Patients and Methods:** We retrospectively reviewed all cases of ALF referred to our Hospital in the last 16 years. ALF was defined by high transaminases, INR≥2.0 regardless of encephalopathy, and children. If treatment is initiated promptly, AI-ALF was diagnosed in no previously recognised liver disease. AI-ALF was diagnosed in children with AI-ALF who were compared to non-AI-ALF patients and a diagnostic score was built on the basis of statistically significant differences between the two groups.

**Results:** We identified 46 children with ALF; 10/46 (21.7%, M/F=6/4, median age of 6.4 years, range 1.3–15.1, Group 1), had AI-ALF (AIH1=4; AIH2=6); 36/46 (78.3%, M/F=20/16, median age 2.4 years, range 0.2–13.7, Group 2), had AI-ALF due to increased levels of low IL-12 levels leads to long-term increased IFN-γ levels and to the break of the immunologic tolerance giving rise to the development of severe erosive necrosis with significant CD4+ and CD8+ infiltration. This scenario mimics human type I AIH and constitutes the first reported model that does not require the use of transgenic animals. This model is easily reproducible and will be useful for new treatment development and the study of the molecular mechanisms underlying AIH.

**Conclusions:** AAV-mediated sustained hepatospecific expression of low IL-12 levels leads to long-term increased IFN-γ serum levels and to the break of the immunologic tolerance giving rise to the development of severe erosive necrosis with significant CD4+ and CD8+ infiltration. The histopathological analysis of the livers of mice expressing IL-12 revealed the presence of a strong inflammatory infiltrate and the development of severe erosive necrosis with a concomitant rise in transaminases serum levels. Animals treated for 30 days showed presence of anti-nuclear (ANA) and anti-smooth muscle actin (ASMA) antibodies in serum, but no anti-mitochondrial or anti-LKM antibodies. Studies of intrahepatic populations revealed an increase in the number and activation of CD8+ and CD4+ T cells suggesting their active role in the hepatic injury. Despite the detection of increased IFN-γ serum levels, in IFN-γ−/− mice we could not detect any other of the features observed in WT mice.

**Conclusions:** The histopathological analysis of the livers of mice expressing IL-12 revealed the presence of a strong inflammatory infiltrate and the development of severe erosive necrosis with a concomitant rise in transaminases serum levels. Animals treated for 30 days showed presence of anti-nuclear (ANA) and anti-smooth muscle actin (ASMA) antibodies in serum, but no anti-mitochondrial or anti-LKM antibodies. Studies of intrahepatic populations revealed an increase in the number and activation of CD8+ and CD4+ T cells suggesting their active role in the hepatic injury. Despite the detection of increased IFN-γ serum levels, in IFN-γ−/− mice we could not detect any other of the features observed in WT mice.

**95 JNK1-DEPENDENT MODULATION OF miR-34a CONTRIBUTES TO APOPTOSIS INDUCED BY DEOXYCHOLIC ACID IN PRIMARY RAT HEPATOCYTES**

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**Background and Aims:** MicroRNAs (miRNAs or miRs) have been increasingly implicated in the pathogenesis of human liver diseases. In this regard, we have recently shown that miR-34a expression correlates with non-alcoholic fatty liver disease
severity and apoptosis. miR-34a-induced apoptosis is, at least partially, dependent on p53 and Sirtuin1 (SIRT1) that regulate apoptosis in response to oxidative and genotoxic stress. In turn, hydrophobic bile acids, such as deoxycholic acid (DCA), are known to modulate the expression of several apoptosis-related proteins, including c-Jun N-terminal kinases (JNK), and enhance the generation of cellular reactive oxygen species, leading to cell death. The purpose of this study was to evaluate if DCA-induced apoptosis of primary rat hepatocytes is regulated by miR-34a-dependent pathways.

**Methods:** Primary rat hepatocytes were incubated with 100 microM DCA, and transfected with a specific miRNA-34a precursor and/or with a p53 overexpression plasmid. p53 transcriptional activity was assessed in nuclear extracts and by using target reporter constructs. JNK function was evaluated by immunoblotting and silencing experiments. Viability, caspase-3 activity and apoptosis were determined by the ApoTox-Glo™ Triplex Assay and Hoechst staining.

**Results:** Our results show that DCA enhances the miR-34a/SIRT1/p53 pro-apoptotic signaling in cultured primary rat hepatocytes. miR-34a overexpression further potentiated the effect of DCA on miR-34a-dependent signaling and, ultimately, apoptosis. Modulation of SIRT1 by DCA was mostly miR-34a-dependent. In addition, p53 overexpression activated miR-34a/SIRT1/p53 pathway, which was further activated by DCA. DCA increased general p53 activity, as well as specific transcriptional activation of PUMA, p21 and miR-34a itself, providing a functional mechanism for miR-34a activation. Finally, JNK1 was shown to be a key target of DCA, upstream of p53, in activating the miR-34a/SIRT1/p53 pathway and engaging apoptosis.

**Conclusion:** Our results support a link between liver cell apoptosis, miR-34a/SIRT1/p53, and JNK signaling. JNK1-mediated activation of p53 is the key mechanism behind induction of miR-34a by DCA. The miR-34a/SIRT1/p53 pro-apoptotic pathway may represent an attractive pharmacological target for the development of new drugs to arrest apoptosis-related liver pathologies.


**96 KNOCKOUT OF THE PSC RISK GENE FUT2 SENSITIZES MICE TO HEPATO-BILIARY BILE ACID TOXICITY**

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**Background and Aims:** The pathogenesis of chronic cholestatic liver diseases is still enigmatic, but bile acid toxicity is considered one major contributor to disease development. A common variant in the fucosyltransferase 2 (FUT2) gene has been shown in genome wide association studies to confer risk for primary sclerosing cholangitis (PSC) and to be associated with serum levels of alkaline phosphatase (ALP). FUT2 is involved in glycocalyx formation. We recently demonstrated that an intact apical cholangiocyte glycocalyx is essential for stability of the biliary HCO3- umbrella, protecting cholangiocytes against bile acid toxicity in vitro (Hepatology 2012;55:173). The aim of this study was to assess in vivo a potential role for Fut2 in hepatobiliary protection against toxic bile acids.

**Methods:** Fut2-/- mice and wildtype (wt) littermates were fed control or glycochenodeoxycholate (GCDC; pH, 4.2)-containing diets for up to 3 months. Serum liver tests, bile acid metabolism, bile flow and liver histology were studied.

**Results:** Fut2-/- in comparison to wt mice showed elevated plasma bile salt levels (mean 40.1 vs. 0.7 µmol/L, p < 0.05), irrespective of diet, and lower bile flow (7.9 vs. 9.7 µL/min/100 g b.w., p < 0.05), despite a preserved biliary bile salt output. Elevation of plasma bile salt levels was correlated with an aberrant morphology of portal triads characterized by thickened vessel walls, disturbed proportion between veins and other structures in the triad, lymphatic edema, and bile duct inflammation. Fut2-/- mice, compared to wt, developed substantial weight loss (-24±3%, n=3) and increased levels of serum liver tests (ASAT 960 vs. 267 U/L, p < 0.001; ALAT 1048 vs. 143 U/L, p < 0.01; ALP 252 vs. 127 U/L, p < 0.01) upon feeding a 0.3% GCDC-containing diet for up to 14 days. Liver histology revealed areas of parenchymal necrosis and signs of cholangiocyte proliferation. Comparable liver damage was not observed upon feeding a 0.1% GCDC-containing diet at different intervals (n=3-8) for up to 3 months.

**Conclusion:** Fut2-/- mice show hepatobiliary abnormalities, increased plasma bile salt levels, impaired bile flow, and are sensitive to GCDC-induced hepatobiliary damage. Our data suggest that Fut2 may play a critical role in hepatobiliary protection against toxic human bile acids.

97 BILIARY STRICTURES AND RECURRENT DISEASE AFTER LIVER TRANSPLANTATION FOR PRIMARY SCLEROSING CHOLANGITIS – A LARGE, MULTICENTER COHORT ANALYSIS WITH LONG-TERM FOLLOW-UP


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**Background and Aims:** Liver transplantation (LT) is the only definitive treatment for patients with end-stage liver disease due to primary sclerosing cholangitis (PSC). Although the long-term recipient survival is favorable for this indication, a higher rate of biliary strictures as well as of recurrent disease has been reported from various transplant centres. In this study we analyzed a large patient cohort with a very long follow-up in order to re-evaluate the incidence of and risk factors for biliary complications after LT for PSC.

**Methods:** We retrospectively collected clinical, surgical and laboratory data, data on inflammatory bowel disease (IBD), on immunosupression, on recipient and graft outcome and on biliary complications (based on cholangiography and histology) of all
patients who underwent LT for PSC in 10 German transplant centers between 01/1990 and 12/2006. Statistical analyses were performed using Mann–Whitney- and chi-square-test.

**Results:** 335 patients (68.4% men, mean age 38.9 years, 73.5% with IBD) were transplanted 8.5 years (mean) after initial PSC diagnosis and followed for 98.6 months (mean, range 0–266). The one-year, five-year- and overall recipient and graft survival was 90.7%, 84.7%, 76.1% and 79.1%, 69.0%, 59.4% respectively. After exclusion of patients with a graft follow up <6 months (n=68), with missing clinical information (n=22) and with recurrent cholangiocarcinoma (n=6) we found that 44% of the remaining 239 patients developed biliary strictures. At further exclusion of 48 patients with arterial complications, chronic ductopenic rejection, pure anastomotic strictures or early (<90 days) interhepatic strictures we diagnosed recurrent PSC in 32% (18.5%) of the overall study cohort. We identified the following significant (p<0.05) risk factors of recurrent PSC: higher MELD- or Mayo-risk-Score at LT, male sex, IBD, active colon-inflammation following LT, more than one episode of acute rejection. Furthermore arterial complications and a chronic ductopenic rejection were significantly associated with overall biliary strictures.

**Conclusions:** This so far largest study on the long-term outcome of LT for PSC confirms some of the clinical risk factors of PSC recurrence like acute cellular rejections and colon inflammation and identifies additional new risk factors. These findings might help to optimize the pre- and post-LT-management of PSC.

98 norUDCA AMELIORATES CHOLEMIC NEPHROPATHY IN LONG-TERM COMMON BILE DUCT LIGATED MICE

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**Background and Aims:** Acute kidney injury commonly occurs in patients with advanced cholestatic liver disease. The structural renal changes in patients with cholestasis became known as cholemic nephropathy. Moreover, renal function is a major renal change in patients with cholestasis became known as cholemic nephropathy. The structural background and aims of the current study are to test the hypothesis that treatment with hydrophilic norursodeoxycholic acid (norUDCA), which undergoes excretion of bile acids (Fickert P, J Hep 2011; 54 (Suppl 1): 244). We therefore aimed to test the hypothesis that treatment with hydrophilic norursodeoxycholic acid (norUDCA), which undergoes extensive renal excretion, protects long-term CBDL mice from cholemic nephropathy.

**Methods:** Five days norUDCA (0.125% w/w) – and chow-fed CL57/BL6 mice (controls) were subjected to CBDL and diets were continued until harvesting 8 weeks thereafter. Body weight course, kidney weight, serum urea levels, cytoclogic urinalysis, and kidney morphology using H&E and Sirius Red stained kidney sections were compared. Renal hydroxyproline content was measured for quantification of kidney fibrosis together with key genes of inflammation and fibrogenesis.

**Results:** Controls developed substantial impairment of renal function with significantly elevated serum urea levels (98.4±37.2 mg/dl) compared to normalized renal function in norUDCA-treated CBDL mice (40.6±8.5 mg/dl, p<0.05). Controls showed renal tubular epithelial cells and cylinders on urine cytology together with pronounced tubulointerstitial nephritis and fibrosis as reflected by significantly increased renal hydroxyproline content (797±160 µg/g), whereas norUDCA significantly ameliorated cholemic nephropathy as demonstrated by normal urine cytology and significantly reduced hydroxyproline content (466±107 µg/g, p<0.05 vs. controls). H&E and Sirius Red staining revealed significantly reduced degree of nephritis and extracellular matrix deposition in norUDCA-fed CBDL mice compared to controls (Figure 1).

Figure 1.

**Conclusion:** norUDCA is highly effective in ameliorating cholemic nephropathy in long-term CBDL mice and may therefore represent a novel therapeutic option for cholemic nephropathy.

99 BASELINE PREDICTIVE FACTORS OF URSODEOXYCHOLIC ACID RESPONSE IN PRIMARY BILIARY CIRRHOSIS

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**Background:** Biochemical response to ursodeoxycholic acid (UDCA) strongly predicts long-term prognosis in patients with primary biliary cirrhosis (PBC). Factors predicting UDCA response might therefore be extrapolated to predict liver transplant (LT)-free survival. In this study, we aimed to identify baseline clinical variables associated with UDCA response, which might be used for risk-stratification.

**Methods:** This was an observational study of 1379 PBC patients recruited to the UK-PBC Project. Clinical data were obtained from case notes. Response to UDCA was evaluated according to the Barcelona, Paris I, Toronto and Paris II criteria. Time-to-event analysis was used to evaluate these criteria in our cohort. Logistic regression was used to identify variables associated with UDCA response.

**Results:**
**Results:** Complete data were available for 1370 PBC patients, including 70 LT recipients. Median follow-up was 72.2 months. Cox regression showed that survival was best predicted by the Paris I criteria (HR=8.4; P=5.10E-12); these criteria were used to define treatment response in subsequent analyses. Overall, 75% of patients responded to UDCA. Multivariate analysis showed that treatment response was predicted by female sex, older age, increased creatinine, lower bilirubin, lower alkaline phosphatase and the absence of splenomegaly at baseline (Table 1).

<table>
<thead>
<tr>
<th>Baseline variable</th>
<th>Odds Ratio</th>
<th>L95</th>
<th>U95</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male versus female)</td>
<td>0.897</td>
<td>0.829</td>
<td>0.971</td>
<td>7.38 \times 10^{-3}</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.006</td>
<td>1.004</td>
<td>1.008</td>
<td>1.56 \times 10^{-7}</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.002</td>
<td>1.001</td>
<td>1.002</td>
<td>7.48 \times 10^{-4}</td>
</tr>
<tr>
<td>LN Bilirubin</td>
<td>0.841</td>
<td>0.811</td>
<td>0.873</td>
<td>&lt;2 \times 10^{-16}</td>
</tr>
<tr>
<td>LN ALP</td>
<td>0.873</td>
<td>0.848</td>
<td>0.900</td>
<td>&lt;2 \times 10^{-16}</td>
</tr>
<tr>
<td>Spleen &gt;12cm</td>
<td>0.892</td>
<td>0.830</td>
<td>0.959</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**Conclusions:** We have identified baseline factors that predict UDCA response, which might also predict long-term prognosis. These factors might be used to prioritise high-risk patients for intensive follow-up and early use of second-line therapies. It is interesting that sex and age at presentation influence outcome. This is a novel and intriguing observation which demands further study. Finally, we have found in the largest-ever series of PBC patients that of the UDCA-response criteria evaluated, all have strong prognostic value. However, the Paris I criteria performed best in our cohort.

**100 COMBINATION OF CALCINEURIN INHIBITORS AND GENOTYPES AT THE IL12A LOcus INFLUENCES RISK OF RECURRENT PRIMARY BILIARY CIRRHOSIS AFTER LIVER TRANSPLANTATION**

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**Background:** PBC is a complex disorder, resulting from the interaction of genetic and environmental factors. GWAS and iCHIP-association studies of PBC have corroborated the well-established HLA association and identified many non-HLA risk loci, including the IL12A locus. PBC represents a major indication for liver transplantation (LT). LT for PBC is effective but the disease recurs in up to 35% of PBC LT recipients and may lead to graft failure. The only well-established risk factor for recurrent PBC (rPBC) is the use of calcineurin-inhibitors (CNI) after LT. The role of genetic factors in rPBC has not been thoroughly investigated. In this study, we sought to determine whether established PBC risk loci influence the time to rPBC in the liver allograft.

**Patients and Methods:** We identified 248 PBC LT recipients for whom genotype data were available from the Wellcome Trust Case–Control Consortium (WTCCC3) iCHIP study of PBC. We undertook time-to-event analysis using the Cox proportional hazards model and Kaplan–Meier (K-M) method.

**Results:** One-hundred-and-five (42.3%) LT recipients developed rPBC with a median time to recurrence of 61.8 months (25–75% IQ: 16.7–103.8). In univariate analysis we found that CNI at induction, CNI at one year, and the SNPs rs62270414 and rs668998 at 3q25 (IL12A locus) and rs11117433 at 16q24 (IRF8 locus) influenced the risk of rPBC. In multivariate analysis using the Cox model, only CNI at one year (Tacrolimus vs. Cyclosporin, Hazard Ratio [HR]=2.70, P=2.7x10^{-5}) and rs62270414 (risk allele G, HR=1.65, P=0.008) remained significant. Figure 1 shows that risk of rPBC is greatest with a combination of Tacrolimus at one year and rs62270414 genotype AG, and least with a combination of Cyclosporin at one year and rs62270414 genotype AA.

**Conclusions:** This study shows that the rate of rPBC is influenced by a combination of genetic and acquired factors (i.e. genotypes at the IL12A locus and CNI at one year). Functional aspects of an interaction between IL-2 and IL-12 pathways need further exploration.
101 TENOFOVIR DF (TDF) COMPARED TO EMTRICITABINE (FTC)/TDF IN HBeAg-POSITIVE, CHRONIC HEPATITIS B (CHB) VIRUS-INFECTED PATIENTS IN THE IMMUNE TOLERANT (IT) PHASE

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Background and Aims: Current guidelines do not recommend treatment with oral antiviral agents (OAV) for IT, CHB patients. We evaluated the efficacy and safety of TDF compared to FTC/TDF in this population.

Methods: Phase 2, prospective, randomized (1:1), double-blind study in HBeAg-positive patients with HBV DNA ≥10⁸ copies/mL and ALT ≤ULN. Patients were assessed for efficacy, safety, and resistance at frequent intervals over 192 weeks.

Results: Of 126 randomized and treated patients, 52/64 (81%) and 52/62 (84%) in the TDF and FTC/TDF groups, respectively, completed 192 weeks. Key results (ITT, Missing=Failure) are summarized in the table.

Table: Baseline characteristics and efficacy results

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TDF (N=64)</th>
<th>FTC/TDF (N=62)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs, mean (range)</td>
<td>33 (18, 62)</td>
<td>33 (18, 58)</td>
<td>0.664</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>31 (48% )</td>
<td>31 (50%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Asian, n (%)</td>
<td>56 (87% )</td>
<td>56 (90%)</td>
<td>0.463</td>
</tr>
<tr>
<td>Genotype B/C, n (%)</td>
<td>33 (52%)</td>
<td>32 (52%)</td>
<td>0.626</td>
</tr>
<tr>
<td>HBV DNA log₁₀ copies/mL, mean (SD)</td>
<td>5.18 (0.40)</td>
<td>5.06 (0.35)</td>
<td>0.878</td>
</tr>
<tr>
<td>Efficacy results:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV DNA &lt;400 cp/mL, n (%)</td>
<td>35 (55%)</td>
<td>47 (76%)</td>
<td>0.016</td>
</tr>
<tr>
<td>HBV DNA &lt;400 cp/mL, mean (SD)</td>
<td>6.32 (1.46)</td>
<td>6.70 (0.91)</td>
<td>0.070</td>
</tr>
<tr>
<td>HBeAg seroconversion, n (%)</td>
<td>3/33 (9%)</td>
<td>6/62 (10%)</td>
<td>0.244</td>
</tr>
</tbody>
</table>

*p value if α = 0.05

No subject had HBSAg loss over 192 weeks. Both treatments were well tolerated with 2% overall (2/126; both in FTC/TDF group) discontinuing for an AE. One patient experienced an ALT flare on TDF associated with nonadherence. No patients had a confirmed increase from BL in serum creatinine of ≥0.5 mg/dL or decrease in CrCl <50 ml/min; 1 TDF patient had a transient serum phosphorus <2 mg/dL. No mutations associated with TDF resistance were detected by genotypic analysis through 192 weeks.

Conclusions: In IT CHB patients, a higher proportion achieved HBV DNA suppression with FTC/TDF; no differences in biochemical or serological responses were seen. Treatments were well tolerated through 192 weeks and no resistance to TDF was detected. It remains to be established whether patients would benefit from OAVs in the IT phase.

102 THE ART OF DECISION MAKING: RETREATMENT WITH TACE IN PATIENTS WITH HEPATOCELULAR CARCINOMA

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Background: We aimed to establish an objective point score to guide the decision for retreatment with transcatheter chemoembolization (TACE) in patients with hepatocellular carcinoma (HCC).

Methods: 222 patients diagnosed with HCC and treated with multiple TACE cycles between 1/1999 and 12/2009 at the Departments of Gastroenterology/Hepatology of the Medical Universities of Vienna (training cohort) and Innsbruck (validation cohort) were included. We investigated the effect of the first TACE on parameters of liver function and tumor response and their impact on overall survival (OS, log rank test) and developed a point score (ART-score: Assessment for Retreatment with TACE) in the training cohort (n = 107, Vienna) by using a stepwise Cox regression model. The ART-score was externally validated in an independent validation cohort (n = 115, Innsbruck).

Results: The increase of AST by >25% (Hazard Ratio (HR) 8.4; p < 0.001), the increase of Child–Pugh score of one (HR 2.0) or ≥2 points (HR 4.4) (p < 0.001) from baseline and absence of radiologic tumor response (HR 1.7; p = 0.026) remained independent negative prognostic factors for OS and were used to create the ART-score. The ART-score differentiated 2 groups (0–1.5 points; ≥2.5 points) with distinct prognosis (median OS: 23.7 vs. 6.6 months; p < 0.001) and a higher ART-score was associated with major adverse events after the second TACE (p = 0.011). These results were confirmed in the external validation cohort and remained significant irrespective of Child–Pugh stage and the presence of ascites prior the second TACE.

Conclusion: An ART-score of ≥2.5 prior the second TACE identifies patients with dismal prognosis who may not profit from further TACE sessions.

103 EFFECT OF REGULAR TRAINING ON HEPATOCELULAR CARCINOMA DEVELOPMENT IN HEPATOCYTE-SPECIFIC PTEN-DEFICIENT MICE

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Background and Aims: Modern, unhealthy lifestyles predispose to NASH. Hepatocellular carcinoma (HCC) is a frequent complication of NASH. Steatosis decreases the expression of the tumor suppressor Pten and HCCs present frequently an activation of the downstream mTOR pathway. NASH patients benefit from physical activity. However, it is unknown whether regular exercise reduces the risk to develop HCC.
Methods: Male hepatocyte-specific Pten-deficient mice (AlbCre-PtenFlox/Flox), which develop spontaneously steatohepatitis and HCC, fed Control Diet (CD) or High Fat Diet (HFD) were randomly divided between exercise and sedentary groups. The exercise groups run on a motorized treadmill for 60 min/day, 5 days/week during 32 weeks. At sacrifice, the body and liver weights were measured. The number and the size of nodules were registered. Livers and tumors were analysed by histology; steatosis and NAFLD activity score (NAS) were graded.

Results: After 32 weeks of regular exercise, body weight, but not liver weight, was significantly reduced by exercise in CD-fed mice. No difference was observed in HFD-fed mice. Under CD, 71% of exercised mice developed nodules larger than 10 mm³ vs. 100% in the sedentary group. The mean number of HCCs per liver was reduced by exercise (1.8±0.8 vs 2.8±2.3), as well as the total tumor volume per liver (444±551 vs 945±1007 mm³). Under HFD, 50% of exercised and sedentary mice developed nodules. The mean number and the total number of HCCs per liver were similar in both groups (2.8±0.8 vs 2.8±0.8 and 484±474 vs 480±317 mm³ respectively). Exercise did not affect steatosis in CD-fed mice (1.4±0.7 vs 1.6±0.8), but exercise reduced steatosis in HFD-fed mice (1.3±0.7 vs 2.3±0.7, p<0.01). Exercise decreased the NAS in HFD-fed mice (2.1±1.2 vs 4.7±1.9, p=0.03), but this effect was absent in CD-fed mice (2.2±0.1 vs 2.8±1.4).

Conclusion: These data show a benefit of regular exercise on the development of HCC in an experimental model of NASH characterized by overactivation of the mTOR pathway. This beneficial effect was lost under high fat diet and independent of the improvement of NASH histology. The mechanisms underlying these effects remain to be determined.

This research received funding from the European Community’s Seventh Framework Programme (FPZ/2007–2013).

104 A CANDIDATE-GENE APPROACH TO VALIDATION OF GENETIC MODIFIER ASSOCIATIONS USING A LARGE COHORT WITH HISTOLOGICALLY CHARACTERISED NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction: Inter-patient genetic variation and environment determine severity and progression of non-alcoholic fatty liver disease (NAFLD). Previous genome-wide association studies (GWAS) have provided important insights into modifier genes influencing radiologically determined steatosis (Romeo, 2008; Speliotes, 2011) and clinical biochemistry (Chambers, 2011) but have been unable to assess effect on steatohepatitis and fibrosis. Using data drawn form a large GWAS study of patients with histologically characterised NAFLD, we adopted a candidate-gene approach to re-examine the broader validity of these associations.

Methods: Genetic variation in a cohort of 1,125 European and North American Caucasian patients with histologically characterised NAFLD from the FLIP Consortium and the NASH CRN was determined using the Illumina OmniExpress platform and targeted TaqMan genotyping for the PNPLA3 rs738409 SNP. Genotyping data relating to genetic variations in 6 genes previously associated with radiologically determined NAFLD (PNPLA3, NCAN, LYPLAL1, GCKR, PPP1R3B) and 1 gene associated with persistent elevation in ALT (TRIB1) were extracted from this recently reported GWAS dataset and compared to 6,000 population controls drawn from the WTCCC and POPSRES cohorts.

Results: In a multivariate model adjusted for age, body mass index, presence of diabetes/insulin resistance and recruitment centre, we identified variants within 4 genes significantly associated with specific histological features of NAFLD after correction for multiple testing. Specifically, Steatosis (PNPLA3 rs738409, GCKR rs780094, PPP1R3B rs11777327, TRIB1 rs2385114); Steatohepatitis (PNPLA3, GCKR, TRIB1); and Fibrosis (PNPLA3, GCKR, TRIB1). However, NCAN and LYPLAL1 did not associate with any aspect of the NAFLD phenotype.

Conclusions: This candidate-gene study across the histological spectrum of NAFLD has further established the overwhelming significance of the chromosome 22 PNPLA3 locus to all aspects of NAFLD and has, for the first time, demonstrated that variations in some loci previously associated with steatosis or mild biochemical abnormalities may have broader pathological significance influencing inflammatory disease and progression to fibrosis in NAFLD.
and glycosylated hemoglobin (p = 0.030) levels than non-NASH patients. With respect to P-MRS, NAFLD patients had increased [PME+PDE]/TP and PDE/TP (p < 0.001). NASH patients showed decreased total ATP/TP and α-ATP/TP (p < 0.004) but normal PME/[PME+PDE] and GCP/[PME+PDE], whereas non-NASH showed normal ATP/TP and α-ATP/TP, decreased PME/[PME+PDE] (p = 0.026) and increased GCP/[PME+PDE] (p = 0.0009). Area under the receiver-operating characteristics curve (AUROC) for total ATP/TP was 0.68 (95% confidence interval CI, 0.58–0.75), and cutoff values of 0.4046 and 0.3173 yielded 91% sensitivity and specificity for diagnosing NASH. AUROC for α-ATP/TP was 0.71 (95%CI, 0.62–0.79), with 91% sensitivity and specificity using thresholds of 0.1636 and 0.1057, respectively.

**Conclusion:** P-MRS shows distinct alterations in ATP and PDE levels which are concordant with mitochondrial dysfunction and endoplasmic reticulum stress, considered to be key players in the progression of NAFLD. P-MRS shows fair diagnostic accuracy for NASH.

### 106 SUSTAINED VIRAL RESPONSE AND SAFETY OF MK-7009 IN CIRRHOTIC TREATMENT-EXPERIENCED PATIENTS WITH GENOTYPE 1 HCV INFECTION WHO HAVE FAILED PREVIOUS PEGYLATED INTERFERON AND RIBAVIRIN TREATMENT


**Background:** MK-7009 is an oral HCV-NS3/4A protease inhibitor being assessed in combination with peginterferon alfa-2a (P) 180 μg QW and ribavirin (R) 1000–1200 mg/d for the treatment of chronic hepatitis C infection.

**Methods:** This is a study in HCV genotype 1 cirrhotic patients who have failed previous PR treatment evaluating MK-7009 in four treatment regimens as shown in the Table. Stratification was by prior response to PR: Null response, partial response, breakthrough and relapse. A minimum of 33% of patients were prior null responders. The majority of patients had baseline HCV RNA greater than 800,000 IU/mL. The primary endpoint of the study was sustained virologic response at 24 weeks after treatment completion (SVR24).

**Results:** 74 compensated cirrhotic patients were enrolled. Of 42 genotyped patients, 71% were IL28B non-CC. 5.4% were African-Americans. All MK-7009 treatment regimens showed statistically significantly superior SVR24 rates vs control: 60.0%, 69.2%, 76.9% and 53.3% in the MK-7009 regimens 1 through 4 relative to 14.3% in control. Patients in MK-7009 treatment regimens exhibited higher rates of GI AEs (nausea, vomiting, diarrhea) compared with control; all cases were mild to moderate. There were no significant differences in rates of rash or grade 1 anemia between the MK-7009 treatment regimens and control. 3 of 60 patients (5%) on MK-7009 reported grade 2 (7.5–8.4 gm/dL) anemia vs none in control. Resistance-associated amino acid variants were predominantly observed at positions 155, 156 and 168 in non-SVR patients.

**Conclusion:** MK-7009 when combined with PR more than tripled the SVR compared with PR control in this trial. MK-7009 is safe for use for up to 48 weeks of therapy in compensated cirrhotic patients. The phase III studies in the treatment-naive and treatment-experienced patients in Japan are ongoing.

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### Parallel Session: HEPATOCELLULAR CARCINOMA – CLINICAL

#### 107 A RANDOMISED CONTROLLED TRIAL OF MELOXICAM, A COX-2 INHIBITOR, TO PREVENT HEPATOCELLULAR CARCINOMA RECURRENT AFTER INITIAL CURATIVE TREATMENT

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**Background and Aims:** Because the recurrence rate of hepatocellular carcinoma (HCC) is high, even after curative treatments such as hepatic resection and microwave ablation, chemopreventive agents that can effectively suppress HCC recurrence are required. Cyclooxygenase-2 (COX-2) was recently found to be overexpressed in HCC. Therefore, COX-2 inhibitors may offer a chemopreventive therapy for HCC. This randomised controlled trial (RCT) investigated the potential for meloxicam, a clinically used COX-2 inhibitor, to prevent HCC recurrence after initial curative treatment.

**Methods:** 232 consecutive patients underwent hepatic resection and/or microwave ablation as initial therapy for HCC at our institute between July 2008 and April 2011. 8 patients were excluded because of poor renal function, history of non-steroidal anti-inflammatory drug-related ulceration, or multiple cancers. The remaining 224 patients were randomised to a control group (CG; n = 113) or a meloxicam group (MG; n = 111). At 7 patients in the CG received meloxicam after the RCT, 106 patients who did not receive meloxicam were analysed as the true control group (TCG). 30 patients in the meloxicam group discontinued meloxicam because of a decline in renal function, gastric ulceration, and poor compliance, so 111 patients were analysed as the true meloxicam group (TMG). The overall survival (OS) and disease-free survival (DFS) rates were determined.

**Results:** The 1-, 2-, and 3-year OS rates were 96.2%/98.2%, 89.5%/90.3%, and 83.8%/85.2% in CG/MG, respectively (P = 0.7447). The corresponding DFS rates were 88.9%/88.0%, 70.6%/73.8%, and 59.3%/42.7% (P = 0.4539). Intent-to-treat analysis showed no differences in OS or DFS between the GC and GM groups. Among patients with good liver function (Child–Pugh class A), per-protocol analysis showed that the 1-, 2-, and 3-year OS rates for the TMG (n = 70)/TCG (n = 82) were 98.5%/96.8%, 96.0%/95.2%, and 96.0%/91.3% (P = 0.3201), respectively, while the corresponding DFS rates were 97.0%/87.4%, 87.4%/77.4%, and 73.4%/48.7% (P = 0.0160). DFS was significantly different between the TMG and TCG. There were no significant differences in OS or DFS in other patient groups, including those with Child–Pugh class B or hepatitis C virus infection.

**Conclusion:** Administration of the COX-2 inhibitor meloxicam may suppress HCC recurrence after initial curative treatments in patients with good liver function (Child–Pugh class A).

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**Journal of Hepatology 2013 vol. 58 | S45–S61 S47**
A NOVEL MODEL OF PRIORITY ASSESSMENT FOR PATIENTS WITH AND WITHOUT HEPATOCELLULAR CARCINOMA ON A COMMON LIVER TRANSPLANT WAITING LIST: A MULTICENTRE, COHORT STUDY

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BACKGROUND: Liver transplantation (LT) priority assessment strictly based on 3-months dropout estimation creates both an unbalance between patients with and without hepatocellular carcinoma (HCC), and penalizes post LT outcomes. The aim of this study was to describe an alternative model able to re-establish allocation equity between HCC and non-HCC patients using 5-year transplant benefit as the common endpoint.

METHODS: We enrolled consecutive adult patients with chronic end-stage liver disease entering the waiting list (WL) for LT (WL group = 2697) and undergoing LT (LT group = 1702) during the period 2004–2009 in the North Italian Transplant program area. Two independent multivariable regressions (WL and LT models) were created to measure the prognostic power of model for end stage liver disease (MELD) in patients with and without HCC. The models were also adjusted for the following covariates: recipient age, sex, and aetiology, re-transplant, donor age. For the WL model we used competing risk multivariable analysis. Hazard ratio (HR, 95% confidence intervals) were finally included in a Markov model to calculate 5-year survival benefit in different subgroups.

RESULTS: WL competing risk model: MELD significantly predicted survival in both HCC (1.075, 1.043–1.110) and non-HCC (1.061, 1.053–1.080) patients.

LT Cox model: MELD significantly predicted survival in both HCC (1.075, 1.043–1.110) and non-HCC (1.061, 1.053–1.080) patients.

Benefit model: the survival benefit of LT at each MELD point was higher in HCC than non-HCC patients (Figure 1).

Figure 1.

Using two-way sensitive analysis (Figure 2) we calculated the following benefit MELD (bMELD) equation: bMELD = 9.16 + 1.32 MELD in HCC patients with MELD score ≤22; bMELD = 40 for HCC patients with MELD >22.

Figure 2.

Conclusion: We obtained a new bMELD score to weigh the priorities of HCC and non-HCC on a common waiting list.

CLINICAL VALIDATION OF A SUB-STAGING PROPOSAL OF PATIENTS WITH INTERMEDIATE HCC (BCLC-B)

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BACKGROUND AND AIMS: The intermediate stage of BCLC staging system for hepatocellular carcinoma (HCC) comprises patients with Child–Pugh A and B, with a single unresectable HCC >5cm or >3 HCC regardless of size, or 2–3 HCC >3cm, without extrahepatic spread or vascular invasion and with performance status ECOG 0. Prognosis is likely highly variable within the intermediate stage. For this reason and to tailor treatment allocation, a sub-staging of BCLC-B has been recently proposed by Bolondi et al. based on literature and expert opinion as reported in the following table, adding in greater detail tumor burden (IN or OUT of the Up-to-seven criterion) and Child–Pugh score (A5 to B9).

Table 1.

<table>
<thead>
<tr>
<th>BCLC sub-stage</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT score</td>
<td>5–6–7</td>
<td>5–6</td>
<td>7</td>
<td>8–9</td>
</tr>
<tr>
<td>Tumor burden Ut7</td>
<td>IN</td>
<td>OUT</td>
<td>OUT</td>
<td>ANY</td>
</tr>
<tr>
<td>ECOG-PS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0–1</td>
</tr>
<tr>
<td>Portal thrombosis</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

Since no validation of the prognostic capability of this new substaging system exists, this study aims to validate its prognostic capacity in a large Italian database (ITALICA).

METHODS: 391 patients, included in the already existing ITALICA (Italian Liver Cancer) database, update end 2008, affected by HCC in BCLC-B stage, were divided in four subgroups (B1–B4) according to the sub-classification. The survival of each group was assessed and compared using Kaplan–Meier method and log-rank test, after a follow-up of 60 months.

RESULTS: Number of patients in BCLC subgroups was B1 = 162, B2 = 136, B3 = 28, B4 = 65. Each stage was associated with different median overall survival (p < 0.0001 among groups), namely B1 = 34m, B2 = 24m, B3 = 15m, B4 = 12 months. The 5y survival were: B1 = 39.5%; B2 = 32.4%; B3 = 10.7%; B4 = 13.8% (p < 0.001).

CONCLUSIONS: The new substaging proposal of intermediate patients according to up-to-seven criteria and specific Child–Pugh numeric
score is able to refine prognostic prediction capacity in the intermediate HCC stage.


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URINARY METABOLIC PROFILE DISCRIMINATES HEPATOCELLULAR CARCINOMA BETTER THAN SERUM ALPHA FETOPROTEIN IN WEST AFRICANS

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Background and Aims: Although effective therapies for hepatocellular carcinoma (HCC) exist, current screening tools (serum alpha fetoprotein [AFP]) have low sensitivity and specificity. We have published preliminary data demonstrating the performance of urinary metabolites in HCC diagnosis. We aimed to validate and further characterise urinary metabolites for screening HCC in West Africa.

Methods: Urine samples were collected from 4 subject groups, at two sites in West Africa on the case–control platform of PROLIFICA, “Prevention of Liver Fibrosis and Carcinoma in Africa” as follows: patients with HCC (n=65), cirrhosis (Cir, n=36), non-cirrhotic liver disease (DC, n=110) and healthy controls (NC, n=91). HCC patients were diagnosed using EASL guidelines. Nuclear magnetic resonance (NMR) spectroscopy was utilised to acquire one-dimensional (1D) spectral data from the urine samples, using a standard 1D NMR pulse sequence with presaturation of the water peak. Spectral data were analysed using both unsupervised principal components analysis (PCA), and supervised orthogonal partial least squares discriminant analysis (OPLS-DA), using MATLAB v 7.0 and SIMCA v 13.0. The diagnostic accuracy of metabolic profiles as well as individual metabolites to discriminate HCC from Cir, DC and NC were examined using Area under the Receiver Operating Characteristic (AUROC) curves.

Results: Multivariate analyses of urinary NMR spectra showed a distinct profile for urine of patients with HCC compared to Cir, DC and NC with sensitivity (95%CI)/specificity (95%CI) of 87% (76–94)/81% (63–93), 86% (74–94)/93% (87–97) and 97% (89–100)/99% (94–100) respectively; which outperformed serum AFP (cut-off=20ng/mL) that differentiated HCC from these groups by 79% (58–93)/53% (28–77), 75% (53–90)/75% (63–85) and 76% (55–91)/100% (59–100) respectively. The metabolites that were significantly increased (p<0.001) in HCC patients compared to all groups of control were methionine, acetylcarnitine, carnitine, 2-oxoglutarate, indole-3-acetate, and creatine; whereas creatinine was significantly lower in HCC than controls. Citrate, 4-cresol sulfate and trimethylamine N-oxide were also significantly lower in HCC compared to NC. Urinary metabolite panel performed better than AFP in discriminating HCC from other non-HCC liver conditions {AUROC: HCC vs. DC (both sexes); metabolites [0.96] vs. AFP [0.85], p=0.018; HCC vs. Cir (men); metabolites [0.92] vs. AFP [0.64], p=0.028}.

Conclusions: Our findings validate urinary metabolic profiling as a potential screening tool for HCC, with superior diagnostic accuracy to serum AFP.

111 DEVELOPMENT OF A SCORING SYSTEM TO PREDICT RISK OF HEPATOCELLULAR CARCINOMA IN A COHORT OF PATIENTS WITH CIRRHOSIS

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Background and Aims: Current guidelines recommend biannual screening imaging for all cirrhotic patients. However, the risk of HCC varies considerably for different groups of cirrhotic patients, making risk stratification difficult. The current study aims to develop a scoring system to predict 5- and 10-year risk of HCC among a large cohort of cirrhotic patients.

Methods: Retrospective review was conducted on a cohort of cirrhotic patients followed over a 10-year period (January 2000 – December 2009) to determine the time to diagnosis of HCC. Risk factors for HCC were determined using Cox Proportional Hazards regression. For each risk factor, HCC incidence was evaluated using the Kaplan–Meier method and appropriate threshold values were determined. Based on the Hazard Ratio (HR) for HCC in the multivariable (MV) Cox regression model, each variable was assigned a point value. The sum of all point categories was used to estimate the risk of HCC.

Results: 2229 patients with cirrhosis (62% male) were identified with median follow-up of 5.6 years (Range 0.5–19.7). 374 patients were diagnosed with HCC. The etiology of cirrhosis was: HBV (20%), HCV (43%), Autoimmune (AIH, PBC, PSC – 12%), Steatohepatitis (NASH, Alcohol – 16%) and Other (9%). By MV stepwise Cox regression, age, sex, etiology of cirrhosis and AST-Platelet Ratio Index (APRI) were significantly associated with development of HCC. Points were assigned for each covariate in proportion to the HR from the MV model: Age (<45 (0), 45–60 (4), >60 (8)); Sex (Female (0), Male (5)); Etiology (Autoimmune (0), Other (3), Steatohepatitis (6), HCV (9), HBV (12), APRI (<1.5 (0), 1.5–4 (4), >4 (8). The sum of points for each variable determined HCC risk (Figure 1). No patients with a combined score of <12 points developed HCC during follow-up whereas patients with >32 points had an HCC incidence of 48% by 10 years (p<0.0001).

Conclusions: Using a combination of routine laboratory and clinical measures (age, gender, APRI, etiology), a risk score was developed to accurately distinguish cirrhotic patients with low, intermediate and high risk for HCC development.

Figure 1: HCC incidence by point score.

Results: 2229 patients with cirrhosis (62% male) were identified with median follow-up of 5.6 years (Range 0.5–19.7). 374 patients were diagnosed with HCC. The etiology of cirrhosis was: HBV (20%), HCV (43%), Autoimmune (AIH, PBC, PSC – 12%), Steatohepatitis (NASH, Alcohol – 16%) and Other (9%). By MV stepwise Cox regression, age, sex, etiology of cirrhosis and AST-Platelet Ratio Index (APRI) were significantly associated with development of HCC. Points were assigned for each covariate in proportion to the HR from the MV model: Age (<45 (0), 45–60 (4), >60 (8)); Sex (Female (0), Male (5)); Etiology (Autoimmune (0), Other (3), Steatohepatitis (6), HCV (9), HBV (12), APRI (<1.5 (0), 1.5–4 (4), >4 (8). The sum of points for each variable determined HCC risk (Figure 1). No patients with a combined score of <12 points developed HCC during follow-up whereas patients with >32 points had an HCC incidence of 48% by 10 years (p<0.0001).

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Conclusions: Using a combination of routine laboratory and clinical measures (age, gender, APRI, etiology), a risk score was developed to accurately distinguish cirrhotic patients with low, intermediate and high risk for HCC development.
INCIDENCE AND PREDICTIVE FACTORS OF HEPATOCELLULAR CARCINOMA AND COMPLICATIONS IN HBV- OR HCV-RELATED COMPENSATED CIRRHOSIS. A MULTICENTER PROSPECTIVE COHORT IN 1653 PATIENTS (ANRS CO12 CirVir)

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Background and Aims: The aim of this cohort was to assess the incidence and predictive factors of complications, mainly hepatocellular carcinoma (HCC) in HBV- or HCV-related compensated cirrhosis.

Methods: This study involved 35 French centres. Inclusion criteria were histologically proven HCV- or HBV-related cirrhosis, Child–Pugh A, no previous hepatic complication including HCC. Patients were prospectively screened for HCC. A sequential biobank was collected at inclusion and annually.

Results: A total of 1653 eligible patients were consecutively enrolled from March 2006 to June 2012, of whom 31 HCV-HBV co-infections were excluded from this analysis. Of the 1622 patients [mean age 56 yrs, males 67%; HCV 1306, HBV 316], alcohol consumption and metabolic syndrome were more frequent in HCV than HBV patients: 30.7% vs 9.8% (P<0.0001) and 16.8% vs 8.6% (P=0.0002), respectively. Based on a median follow-up of 30 months, liver nodule(s) occurred in 343 patients, diagnosed as HCC in 108 (2-yr cumulative incidence, cum: 4.3%) and cholangiocarcinoma in 2. The cumulative incidence of HCC was higher in HBV patients than in HBV patients (2-yr cum: 4.6% vs. 2.9%), though non-significantly (P=0.06). Other hepatic complications occurred more frequently in HCV than HBV patients (2-yr cum: 10.4% vs. 3.5%, P=0.0003). Eighty deaths occurred, more frequently in HBV than in HBV patients (2-yr OS: 99.6% vs. 97.2%, P=0.0003), attributable to liver disease in 36 (45%) and to extra-hepatic causes in 44 (55%). Viral control was obtained in 370 HCV (28.3%) and 223 (71%) HBV patients. In multivariate analyses, predictive factors of HCC were low platelet count (HR=1.007 [95% CI: 1.002–1.01], P=0.008) and esophageal varices (HR = 1.69 [95% CI: 1.02–2.8], P=0.04).

Conclusion: Early results of this prospective cohort are:

a. only 31% of detected liver nodules were confirmed as primary liver cancer;

b. complications were more frequent in HCV than HBV patients in whom viral infection control was higher;

c. after a follow-up of 2.5 yrs, non liver-related mortality still concerned more than one half of deaths.

This multicenter cohort constitutes the backbone permitting precise study of HCC and other complications of cirrhosis, particularly through subsequent follow-up and nested studies exploiting high quality clinical data and biobank.

A PROSPECTIVE RANDOMIZED TRIAL COMPARING HDR-BRACHYTHERAPY AND TRANSARTERIAL CHEMOEMBOLIZATION IN HEPATOCELLULAR CARCINOMA

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Aim: To compare image-guided HDR-brachytherapy (BT) with transarterial chemoembolization (TACE) in intermediate and advanced stage hepatocellular carcinoma.

Design: Single-center randomized controlled trial.

Material and Methods: In this single-center randomized controlled trial 75 patients were allocated to either BT (n = 38) or TACE (n = 37). Eligibility criteria included BCLC A-C, Child Pugh ≤9 points, no PVT and no extrahepatic disease. Primary endpoint was time to progression (TTUP) after a median follow-up of 2 years. BT was performed by HDR-brachytherapy (BT) with a standardized mixture of Cisplatin, Doxorubicin and Lipiodol. Cross over was allowed after the primary endpoint was reached.

Results: Mean patient age was 69.9 and 67.1 years (BT/TACE group, respectively). 26 patients were classified as BCLC A-C, Child Pugh ≤9 points, no PVT and no extrahepatic disease. Primary endpoint was time to progression (TTUP) after a median follow-up of 2 years. BT was performed by HDR-brachytherapy (BT) with a standardized mixture of Cisplatin, Doxorubicin and Lipiodol. Cross over was allowed after the primary endpoint was reached.

Conclusion: TTUP and TTP were significantly longer in the BT group as compared to TACE. Failed demonstration of improved OS may be attributed to the cross-over design as well as to the low patient number. These data suggest BT should be further validated for possible inclusion in future HCC treatment concepts.
Background and Aims: TACE is the standard-of-care treatment for intermediate-stage HCC (BCLC-B) or those who are poor candidates for radioembolisation (SIRT) and has a median overall survival (OS) of 12 months. A recent comparison of TACE vs radioembolisation demonstrated a trend towards a survival benefit for radioembolisation, and a significantly higher response rate for radioembolisation. Radioembolisation using yttrium-90 microspheres (SIRT) is more commonly used to treat patients with intermediate-stage HCC (BCLC-B), while SIRT (also termed transarterial radioembolisation) is more commonly used to treat patients with advanced-stage HCC (BCLC-C) or those who are poor candidates for radioembolisation.

Methods: Patients 18 years or older with unresectable HCC, established according to the EASL criteria, and with good liver function (Child–Pugh ≤B7), ECOG performance status ≤2 and ≤5 lesions (≤20 cm total maximum diameter) were included. After stratification by treatment centre, patients were randomised (1:1) by study procedure and followed-up for a minimum of 12 months or until death. TACE with epirubicin 50 mg, lipiodol and embolising agent (Embospheres; BioSphere Medical) was administered at 6-weekly intervals as indicated; SIRT using Yttrium-90 (90Y) resin microspheres (SIR-Spheres; Sirtex Medical) was administered as either a whole-liver, lobar or segmental procedure in a single treatment session. Treatment response was assessed by local (using RECIST 1.0) and independent central (RECIST 1.1 and mRECIST) review.

Results: Twenty-eight patients (median age: 65.6 years; ECOG 0/1: 78.6%/21.4%) received either TACE (n=15) or SIRT (n=13). Patients received a mean of 3.4 TACE interventions (median 2; range 1–11) or 1 SIRT procedure. Best overall response rates of target lesions by RECIST 1.0/11/mRECIST were 13%/20%/33.3% for TACE and 30%/30%/23.1% for SIRT; and disease control rates were 73.3%/67.7%/67.7% and 76.9%/84.6%/69.2%, respectively. Median progression-free survival (mRECIST) was 5.5 months (95% CI: 1.6–not reached) for TACE and 4.1 months (95% CI: 2.3–9.9) for SIRT (p=0.411). Overall survival did not differ by procedure (p=0.244). Mean (±SD) hospital admission days for TACE and SIRT were 13.8 (±13.2) and 11.6 (±7.3), respectively. Of 10 Grade ≥3 adverse events reported, 4 were in the TACE group and 6 in the SIRT group (p=0.433).

Conclusions: In a typical TACE cohort, single-session SIRT appeared to be as safe and effective as multiple sessions of TACE. These results underscore the problems of designing a phase III trial for intermediate-stage HCC.
alcoholic cirrhosis (AH). The hepatic lymphocytes are particularly rich in natural killer (NK), NKT and NK-like CD8+ T cells. NKG2D is a central activation receptor expressed on these cytotoxic cells. Receptor ligands are induced in response to cellular stress, injury as well as infections and activate the cytotoxic cells to kill target cells. As products of cell damage are known to hold immunogenic potential, defective clearing of injured cells may lead to chronic inflammation. Dysfunctional cytotoxic cells may, thus, participate in the sustained immune activation in AH and also in the decreased resistance towards infections. Our aim was, therefore, to evaluate the frequency and activation state of NK, NKT and NK-like CD8+ T cells in patients with severe AH.

Methods: We analysed blood samples from 20 consecutive patients with severe AH. As control group, we included 10 patients with stable alcoholic cirrhosis (AC) and 10 healthy controls (HC). The expression of NKG2D by flow cytometry is presented as median fluorescence intensity.

Results: In AH patients compared with HC, there was no difference in the frequency of NK cells (CD3+CD56+) (median:IQR: 10.4%±12.4 vs. 14.0%±10.1) or NKT cells (CD3+CD56+) (2.9%±3.9 vs. 2.0%±6.4), yet the frequency of NK-like CD8+ T cells (CD3+CD56+CD8+) was halved (27.0%±29.9 vs. 56.6±28.1, p=0.005). Despite little difference in cell frequencies, the expression of NKG2D was clearly lower in NK cells of AH patients compared with both AC (903±584 vs. 1031±176, p=0.05) and HC (1179±356, p=0.02) and the same was seen in NKT cells compared with both control groups (903±656 vs. 1417±450, p=0.01; 1296±386, p=0.03). A similar but not significant tendency was evident also for NK-like CD8+ T cells (Figures 1–3).

Conclusion: The functionality of NK, NKT and NK-like CD8+ T cells has never previously been investigated in AH. Our results, however, suggest dysfunctional activation of these cells in AH, which is likely to contribute towards both the sustained inflammatory state and the patients' susceptibility to infections.

117 SPONTANEOUS DECREASE IN LIVER INJURY DOES NOT STRONGLY PREDICT EVOLUTION OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS TREATED WITH CORtICOSTERoids

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Early improvement in liver function under treatment with corticosteroids for severe alcoholic hepatitis is related to survival. One can suppose that spontaneous improvement before therapy may predict outcome.

Aims: To evaluate
1. spontaneous improvement in liver function from the admission to corticosteroid treatment
2. the impact of such improvement on survival and therapeutic response.
Methods: Modification in liver parameters was prospectively registered from the admission up to the beginning of corticosteroids and thereafter.

Results: Characteristics of the 339 included patients at admission were: age 49.6 years, male 58.6%, ascites 73.9%, encephalopathy 24.6%, bilirubin 162 mg/dL, Maddrey function 59.8. After infection screening and liver biopsy, corticosteroids were started after 7 days (median). Both bilirubin [evolution of 0 mg/dL (95% CI: −1 to 4.4)] and DF (0.31, 95% CI: 0−1.32) remained stable from admission to corticosteroid onset. Thus, 50% of patients decreased their bilirubin and DF, so-called spontaneous responders (SR), in contrary to spontaneous non-responders (SNR): −16 vs. +5.5 mg/dL, p < 0.001 and −7.6 vs. +6.9, p < 0.001. SR and SNR were not different for DF at admission: 57.3 vs. 61.1, p = 0.3. On overall patients after 7 days of corticosteroids, bilirubin decreased by 22 mg/dL (15.2−30), Lille model was 0.34 (0.27−0.39) and 61.4% of patients were responders to corticosteroids (Lille model <0.45). Survival was higher in SR: 69.7±3.7 vs. 55.8±4.1%, p = 0.0006. Leukocytes, encephalopathy, ascites, MELD score, spontaneous response and Lille model predicted 6-month survival in univariate analysis. In multivariate analysis, ascites (RR 2.12, p = 0.007), MELD score (RR 1.03, p < 0.001) and Lille model (RR 44.5, p < 0.001) were independent prognostic factors, whereas encephalopathy (p = 0.13), leukocytes (p = 0.46) and spontaneous response (p = 0.08) were not. After 7 days of corticosteroids, SR and SNR decreased their bilirubin level: −23.9 vs. −26.9 mg/dL, p = 0.14. SR were more frequently responders to corticosteroids (72.8 vs. 52.7%, p < 0.001) and had lower liver injury at corticosteroid onset: DF 51.9 vs. 67.6, p < 0.001.

Conclusion: Spontaneous improvement is observed in 50% of patients with severe alcoholic hepatitis but does not predict survival. Probability of response to corticosteroids is the highest predictor of outcome and is more frequent in spontaneous responders.

118 METABOLIC FATTY-LIVER DISEASE INCREASES THE RISK OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH ALCOHOLIC CIRRHOSIS LISTED FOR LIVER TRANSPLANTATION

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Fatty liver and alcohol are risk factors for HCC but their synergism in alcoholic cirrhosis is unknown.

Aim: To analyze the impact of fatty liver and coexistent metabolic risk factors (MRF) on HCC development in patients with alcoholic cirrhosis listed for liver transplantation (LT).

Methods: One-hundred patients transplanted for alcoholic ESLD were studied for: length of abstinence before LT, steatosis (≥10% on the native liver) and previously diagnosed MRF (overweight/obesity or type-2 diabetes) in relation to the presence of HCC.

Results: Indications for LT were: 77% decompensated cirrhosis without HCC, 23% HCC. Mean age was 55 yrs, mean abstinence 2.2 yrs (≥6 months in 71%). HCC was not confirmed in 3 patients and incidentally found on liver explant in eight patients; thus 28 patients had HCC.

Thirty-two patients had neither steatosis nor MRF (NONE); 24 steatosis alone (STEAT); 20 steatosis coexisting with MRF (STEAT+MRF); 24 MRF but no steatosis (MRF). Mean age increased gradually between these categories. Patients in the MRF and STEAT+MRF groups were older than those in the NONE and STEAT groups (56.8 vs. 53.7 yrs, respectively, p < 0.04) but had similar length of abstinence (≥6 months in 80% vs. 64%, p = 0.12).

HCC patients were older (57.4 vs. 54.2 yrs, p = 0.05), have been more frequently overweight (54% vs. 14%, p < 0.001) or diabetic (43% vs. 22%, p < 0.04) than those without HCC. There was no difference in disease duration, length of abstinence, waiting time before LT or proportion of steatosis between HCC and non-HCC. However, the proportion of HCC was highest among the STEAT+MRF group (50%) and declined to 6% in the NONE group. Only 13% of patients in the NONE/STEAT groups had HCC vs. 48% in the MRF/STEAT+MRF groups (p = 0.0001). Results were similar in patients with >6 months abstinence. After adjusting for sex, age and alcohol, history of overweight/obesity or diabetes remained significantly associated with HCC (p < 0.01 and <0.04, respectively).

Conclusion: The risk of HCC was significantly increased by previous exposure to MRF and concurrent steatosis, even in patients with >6 months alcohol abstinence. Concurrent metabolic fatty-liver disease could be a carcinogenic cofactor in alcoholic cirrhosis.

ORAL PRESENTATIONS

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Background and Aims: Carbohydrate Responsive Element Binding Protein (ChREBP) is a key transcription factor involved in the control of hepatic lipogenesis under physiological and physiopathological conditions. Ethanol-induced fatty liver is a worldwide health problem without effective therapeutic method. In the current study, we investigated the contribution of ChREBP to alcohol-induced steatosis in a binge-drinking model.

Methods: C57BL/6J male mice were fasted for 4 hours before receiving 33% (vol/vol) ethanol at a total accumulating dose of 3.5 g/kg body weight by 3 equally divided gavages in 30 minutes intervals. Control mice received the same volume of dextrin-maltose (DM) in order to reach similar caloric value. Ten hours after the first gavage, mice were sacrificed. To determine the specific role of ChREBP, a shRNA directed against ChREBP (shChREBP), was injected 5 days before the binge drinking protocol. In parallel, primary cultures of mouse hepatocytes incubated with 50 mM ethanol in the presence or not of the shChREBP were performed.

Results: Binge-drinking markedly raised circulating ethanol concentrations. In liver of ethanol-treated mice, a significant decrease of the NAD/NADH ratio was observed suggesting that the activity of NAD-dependent deacetylases, such as SIRT1, was reduced. Indeed we observed that global and ADH (Alcohol Dehydrogenase) acetylation, the first enzyme which catalyses ethanol oxidation, was significantly induced under binge-drinking conditions. We also observed that ChREBP acetylation as well as its recruitment on its target genes was significantly increased leading to hepatic steatosis development with a 3-fold increase in triglycerides (TG) concentrations compared to DM-treated mice. Experiments performed in primary cultures of mouse hepatocytes confirmed that ethanol-induced TG accumulation could be prevented by ChREBP silencing. In vivo ChREBP knockdown, by reducing lipogenic gene expression, significantly prevented alcohol-induced TG accumulation. Interestingly, under binge-drinking conditions, the expression of SIRT1, a direct negative target of ChREBP, was re-induced upon ChREBP knockdown. As a consequence, global and ADH acetylation was decreased suggesting that ethanol oxidation may be reduced under ChREBP inhibition.

In conclusion: Our results reveal the contribution of ChREBP to alcohol-induced hepatic steatosis under binge drinking conditions and underline the importance of ChREBP in controlling SIRT1 expression in liver.
120 SEVERE VITAMIN D DEFICIENCY IS ASSOCIATED WITH COMPLICATIONS OF PORTAL HYPERTENSION AND A WORSE PROGNOSIS IN ALCOHOLIC CIRRHOSIS

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Background and Aims: Vitamin D deficiency has been reported in various chronic liver diseases especially at the cirrhosis stage. However, its influence on the severity of alcoholic cirrhosis (AC) has been poorly elucidated. We investigated the association of vitamin D levels with clinical, biological and survival in AC patients.

Methods: 254 AC patients were retrospectively tested for 25-hydroxyvitamin D [25(OH)D] serum levels. The diagnosis of cirrhosis was based on liver biopsy (97.6%) and/or an unequivocal clinico-biochemical profile, highly suggestive endoscopic exam. Hepatic venous pressure gradient (HVPG) measurement was performed in 91% of patients. We tested the association between 25(OH)D concentration and 1) the severity of the disease (assessed by MELD and Child–Pugh scores and presence of alcoholic hepatitis [AH]); 2) complications of portal hypertension (ascites, encephalopathy, spontaneous bacterial peritonitis [SBP], alcoholic hepatitis [AH]); 2) complications of portal hypertension (assessed by MELD and Child–Pugh scores and presence of alcoholic hepatitis); 3) early mortality at one year.

Results: 254 patients (median follow-up of 5 months) were included (64% males, mean age 54.8±9.0 years, 63% with a BMI >25 kg/m2, 77% drank >80 g/day, and 23% had AH, median MELD score 12.0 [7.3–17.9], median Child–Pugh score 9 [6–11]. Mean hepatic venous pressure gradient (HPVG) 16.6±6.0 mmHg, median vitamin D level 8.8 [5.0–15.5]. Severe deficiency in 25(OH)D (<10 ng/ml) was significantly associated with increased HVPG (p <0.001), a higher rate of AH (p =0.001), MELD (p <0.001) and Child–Pugh scores (p <0.001). Furthermore, severe 25(OH)D deficiency was significantly associated with presence of ascites (p <0.001), encephalopathy (p =0.001), SBP (p =0.033) and HRS (p <0.001). However, although bleeding was more prevalent in the low vitamin D concentration group, this difference was not statistically significant (22.8% vs. 16.1% p =0.152). Finally 25(OH)D <10 ng/ml was associated with a higher mortality at one year (HR =4.33, 95%CI:1.47–12.78, p =0.008) independently of age, sex, BMI >25 kg/m2, and the MELD score.

Conclusions: Severe vitamin D deficiency is associated with higher cirrhosis severity, most complications of portal hypertension, and early mortality in AC patients. Vitamin D may well represent both a biomarker of severity and prognosis in AC. These results may also advocate for vitamin D supplementation in AC.

121 HIGH LIVER EXPRESSION OF SPINK-1 IS ASSOCIATED WITH PROGENITOR CELL AND HEPATOCYTE PROLIFERATION AND DETERMINES MELD SCORE IMPROVEMENT IN DECOMPENSATED ALCOHOLIC LIVER DISEASE

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Background and Aims: The prognostic significance of liver progenitor cell (LPC) and macrophage expansion in the regeneration of decompensated alcoholic liver disease (ALD) remain ill defined. In a well-characterized population of patients with acutely decompensated ALD (Hepatology 2011, A62), we analysed macrophage infiltration, proliferative LPC and differential expression of hepatic genes at baseline in relation to outcome at 3 months follow up.

Methods: Fifty-eight patients (MELD 20) were included. Liver biopsy at inclusion was used for (1) immunohistological analysis of proliferative LPC (MB1/Ck7+), proliferative hepatocytes (MB1/Ck7+ parenchymal cells), morphometric analysis of macrophage infiltration (CD68) and LPC expansion (Ck7), and (2) transcriptome profiling using Affymetrix GeneChip Human arrays. Serum levels of HGF were determined by immunoassays. A ≥3 points decrease in MELD at 3 months as compared to baseline defined the improvers. Fifteen abistent cirrhotics served as controls. CD68 and SPINK3 mRNA expression was determined in various mice models of liver injury.

Results: At baseline, patients with decompensated ALD presented a significant expansion of CD68+ macrophages and Ck7+ cells compared to abistent cirrhotics. Patients who will improve (n =34) were characterized at baseline by a higher number of Ck7+/MB1+ cells (1.5±1.5 versus 0.9±0.9 cells/field, p <0.01), MB1+ hepatocytes (4.1±3.6 versus 1.8±1.4 cells/field, p <0.01), an increased expansion of liver macrophages (4.4% versus 3.3% of surface area, p <0.05) and a higher level of serum HGF (p <0.05), compared to those who will not (n =24). Transcriptome analysis revealed that the first pathways upregulated in improvers were related to cell cycle and a 7-fold increase of liver serine peptidase inhibitor Kazal type 1 (SPINK1) compared with non-improvers (p =0.005). SPINK1 liver expression positively correlated with CD68 (r =0.46) and cyclinE1 (r =0.6). In mice, a 20-fold increase in liver SPINK3 expression, the homolog of human SPINK1, was evidenced following partial hepatectomy, concurrent with hepatocyte proliferation.

Conclusions: Baseline markers of liver macrophages and liver cell proliferation predict the clinical outcome in decompensated ALD. This study reveals an unexpected implication of SPINK1, an acute phase reactant, in liver regeneration and human ALD.

122 NON-SELECTIVE BETA-BLOCKERS MIGHT DECREASE SURVIVAL IN SEVERE ALCOHOLIC CIRRHOSIS

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Background and Aim: The effect of beta-blockers (BB) on survival in cirrhotic patients with poor liver function, especially with refractory ascites, is debated. Recently, the window hypothesis has suggested 3 phases: no effect, improved survival, decreased survival by BB. Therefore, we evaluated the BB effect on survival according to liver severity.

Methods: Consecutive patients with alcoholic cirrhosis and no history of hepatocellular carcinoma were prospectively included in...
a single tertiary center. The date and cause of death were recorded and checked using the national death registry.

**Results:** Baseline characteristics of 361 included patients were: age: 60.0 ± 9.8 yrs, male: 71.7%, Child Pugh score: 6.1 ± 1.8 (A: 69.7%, B: 23.3%, C: 7.0%), Meld score: 11.3 ± 4.5, follow-up: 3.1 ± 1.4 yrs, death rate: 28.8% (liver related death in 62.5%). The 144 patients with non-selective BB were compared to the 190 controls without BB; 27 patients with selective BB were excluded. At baseline, there were higher frequencies of esophageal (p < 0.001) or gastric varices (p = 0.019) and alcohol withdrawal (p = 0.001) in the BB group. Child–Pugh (p = 0.56) and Meld (p = 0.25) scores did not differ. The independent predictors of overall survival were Meld (p < 0.001) and age (p = 0.028) by Cox model. The independent predictors of liver related survival included Meld (p < 0.001) and gastric varices (p = 0.026), and a significant interaction between Meld and BB (p = 0.035). Therefore, we assessed the BB effect according to baseline Meld score: in the lowest tertile, BB group had an improved overall survival as compared to controls (log-rank: p = 0.071); a beneficial BB effect was also observed in the second tertile until 4 years of follow-up; in contrast, in the highest tertile, patients treated with BB had decreased survival beyond 2 yrs (p = 0.095). Liver survival showed the same trends in the first (p = 0.140) and second (p = 0.306) tertiles; in the highest tertile, the poorer survival in BB group was more pronounced than for overall survival (p = 0.019).

**Conclusion:** The BB effect on survival differed according to liver disease severity in alcoholic cirrhosis: in good condition patients, BB tended to improve survival during the first years whereas there was a significantly decreased liver survival in the most severe patients.

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**Parallel Session: HEPATITIS B & D – EXPERIMENTAL**

**123 STRUCTURE OF THE HEPATITIS B VIRUS RNase H, A TARGET FOR NEW ANTIVIRAL DRUG DEVELOPMENT, UNRAVELED BY ULTRA-DEEP PYROSEQUENCING AND MOLECULAR MODELING**

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Last-generation nucleoside/nucleotide analogues are potent and have a high barrier to resistance. They help control viral replication in the short- to mid-term in a large proportion of treated patients. However, delayed responses have been observed in patients already exposed to other drugs of the same class, long-term resistance is possible, and cure of infection cannot be achieved with these therapies, emphasizing the need for other therapeutic approaches with different viral targets. Among them, the HBV RNase H represents an interesting target because its enzyme activity (cleavage of RNA/DNA heteroduplexes) is essential to the HBV life cycle. The goal of our study was to characterize the molecular structure of HBV RNase H. We generated a new predict 3D molecular model of HBV RNase H, derived from E. coli RNase H, using quasispecies sequences from patients infected with different HBV genotypes available in public database, and from a homogenous population of 73 treatment-naïve patients infected with HBV genotype D generated by means of ultra-deep pyrosequencing (454, Roche-Molecular-Systems). In the latter experiments, 958,000 sequences were generated, i.e. on average 12,900 sequences per patient, with an average sequence length of 302 base pairs. The new model revealed the following specificities of the HBV enzyme compared to other RNases H:

i. among the 4 residues of the HBV RNase H catalytic site, one presents variability and additional one has been observed in one patient; variability at this position is always silent in the overlapping HBx gene and appears to have no impact on the levels of viral replication;

ii. the basic protrusion containing C-helix, which is required to guide the RNA/DNA heteroduplex into catalytic site, displays a highly conserved additional domain in regards to E. coli; this domain could be used to target RNase H inhibitors that are specific for the HBV enzyme, without cross-species activity.

**Conclusions:** By means of ultra-deep pyrosequencing and molecular modeling, we have highlighted key features HBV RNase H. The model shows substantial differences with other known RNases H, and paves the way for the development of new inhibitors of HBV cell cycle specifically targeting RNase H activity.

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**124 HEPATITIS DELTA INFECTION IN HUMANIZED UPA/SCID-MICE LEADS TO PRONOUNCED INDUCTION OF HUMAN-SPECIFIC INNATE IMMUNE RESPONSES AND CYTOKINE EXPRESSION IN COMPARISON TO HBV MONO-INFECTION**

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The induction of interferon-stimulating genes (ISGs) is the hallmark of antiviral immunity. While ISG activation is known to play a fundamental role in counteracting viral infection, elevated pre-treatment ISG levels have been associated with weaker responses to IFN-α treatment and may even support viral replication. The limited availability of hepatitis delta virus (HDV) infection models has hindered studies of interactions between HDV and infected human hepatocytes. Aim of the study was to investigate the antiviral state of the human hepatocytes in the setting of chronic HBV/HDV co-infection compared to HBV mono-infection using the uPa/SCID mouse model.

**Methods:** Viremia and induction of human ISGs and cytokines were determined by qRT-PCR and immunohistochemistry in HBV/HDV infected uPa/SCID mice and compared to uninfected and HBV mono-infected animals.

**Results:** Upon HBV/HDV inoculation all mice developed HBV and HDV viremia, reaching a maximum at week 8 post infection (median 1.467 HBV-DNA copies/ml and 6.767 HDV-RNA copies/ml). Viremia development was accompanied by a clear induction of human ISGs, which was ascertained both at RNA and protein levels. While HBV mono-infection led to only moderate ISG elevations (fold inductions: 2.7x MxA; 4.0xOAS1; 2.5xH4LA-E; 2.8xUSP18) compared to uninfected mice, enhancement of innate defence mechanisms in human hepatocytes was more prominent in the setting of HBV/HDV co-infection (fold inductions: 6.8xMxA; 6.4xOAS1; 6.0xH4LA-E; 4.4xUSP18). The most remarkable expression change regarded the level of human ISG15 with a 17.7-fold induction in HBV/HDV co-infected mice in comparison to a 2.6-fold-induction in HBV mono-infected animals. Interestingly, a clear induction of human-specific cytokine (TGF-β, IFN-β and IFN-λ) expression was determined in the setting of HBV/HDV co-infection, while levels remained lower or below detection in uninfected and HBV mono-infected mice.

**Conclusions:** Establishment of HDV infection provoked a clear enhancement of human innate defence mechanisms in chimeric...
mice, in the absence of adaptive immune responses. Elevated pre-treatment ISG and interferon levels may directly contribute to liver damage and inflammation, providing a rationale for the more severe course of HDV-associated liver disease. The antiviral state induction might also explain the lower levels of HBV activity frequently found in co-infected hepatocytes.

125 HEPATITIS DELTA VIRUS (HDV) INDUCES SPECIFIC DNA METHYLATION EVENTS TOWARDS MALIGNANCY IN HEPATOMA CELLS

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Background and Aims: Hepatitis delta virus (HDV) is a small, defective RNA virus that can infect only individuals carrying hepatitis B virus. HBV/HDV co-infection results in more severe liver disease than HBV single infection and more rapid progression to cirrhosis and hepatocellular carcinoma (HCC). The epigenetic events involved in hepatocyte transformation towards malignancy in this context are poorly known.

Materials and Methods: DNA methyltransferases (DNMT1 and DNMT3b) expression levels were evaluated by qRT-PCR and immunoblot detection, in an in vitro model of Huh-7 cells expressing the HDV antigens. Methylation levels of 24 genes involved in HCC were assessed using the EpiTect Methyl quantitative PCR array.

Results: DNMT3b mRNA and protein expression levels were higher in HDV expressing cells as compared to control cells (p = 0.01). Conversely, no significant changes were observed for the mRNA expression of DNMT1. Furthermore, we present evidence that HDV expression induces DNMT3b overexpression through STAT3 as demonstrated by NSC74859 treatment, and is associated to increased methylation levels of E2F1. In turn, this leads to a decreased mRNA expression of E2F1 that can be reversed by azacitidine treatment.

Conclusions: This is the first report showing that HDV induces DNMT3b expression and hampers E2F1 transcription factor, which plays a crucial role in the control of cell cycle and action of tumor suppressor proteins. Our findings suggest that HDV could play a role in HCC development at least in part by altering DNA methylation events. A better understanding of the molecular mechanisms involved in HDV-related carcinogenesis could help to identify new therapeutic targets.

126 ESTABLISHMENT OF PERSISTENT HEPATITIS B VIRUS INFECTION IN MICE EXPRESSING HLA-A2 AND HLA-DR1 FOR EVALUATING IMMUNO-THERAPEUTICS FOR CHRONIC HEPATITIS B

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Background and Aims: Hepatitis B virus (HBV) persistence may be due to impaired HBV-specific immune responses being unable to eliminate efficiently or cure the infected hepatocytes. The immune mechanisms that lead to HBV persistence have not been completely identified and no appropriate animal model is available.

Methods: We established a chronic HBV infection model in a humanized mouse strain with human leukocyte antigen (HLA)-A2/-DR1-transgenes and H-2 class I-class II-knockout (HLA-A2/DR1). The liver of these mice was transduced with an adenovirus associated-virus serotype 2/8 (AAV2/8) carrying a replication competent HBV-DNA genome.

Results: In all AAV2/8-transduced mice, hepatitis B surface antigen (# > 2500IU/ml), hepatitis B e antigen (# 44 PE IU/ml), and HBV DNA (3.4–4.6 log10IU/ml) persisted in serum for at least one year. Viral replication intermediates and transcripts were detected in the livers of the AAV-HBV-infected mice. The hepatitis B core antigen was expressed in 60% of hepatocytes, but no significant inflammation was observed in the liver. This was linked to a higher than control number of regulatory T cells in liver and a defect in HBV-specific functional T-cell responses. Despite the substantial tolerance resulting from expression of HBV antigens in hepatocytes, we succeeded in priming functional HBV-specific T cell responses in peripheral tissues, which subsequently reached the liver. However, despite production of IFN-γ by vaccine-activated T cells, these responses were not sufficient to eliminate HBV antigens and stronger vaccine protocols are being tested.

Conclusions: This AAV2/8-HBV-transduced HLA-A2/DR1 murine model recapitulates virological and immunological characteristics of chronic HBV infection in the immune tolerant phase of the disease and it could be useful for the development of new treatments and immune-based therapies or therapeutic vaccines for chronic HBV infections.

127 PROLIFERATION OF HEPATITIS B VIRUS INFECTED HUMAN HEPATOCYTES INDUCES SUPPRESSION OF VIRAL REPLICATION AND RAPID cccDNA DECREASE IN HUMANIZED MICE

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In chronic hepatitis B virus (HBV) infection, the viral genome forms a stable minichromosome, the covalently closed circular DNA (cccDNA), which can persist throughout the hepatocyte lifespan. Immune mediated cell injury and compensatory cell growth, however, may favor cccDNA destabilization and impair cccDNA activity leading to the selection of cccDNA-free hepatocytes.

Aim: Of the study was to investigate the impact of liver regeneration on cccDNA stability and activity in HBV-infected human hepatocytes using the uPA/SCID mouse system.

Methods: Human cell proliferation was triggered either (i) by transplanting HBV-infected primary human hepatocytes (PHH) isolated from one highly viremic (10xE9 HBV-DNA/ml) humanized mouse into 19 naive uPA/SCID mice or (ii) by performing 2/3 partial hepatectomy (PH) in HBV-infected humanized mice. PHH proliferation and viral load changes were determined by performing qRT-PCR and immunohistochemistry.

Results: Isolated PHHs engrafted and strongly proliferated early after transplantation reaching 6.7 cell doublings within 60 days. Although all PHHs appeared HBcAg-positive in the donor mouse, HBcAg was barely detectable already at day 5 after transplantation, at a time when the majority of the PHHs (70% Ki67+) underwent cell division. Not only intrahepatic HBV-RNAs (pgRNA and subgenomic RNAs) levels decreased in the first 30 days post-transplantation, but also cccDNA copy number/PHH dropped dramatically, being <1copy/400PHHs at day 5 and <1copy/400PHHs at day 30. Notably, serological (viremia, HBsAg) and intrahepatic virological markers (HBV-RNAs and cccDNA loads) rebounded in all mice from day 60 on, as PHH expansion repleted. Persisting intrahepatic HBV-DNA sequences were mostly present as replicative intermediates and not as integrated virus. Proliferation-driven suppression of intrahepatic virion productivity (HBV-RNAs, HBcAg) and decrease of cccDNA
copies/PHH (median 30%) was also triggered in mice undergoing PH (3 to 5 days post-PH).

**Conclusion:** In the milieu of a strong liver regeneration, expansion of PHHs induced strong reduction of viral replication and cccDNA levels, leading to a rapid formation of cccDNA-free hepatocytes. Nevertheless, complete cccDNA eradication was not achieved, so that in the absence of antiviral treatment, de novo HBV infection could be re-established in quiescent human hepatocytes.

### 128 INTERFERON-ALPHA ELIMINATES HBV cccDNA VIA BASE EXCISION REPAIR PATHWAY


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**Background and Aims:** Interferon (IFN)-α, the only licensed immunomodulatory drug for hepatitis B therapy, has been used for more than 20 years and is the only drug that may induce virus clearance. Although the effect of IFN-α has been widely studied, its definite mode of action is still unclear. The aim of our study is to reveal the molecular mechanism of this antiviral effect.

**Methods:** Primary human hepatocytes and differentiated HepaRG cells were infected with HBV, and then treated with IFN-α or Lamivudine (LAM). HBeAg was measured by immunoassay. HBV cccDNA was assayed by qPCR and Southern blot and characterized by differential DNA denaturation PCR (3D-PCR). Mutations were confirmed by sequencing. DNA damage response was analyzed by Western blot or qRT-PCR. HBV(x-) HepaRG cells were used to study the transcriptional dependent effect of IFN-α. STAT phosphorylation inhibitor TPCA-1 was used to block JAK-STAT signal pathway, and HIV-IFV, stably transduced by a lentiviral vector, was used to inhibit APOBEC3A/F/G.

**Results:** While LAM inhibited only HBV DNA replication, IFN-α affected replication, transcription and antigen secretion. Although HBeAg secretion partially recovered after termination of IFN-α treatment, it remained significantly below the level of untreated samples. Accordingly, HBV cccDNA was reduced under IFN-α treatment, and 3D-PCR indicated sequence alteration. Sequence analysis revealed C to U hypermutation of the HBV cccDNA minus strand in IFN-α treated samples rendering it sensitive to degradation. In HBV(x-) infection, reduction of cccDNA by IFN-α depended on trans-complementation with HBx, which is required to activate cccDNA transcription. In addition, preferential hypermutation of the HBV minus-strand indicated transcriptional dependence. Upregulation of deaminases of the APOBEC3 family by IFN-α in a time and dose dependent manner and rescue of cccDNA by either JAK-STAT signalling blockade or HIV-IFV expression proved that IFN-α induced cccDNA deamination by APOBEC3 subsequently leading to cccDNA degradation.

**Conclusions:** IFN-α is able to trigger HBV cccDNA hypermutation and subsequent degradation by endonucleases via the base excision pathways. C to U hypermutation is due to APOBEC3 activation and depends on active transcription of HBV cccDNA.

### 129 IDENTIFICATION OF HBx TARGET miRNAs THAT REGULATE HBV REPLICATION BY ChIP-Seq

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**Background:** HBx regulatory protein is required for HBV cccDNA transcription/viral replication and contributes to HBV oncogenicity. Recent evidence indicates that HBx affects the epigenetic control of HBV viral chromatin, by preventing HDACs recruitment onto the cccDNA, as well as of cellular chromatin, by favouring the recruitment of CBP acetyl-transferase on CREB-activated target genes and of the de novo methyl-transferase DNMT3a on repressed genes.

**Objectives:** Aim of this study was to use a broad chromatin immunoprecipitation approach to define the HBx – miRNAs transcriptome.

**Methods:** High-throughput sequencing of anti-HBx ChIP-enriched DNA fragments (ChIPSeq) was performed on an Illumina GAIIx. Chromatin immunoprecipitated from mock, wt and HBx-mt monomeric linear full length HBV DNA cells was analysed by TaqMan real-time PCR using gene (promoter) specific primers. HBx target miRNAs levels were assessed by real-time RT-PCR.

**Results:** ChIPSeq analysis of HBx chromatin recruitment revealed a specific binding to a large number of new and known target sequences. In 4 independent ChIP-seq experiments ~16000 HBx binding sites were identified. 12.8% located within 10 kb of a transcription start site. Several peaks were validated by quantitative PCR (qPCR). Systematic integrative analysis of the ~7000 genes potentially regulated by HBx shows an enrichment in gene involved in cell metabolism, chromatin dynamics and cancer but also HBV replication (Ras, calcium transport, endocytosis, MAPK/WNT pathways, Src, the EGF/HGF family). HBx also binds to 233 potential miRNAs [99 putative miRNA promoters and 133 mirtrons], including mir224, mir21 and several mirRNA deregulated in cancer. ~230 miRNAs are potentially regulated by HBx. Functional analysis shows that: (a) HBx can both upregulate and repress the expression of miRNAs that affect HBV replication and define new regulatory loops (i.e, miR224, miR552, miR3648 and others) and the control of cellular functions (i.e, mir21, mir26b, mir-502); (b) multiple transcription factors mediate HBx binding to its target genomic sequences (NFκB, E2F1, b-catenin, …); (c) HBx binding to miRNAs regulatory regions is accompanied reshuffling of chromatin modifying enzymes binding and histones epigenetic changes.

**Conclusion:** HBx is recruited to several genomic loci to modulate the epigenetic control of target genes and miRNA transcription.

### 130 A MULTIVALENT ADENOVIRUS-BASED IMMUNOTHERAPEUTIC FOR TREATMENT OF CHRONIC HEPATITIS B INDUCES BROAD, ROBUST AND POLYFUNCTIONAL T CELLS IN NAIVE AND HBV TOLERANT MICE

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**Background and Aims:** Current therapies for chronic hepatitis B (CHB) virus infection seldom achieve cure. Cohort studies have shown that cellular-based immunity is crucial for control and/or cure of infection suggesting that T-cell driven immunotherapies represent an attractive novel treatment approach to CHB offering the likelihood of increasing cure rate. We engineered 32 HBV immunotherapeutics based on different antigenic designs and viral platforms. One Ad5-based candidate (labelled TG1050) was selected.

**Methods:** TG1050, encoding a unique fusion protein composed of a truncated Core, a modified Polymerase and HBsAg (Env) domains was in vitro characterized by sequencing and Western Blotting. TG1050 immunogenicity was assessed quantitatively and qualitatively, following single or multiple injections, in five different
Parallel Session: EXPERIMENTAL FIBROSIS

131 CHARACTERIZATION OF FIBROSIS INDUCTION AND REVERSION IN C5 RECEPTOR-DEFICIENT MICE

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Background: Complement factor C5 contributes to hepatic fibrogenesis as C5-deficient mice display less fibrosis after challenge with CCl4 (Hillebrandt et al. 2005). During inflammation C5 is cleaved and the small chemoattractant peptide C5a binds to the receptors C5aR and C5L2, the latter of which was postulated to represent an anti-inflammatory decoy receptor. Our aim was to assess the specific roles of the C5 receptors during CCl4-induced liver injury and fibrosis.

Methods: C5aR−/− and C5L2-deficient mice and wild-type (WT) controls were treated with CCl4 (0.7 ml/kg) for 24 hours (acute, 1 injection), 10 days (short-time, 3 injections; Geerts et al. 1990), and 6 weeks (chronic, 12 injections twice weekly). In addition, mice were challenged with CCl4 for 6 weeks and left untreated for another 6 weeks (regression model). Expression of Th1 and Th2 cytokines was determined by qRT-PCR and hepatic collagen contents were measured via hydroxyproline.

Results: In the acute injury model, C5L2−/− but not C5aR−/− deficient mice showed increased expression of Th2 cytokines (IL12: 16.9±10.8; IL23: 116.7±55.5-fold) in comparison to WT mice. During stellate cell activation by short-time CCl4 exposure, cytokine expression patterns differed, with C5L2 knockout mice displaying lowest expression of IL6, IL10, IL12 and IL23 as compared to C5aR−/− and WT mice. In contrast, chronic fibrosis was least pronounced in C5aR-deficient mice in comparison to the other lines. Of note, 6 weeks after the last injection C5aR-deficient mice developed highest collagen levels, indicating late response and ongoing damage after cessation of fibrotic stimuli. This is resembled by cytokine profiles, with IL6, IL10, IL12, IL23 and IL27 being reduced in mice deficient for C5 receptors in the chronic model but elevated in C5aR−/− mice during fibrosis regression.

Conclusions: C5a receptors C5aR and C5L2 modify both acute and chronic responses to liver injury. Overall, C5L2 seems to be more relevant during the early phase, whereas C5aR is critical during chronic fibrogenesis. The novel observation that fibrosis increases in mice deficient for the C5aR receptor after removal of the fibrotic stimulus points to its critical role during wound healing and fibrosis regression.

132 NLRP3-INFLAMMASOME ACTIVATION IS ESSENTIAL FOR PROGRESSION TO STEATOHEPATITIS AND FIBROSIS

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The inflammasome, a caspase-1 activation platform critical for processing of key pro-inflammatory cytokines has been implicated in the development of steatohepatitis. As sources and mechanisms of inflammasome mediated liver damage remain poorly understood, we hypothesized that NLRP3-inflammasome activation is a central mechanism for innate immune activation and disease progression to NASH.

Methods: 6–8 week-old NLRP3 KO mice and wild type (WT) littermates were placed on a choline-deficient amino acid-defined (CDAA) diet and choline-sufficient control diet (CSAA) for 16 weeks, which results in severe NASH and fibrosis. Additionally to directly assess the effects of NLRP3 inflammasome activation we generated a tamoxifen-inducible mutant NLRP3 knockin mouse and fed the same diet for 4 weeks, a time point that results in early changes of hepatic steatosis. Upon completion of the diet cycles mice were sacrificed and liver tissue and serum were harvested.

Results: After 16 weeks, mice on CDAA diet gained slightly more weight than mice on CSAA diet without significant differences with respect to KO status. As expected, hepatocellular lipid accumulation was greater in the CDAA group, but no difference between WT and KOs was observed. NLRP3 KOs showed less inflammatory response to CDAA diet and correspondingly their ASC (p <0.05) and IL1 beta (p <0.05) mRNA levels were significantly lower. More importantly, NLRP3 KO showed a marked protection from CDAA-induced fibrosis as assessed by morphometric quantitation of Sirius Red Staining and mRNA levels for key fibrogenic genes including aSMA (p <0.05) and COL1A1 (p <0.05). Although there was a higher rate of apoptosis, determined by TUNEL-assay and cleaved Caspase 3 and 8 protein quantification, in animals fed a CDAA diet, this was similar in KO and WT groups. After 4 weeks on the CDAA diet, WT animals showed isolated hepatic steatosis while NLRP3 knockin mice showed severe liver inflammation, high rates of cell death, and early signs of liver fibrosis.

Conclusion: Our study uncovers a crucial role for NLRP3-inflammasome in the development of NASH and fibrosis. These findings may lead to novel therapeutic strategies aimed at halting the progression of hepatic steatosis to the more severe forms of this disease.
Chronic liver damage may eventually progress to end-stage liver cirrhosis and hepatocellular carcinoma. Cytoglobin (Cygb) is a 21 kDa globin expressed in hepatic stellate cells and functions as a hypoxia sensor and a gas carrier. It serves as local peroxidase by degrading H₂O₂. However, its pathophysiological role in vivo remains undetermined. Here, we report the promotion of liver cancer development in Cygb-deficient (KO) mice administrated with either diethylnitrosamine (DEN) or choline-deficient amino acid-defined (CDAA) diet that induces hepatosteatosis.

**Methods:** Cygb KO mice and corresponding wild-type (WT) mice at 8-week-old were treated with either DEN, CDAA, or CSAA diet for 8–32 weeks. Macroscopic and microscopic observations were performed. Gene expressions and intracellular signaling pathways were analyzed. Oxidative stress was determined by the formation of 8-OHdG, DHE, and nitrotyrosine.

**Results:** Model 1: 25 or 0.05 ppm DEN treatment for 25 or 36 weeks induced liver tumor formation in 100% or 44%, respectively, in Cygb KO mice compared to 44% or 0%, respectively, in WT mice. Background liver developed fibrosis together with the augmented expression of mRNA of TGF-beta 3, collagen 1a1, and TIMP-1. Inflammatory gene expressions and augmented oxidative stress formation were evident in KO mice. Model 2: as early as 8 weeks of CDAA treatment, Cygb KO mice exhibited dominant steatohepatitis, which resulted in advanced fibrosis at 16-week-point, compared with WT as assessed by pathological NASH scores, collagen deposition, alpha-smooth muscle actin expression, and hepatic hydroxyproline content. Surprisingly, after 32 weeks under CDAA administration, 100% of both male and female Cygb KO mice developed liver cancers, compared to 0% in corresponding WT mice. Analyses at 32 week-point showed histologically severe inflammatory reactions concomitant with increased mRNA expression of Tnfα, Tgfβ1, II-1β and II-6 in Cygb KO mice compared with WT mice. Cygb KO mice showed increased hepatocyte proliferation (Ki67 staining) and expression of AFP. Oxidative stress and antioxidant defense PCR array identified altered expression of 31 genes involved in the metabolism of reactive oxygen species in Cygb KO mice.

**Conclusion:** Deficiency of Cygb promotes liver cancer development through activating inflammatory reaction and oxidative stress pathway.

**134 HEPcidin Knockout Mice Develop Chronic Liver Injury and Liver Fibrosis as a Consequence of lysosomal Iron Overload**

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**Background and Aims:** Hepcidin is the central regulator of iron homeostasis. Disrupted hepcidin signalling results in hereditary hemochromatosis and iron overload seen in chronic liver disorders. While the association between iron overload and development of end-stage liver disease is well established, the understanding of the underlying mechanisms is hampered by the lack of a suitable animal model. To circumvent this problem, we analyzed hepcidin-knockout (KO) mice as a model of iron-overload associated liver disease.

**Methods:** Hepcidin wild-type (WT) and KO animals fed 3% carbonyl iron-containing chow were compared to mice kept on standard diet. Liver histology and serum parameters were used to assess the extent of liver injury/fibrosis. Iron distribution was determined by subcellular fractionation and electron microscopy.

**Results:** Among mice kept on iron-rich diet, 6 month old hepcidin KOs (vs. WTs) displayed profound hepatic iron overload (2543±114 vs. 1493±136 p<0.005), elevated liver enzyme (AST: KO 261±15, WT 142±34 p<0.05) and serum iron levels, mild hepatocellular inflammation and apoptosis. 12, but not 6 month old KOs fed iron-rich diet developed moderate liver fibrosis as determined by Sirius red staining and increased hydroxyproline levels. The liver injury was accompanied by a marked lysosomal iron overload and lysosomal fragility with release of cathepsins into the cytoplasm, while no major differences were seen in mitochondrial morphology or injury markers. Increased p62 levels as well as elevated lipofuscin pigment suggested a defect in protein degradation. As a potential mechanism leading to lysosomal iron overload, the expression of DMT1 and STEAP3, i.e. the molecules needed for lysosomal iron export, was greatly reduced. Finally, hepcidin KOs exposed to iron-rich diet also displayed an elevated oxidative DNA damage.

**Conclusions:** Hepcidin KOs represent a unique tool to study the mechanism of iron overload-related liver diseases and implicate lysosomal injury as a crucial event in iron toxicity.
Hence, we investigated primary HSCs from Jnk1γ− mice showing reduced transdifferentiation compared with WT and Jnk1γcre−/− mice.

**Conclusion:** Jnk1 in HSCs, but not in hepatocytes, plays a crucial role in the development of liver fibrosis, thus we identify Jnk1 in HSCs as a pro-fibrotic kinase and a promising cell-directed target for treatment of fibrotic patients.

### 136 ENDOGLIN DEFICIENCY IN HEPATIC STELLATE CELLS HAS A DIFFERENTIAL EFFECT ON LIVER FIBROSIS AND TGF-β SIGNALLING IN TWO EXPERIMENTAL MODELS OF MURINE LIVER FIBROSIS

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**Background:** Hepatic stellate cells (HSCs) are the major source for extracellular matrix (ECM) production in liver fibrosis. Endoglin (ENG) is a type III auxiliary receptor for TGF-β that is expressed on proliferating endothelial cells and HSCs. TGF-β is the most profibrotic cytokine expressed in response to liver injury. This study analyzes the role of ENG and TGF-β signalling in two models of experimental liver fibrosis by cell line specific Endoglin deletion in HSCs.

**Methods:** Using the Cre-LoxP genetic recombination system we created HSC specific ENG+− mice by crossing GFAPCre to ENGflox/flox mice. Mice were subjected to liver injury by CCl4 treatment (8 weeks) or bile duct ligation (BDL) (21 days). Liver fibrosis was analyzed by hydroxyproline measurement and Sirius red staining (8 weeks) or bile duct ligation (BDL) (21 days). Liver fibrosis was reduced transdifferentiation compared with WT and Jnk1D− mice. Mice were subjected to liver injury by CCl4 treatment. GFAPCre−/Engflox/flox primary HSCs demonstrated a differential effect of ENG on TGF-β signalling. In the absence of ENG signalling in vitro conditioned media (CM) and cytokines on WEHI265.1-monocyte cholangiocytes (Luminex); c) the effects of cholangiocyte conditioned media (CM) and cytokines on WEHI265.1-monocyte proliferation (MTS), chemoattraction (Boyden chamber) and transdifferentiation into fibrocytes (RT-PCR for COL1(A1)). WT littersmates served as controls.

**Results:** In Pkd1Δ/Δ mice, in spite of progressive fibrosis, portal accumulation of α-SMA+ cells (portal myofibroblasts) was evident only after 9th months. In contrast, we observed an early and important peribiliary recruitment of CD45+ cells (porta myofibroblasts) which is one of the main sources of liver fibrosis that is not visible in WT mice.

**Conclusions:** Endoglin deficiency in hepatic stellate cells has a differential effect on liver fibrosis depending on the cause of injury. Hepatocyte necrosis due to CCl4 treatment leads to strong TGF-β expression Smad signal transduction. Endoglin deficiency leads to amelioration of liver fibrosis, underlined by in vitro results. In contrast cholestatic liver injury is aggravated by endoglin deficiency in HSCs, reflecting a complex interplay of cholestasis, inflammation and toxic injury. Endoglin obviously modulates TGF-β signalling in this periportal-centered injury differentially, leading to significantly more fibrotic changes.
is sufficient to initiate hepatocellular carcinoma (HCC). However, the biologic significance of c-myc in precursor stages such as liver fibrosis is less defined. Liver fibrogenesis involves cell proliferation of hepatic cell populations such as hepatocytes and hepatic stellate cells (HSC). Here, we aimed to determine the potential role of c-myc in this process.

**Methods:** Expression of c-myc was measured in biopsies of patients with liver fibrosis of different etiologies by qPCR and immunohistochemistry. Liver fibrosis in wildtype (WT) and alb-myc<sup>tg</sup> mice was induced by periodic CCl<sub>4</sub> treatment. Primary HSC were isolated from WT and alb-myc<sup>tg</sup> mice and investigated for markers of cell cycle progression and fibrosis by qPCR and immunofluorescence microscopy.

**Results:** In patients with advanced (F3) liver fibrosis, hepatic c-myc was tenfold upregulated compared to healthy controls. Immunohistochemistry revealed an accumulation of c-myc-expressing cells in areas of fiber formation. Similarly, c-myc was also induced in murine WT liver during liver fibrogenesis. In turn, overexpression of c-myc in alb-myc<sup>tg</sup> mice resulted in increased collagen deposition and induction of α-smooth-muscle-actin (α-SMA) expression in liver over time. Most strikingly, primary HSC derived from alb-myc<sup>tg</sup> mice showed:

i. increased basal expression of α-SMA directly after isolation,
ii. premature α-SMA induction, and
iii. enhanced proliferation and accelerated transdifferentiation into myofibroblasts in comparison to WT HSC. Importantly, c-myc expression in HSC of both groups was only transiently detected. We hypothesized that c-myc overexpressing hepatocytes promote fibrosis by triggering the activation of HSC. In agreement with this idea, fibrosis initiation after chronic CCl<sub>4</sub> treatment was detected significantly earlier in alb-myc<sup>tg</sup> mice compared to controls, which was associated with increased expression of pro-fibrotic markers and strong induction of hepatocytes proliferation.

**Conclusion:** Here we provide *in vitro* and *in vivo* evidence that c-myc overexpression in hepatocytes is a molecular trigger of HSC activation and predisposes to liver fibrosis. We propose that high c-myc expression in liver of patients could be a useful predictive fibrosis marker.
139 AN UNFAVORABLE INTERACTION BETWEEN DONOR AGE AND LATENT RECIPIENT CYTOMEGALOVIRUS (CMV) INFECTION AFTER LIVER TRANSPLANTATION (LT): INSIGHTS FROM THE LIVER MATCH STUDY

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CMV infection, a strong up-regulator of alloantigens, is involved in many pathological events after LT. Despite widespread use of antivirals, CMV infection remains a problem, being associated with a 5-fold increase of all-cause mortality. As an interaction of CMV with the development of immunosenescence has been proposed, we investigated the impact of donor age and CMV infection on LT outcomes within Liver Match, an Italian prospective observational study.

Methods: The cohort comprised 1079 first adult LTs performed between June 2007 and May 2009, including 889 (82.4%) anti-CMV (IgG) positive (median age 46, IQR: 27–60) donors. Among recipients, 990 (91.8%) were anti-CMV positive (median age 56, IQR: 40–69) and 84 (7.8%) were negative (median age 54, IQR: 32–67). IgM positive anti-CMV represented <1% in all groups. In 456 (42.3%) LTs grafts from donors >60 years were used. During a current median follow up of 1125 days, 256 graft losses occurred.

Results: LTs were grouped as follows: (1) CMV-negative donor and CMV-positive recipient (CMV:D−/R+, n = 164, 15.2%); (2) CMV-positive donor and CMV-negative recipient (CMV:D+/R−, n = 68, 6.3%); (3) no-mismatch (n = 847, 78.5%). The impact of grafts from donors >60 years was evaluated within each group. Kaplan–Meier graft survival was significantly lower in LTs performed using grafts from older donors in the D−/R+ group (3-year survival 0.59 vs 0.88, p < 0.0001), while no significant differences were observed in the other two groups. The increased risk of graft loss in CMV-positive recipients of livers from CMV-negative donors >60 years (but not from donors <60 years) carry a significant excess risk of graft loss. This suggests a deleterious interaction of donor age with latent recipient CMV infection, which may then reactivate. Further studies on CMV and immunosenescence in the LT setting are warranted.

140 IMPACT OF SARCOPENIA ON MORTALITY AFTER LIVER TRANSPLANTATION IN HIV INFECTED PATIENTS

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Background: Long term results of liver transplantation (LT) for viral hepatitis in HIV-positive patients are inferior to non HIV infected patients, new tools are needed to select candidates.

Aim: To evaluate the impact of sarcopenia on post transplant mortality in HIV coinfected patients.

Patients and Methods: Between January 2007 and December 2011, 56 HIV+ patients (mean age 46.8 ± 5, F/M [8/48], with mean follow-up 35 months [±24], were transplanted for HCV, HBV and HBV/HCV/HDV related cirrhosis (n = 44, 6 and 6 respectively) of those 17 patients had HCC. Cross-sectional areas of the left and the right psoas muscles at the level of the 4th lumbar vertebra were determined in our population. Pre-operative donor and recipient characteristics were analyzed and for continuous variables a cut-off has been determined by a ROC curve.

Results: Overall survival at 1 and 3 years was 77% and 55% respectively. In univariate analysis, risk factor were psoas area <1691 mm2, MELD >17, cirrhosis other than HBV and preoperative urea. In multivariate analysis all those factors but urea, were significantly associated with mortality (p = 0.028, 0.049 and 0.038 respectively). In sarcopenic recipients with MELD score >17 and non HBV correlated cirrhosis overall survival was 22.2% vs 66% of remnant population (OR 11.3 p = 0.001).

Conclusion: In HIV infected patients, mortality after LT is strongly associated with sarcopenia (p = 0.028) especially in HCV+ patients with high MELD score (>17).
POSTERS

141 USE OF ANTI-HBc POSITIVE DONORS IN LIVER TRANSPLANTATION: THE EXPERIENCE OF PADUA LIVER TRANSPLANT CENTRE

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Background and Aims: Liver transplantation (LT) is the treatment of choice for advanced liver diseases. The growing gap between patients in the waiting list and liver donors has led to expand the donor pool with non optimal donors. The aim of the study is to evaluate the experience of the Liver Transplant Centre at the University Hospital Padua with the use of anti-HBc positive grafts.

Methods: Between 2008 and 2011, 256 consecutive cirrhotic patients underwent LT. HbsAg, anti-HBs titer, HBV-DNA, liver biopsies and prophylaxis were performed with an anti-HBc positive graft. Etiology of liver disease before LT and yearly after LT was determined according to etiology of liver disease, presence of hepatocellular carcinoma and MELD at LT. We observed at 12 months after LT a statistically significant higher fibrosis score compared to the other two groups (p = 0.0086), anyway this has not influenced survival. 2/71 (2.8%) patients developed de novo HBV. 6/71 of these donors were both anti-HBc and HbsAg positive and they were all assigned to HBV positive recipients. 6/6 patients receiving a HbsAg positive donor received combined prophylaxis with antiviral drugs and immune globulins. In this group of patients we observed both serological and histological delayed negativization of HbsAg. We have not found recurrence of HBV-related liver disease in these recipients. 1/6 patient developed immune-mediated cirrhosis after LT.

Results: From 1990 to September 2011 71/1010 (7%) were performed with an anti-HBc positive graft. Etiology of liver disease of recipients was HBV in 21/71 (29.5%) patients, HCV in 21/71 (29.5%) patients, other causes of liver disease in 39/71 (41%) patients. 79% of these recipients received de novo HBV prophylaxis after LT; 21% of them didn’t receive any prophylaxis. No statistically significant difference has been observed analyzing survival of anti-HBc positive graft’s recipients according to etiology of liver disease, presence of hepatocellular carcinoma and MELD at LT. We observed at 12 months after LT a statistically significant higher fibrosis score in patients underwent LT for HCV-related liver disease compared to the other two groups (p = 0.0086), anyway this has not influenced survival. 2/71 (2.8%) patients developed de novo HBV. 6/71 of these donors were both anti-HBc and HbsAg positive and they were all assigned to HBV positive recipients. 6/6 patients receiving a HbsAg positive donor received combined prophylaxis with antiviral drugs and immune globulins. In this group of patients we observed both serological and histological delayed negativization of HbsAg. We have not found recurrence of HBV-related liver disease in these recipients. 1/6 patient developed immune-mediated cirrhosis after LT.

Conclusions: The use of anti-HBc positive donors seems a safe way to expand the donors pool. It seems necessary to carefully allocate these grafts to HCV positive recipients.

142 DEFINITION OF SARCOPENIA IN CIRRHOTIC PATIENT BEFORE LIVER TRANSPLANTATION

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Background: End-stage liver disease associated protein malnutrition is responsible of mortality after liver transplantation (LT) but the maximal acceptable amount of malnutrition is unknown. Assessment of survival after LT and measurements of skeletal muscle mass (SMM) on CT-scan before LT should allow us to define this threshold of SMM loss or “sarcopenia”. Our aim was to compare different methods of SMM estimation in order to determine what mean “sarcopenia” in cirrhotic patients undergoing LT.

Methods: Between 2008 and 2011, 256 consecutive cirrhotic patients were transplanted and followed in our department. All had a CT scan in the 4 months before or in the week after LT. Measurements of the psoas muscle area (PMA) and total skeletal muscle area at the level of the 3rd or 4th lumbar vertebra were done. Predictive value after LT was compared between the different types of CT-scan SMM measurements within a ROC curve 1 year survival analysis. Sarcopenia, defined in this way, was compared with others pre or intra-operative predictive factors of post-LT survival in a multivariate analysis.

Results: SMM was associated with gender, weight, height, BMI, presence of ascites but not with MELD score. The PMA, in absolute value or normalized for body surface area or stature, and the 3rd lumbar vertebra skeletal muscle index were prognostic of 1-year survival. PMA was the simplest method and had the strongest predictive value (AUC= 0.757 with p < 0.0001). Cut-off values of 1460mm² in women and 1560mm² in men defined sarcopenia. Sarcopenic patients had worse 1 year survival (58% vs 94%, p < 0.0001). In multivariate analysis, sarcopenia was an independent factor (OR = 18, p < 0.001) of 1 year mortality, as well as cold and warm ischemia time (OR = 10 and 9.5, p = 0.008 and 0.015), hepatitis C virus (OR = 5, p = 0.01) and cytomegalovirus positive graft (OR = 3, p = 0.015).

Conclusions: In cirrhotic patients undergoing LT, sarcopenia was defined by PMA < 1460mm² in women and 1560mm² in men. This parameter was a major independent prognostic factor for mortality after LT in cirrhotic patients.

143 INTERNATIONAL BENCHMARKING IN LIVER TRANSPLANTATION IN EUROPE

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Background: Differences in donor quality and recipient selection for liver transplantation (LT) in European countries may lead to differences in outcome. Comparing outcomes after LT internationally might stimulate cross-national learning. Aim of this study was to compare outcomes in LT recipients in the United Kingdom (UK) and in Italy.

Patients and Methods: Data on 2,339 deceased donor LTs performed in adult recipients in the UK and Italy (01/06/2007—31/05/2009) were obtained from the UK Transplant Registry (n = 859) and the Italian ‘Liver Match’ database (n = 1480). Follow-up data were available for 2335 patients with a median follow up of 2.8 years.

Results: Hepatitis C (HCV) and hepatocellular carcinoma (HCC) were more common as primary indications for LT in Italy compared to the UK (HCV: 22.8% vs. 16.1%; HCC: 42.4% vs. 5.8%). Compared with their UK counterparts, LT recipients in Italy had lower MELD (median: 15 vs. 16, p = 0.0001), lower BMI (median: 25.1 vs. 26, p = 0.001), shorter cold ischemia time (436 min vs. 568 min, p < 0.0001), were more likely to receive grafts from older donors (median age: 56 vs. 47, p < 0.0001) and donors died for trauma (25.8% vs. 13.1, p < 0.0001). Risk factors for graft loss at Cox multivariate regression were different in the two countries: in Italy, risk factors consisted of donor age (HR = 1.007), donor HbcAb status (HR = 1.5), aetiology (HR for HCV = 2.2), bilirubin (HR = 1.18), creatinine (HR = 1.3), portal vein thrombosis (HR = 2.03); in the UK, risk factors consisted of national vs. local allocation (HR = 1.5), recipient creatinine (HR = 1.6) and donor BMI (HR = 1.03). While 90-days unadjusted graft survival was similar in the two countries, differences were observed at 3-years (78% in Italy vs. 84.4% in UK, p = 0.0001). However, after adjusting for donor, graft and recipient significant factors, disease-specific survival was
Conclusions: Risk adjusted-survival analysis provides similar results in both countries, except for those transplanted for ALD; this requires further investigation. Given significant differences in aetiology, donor, recipient characteristics and risk factors, international comparison of LT outcomes must be approached with caution. These data suggest that predictive models developed in one country may not be valid in another.

144 POOR PREDICTIVE ABILITY OF THE AMERICAN DONOR RISK INDEX IN ORTHOTOPIC LIVER TRANSPLANTATION IN EUROPE

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Background: The Donor Risk Index (DRI), developed within the Organ Procurement and Transplantation Network (OPTN), is a continuous scoring system which predicts graft-failure based on donor and transplant characteristics. The DRI has been shown to be an important risk factor for liver transplantation (LT) in the Eurotransplant region; however, its ability to predict outcome has not been assessed. The aim of the study was to validate the DRI and to assess its predictive ability in another, more heterogeneous, European cohort.

Methods: Data on 2,339 deceased donor LTs performed in adult recipients (June 2007-May 2009) in the United Kingdom (UK) and Italy were obtained from the UK Transplant Registry (n=859) and the Italian ‘Liver Match’ database (n=1480). The relationship between DRI and graft survival was investigated using the Kaplan–Meier method. Cox proportional hazards models were used to investigate the risk-adjusted effect of DRI. A concordance statistic of Gönen and Heller was calculated to assess the predictive ability of the models. Follow-up data were available for 2,335 patients with a median follow-up of 2.8 years.

Results: The mean DRI was higher in the European cohort (1.54±0.33) than in OPTN (1.45) in the same time frame. The Kaplan–Meier curves showed reasonably good discrimination between different DRI categories, with inferior graft survival associated with increased DRI (log-rank test: p=0.005). This effect remained apparent after adjusting for recipient creatinine, bilirubin and diagnosis; the change in the log likelihood statistic was highly significant on adding DRI to this Cox model. However, the predictability of the model including DRI and significant recipient factors was poor (c-statistic: 0.60) and there was only a small reduction after the exclusion of DRI (c-statistic: 0.58). Country-specific sub-analysis showed similar predictive ability in both countries.

Conclusions: DRI represents a significant factor influencing graft outcome. However, DRI has limited predictive ability in a heterogeneous European cohort. Other donor/transplant factors, or factors similar to those included in the DRI when different weighting, might better predict outcome in European populations. A donor scoring system tailored to country-specific risk factors and recipient aetiology, ideally used for liver allocation purposes, is the future direction.
EARLY INTRODUCTION OF EVEROLIMUS IN DE NOVO LIVER TRANSPLANTATION: A MULTICENTER RANDOMIZED CLINICAL TRIAL (EPOCAL)

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Introduction: There are no prospective randomized trials analysing safety and efficacy of early introduction of Everolimus in patients undergoing liver transplantation (LT).

Methods: We performed a spontaneous, phase 2, multicenter (7 centers), randomized, open label, clinical trial. Inclusion criteria: first LT, acceptable graft function in postoperative day (POD) 7, cold ischemia time <12 hours. Patients were randomized in POD 7 in two possible arms using a 2:1 ratio, the study group (with Everolimus introduction in POD 8 and Tacrolimus reduction/weaning), and a control group (conventional immunosuppression).

Primary endpoint: incidence of acute rejection and graft loss in the first 3 months after LT. Secondary endpoints: renal function, suspension of Tacrolimus, incidence of adverse events.

Calculated sample size: 117 patients evaluable for the primary endpoint.

We present the preliminary results of an interim analysis carried out in October 2012.

Results: Up to October 2012, 84 patients were enrolled in the study group and 46 in control group (110 patients evaluable at 3 months for the primary endpoint). We did not find any significant difference between the two groups. The primary endpoint in the study group vs. controls has been so far observed: acute rejection, 17% vs. 7% (p < 0.05), graft loss, 2% vs. 8% (p > 0.05).

The number of adverse events was also comparable between the two groups with the exception of infections having a significantly higher incidence in the controls (20% vs 1%, p < 0.01).

The weaning of tacrolimus at 1 month in the study group was successful in 43% of cases. Renal function at 3 months in the two groups was not significantly different.

Conclusions: Everolimus seems safe and effective when introduced early after LT.

HEPATIC ENCEPHALOPATHY IS AN INDEPENDENT RISK FACTOR FOR MORTALITY IN PATIENTS WAITING LIVER TRANSPLANTATION

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Introduction: Hepatic encephalopathy (HE) is a severe complication of liver cirrhosis. HE is not accounted for in the MELD score, which is widely being used for organ allocation. Aim of this study was to assess the impact of encephalopathy on survival of patients awaiting liver transplantation.

Methods: Retrospective analysis of consecutive adult patients listed for liver transplantation between 2007 and 2011 at LUMC, NL. Clinical data were retrieved from patient records and MELD and MELDNa score were calculated. Survival analysis was performed using Kaplan Meier and Cox proportional hazard regression analysis with death as event, censored for liver transplantation or last visit. Log-rank analysis was performed to exclude competing risk of transplantation. Univariate analysis was performed for presence of HE, MELD score, MELDNa score, age, ascites, prior SBP or variceal hemorrhage and hepatocellular carcinoma. Parameters with p < 0.10 were included in multivariate analysis.

Results: 168 Patients were included; 25/51 patients with HE (49%) and 64/117 (54%) patients without HE underwent liver transplantation after a mean of 7.0 ± 7.8 (HE) vs. 9.7 ± 7.8 months (no HE) (p = 0.158). HE patients had a higher MELD score at listing than patients without HE (20 ± 9 vs. 12 ± 5, p < 0.001).

The chance to receive a liver transplantation showed a trend towards earlier OLT in patients with HE (p = 0.063). The presence of HE was independently associated with increased mortality before transplantation (figure 1) (HR 3.702 (95%CI 1.496–9.162), p = 0.005), also after adjusting for MELD and MELDNa score in multivariate analysis. MELD (HR 1.095 (95%CI 1.031–1.163), p = 0.003) and MELDNa score (HR 1.124 (95%CI 1.051–1.202) were also independent predictors of mortality, whereas prior SBP and ascites were not. More severe HE was associated with a higher mortality risk, i.e., grade 2 HR 4.973 (p < 0.001) grade 3–4 HR 28.413 (p < 0.001). Mortality was not increased in patients with HE grade 1 (HR 1.094).

Figure: Kaplan–Meier survival estimate (months) of all patients until death according to the presence or absence of hepatic encephalopathy (HE) in patients listed for liver transplantation.

Conclusion: Hepatic encephalopathy is an independent risk factor for mortality in patients awaiting liver transplantation. Objective biomarkers for assessment of HE are needed as HE patients might deserve higher priority.
LIVER TRANSPLANTATION IN CIRRHOTIC PATIENTS WITH MELD SCORE <18: A NEW PROGNOSTIC MODEL TO PREDICT DROP-OUT FROM THE WAITING LIST

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Background and Aims: Model of End-Stage Liver Disease (MELD) is widely used for organ allocation in liver transplantation (LT), however its prognostic accuracy is better among patients with high score. This study aimed to assess outcome of patients with MELD<18 awaiting LT and find prognostic variables able to identify patients with high drop-out risk.

Methods: We enrolled patients with MELD<18 listed for LT between January 2003 and January 2011. The training set consisted of 277 patients from Modena and Padova Transplant Centers and the validation cohort of 292 patients from Bologna Transplant Center. Competing risk regression analysis taking into account the presence of LT was used for univariate/multivariate survival analysis.

Results: Ascites [sub-hazard ratio (SHR) 3.33, 95%CI: 1.3–8.54], sodium (SHR 0.91, 95%CI: 0.83–0.99), bilirubin (SHR 1.31, 95%CI: 1.1–1.57), albumin (SHR 0.31, 95%CI: 0.14–0.69), and glomerular filtration rate (SHR 0.99, 95%CI: 0.97–0.99) were independently associated with the risk of drop-out at 12 months. Combining these 5 prognostic parameters we calculated a new score named Hepato-Renal-Risk (HRR). The 12-months AUC for HRR (0.891) showed a discrimination power better than CTP (0.818; p = 0.009) and MELD (0.700; p < 0.001). Similarly, Hosmer-Lemeshow test showed a remarkable calibration (p = 0.896), with a R-square value of calibration curve=0.982. These data were confirmed in the validation set both for discrimination (AUC: HRR 0.83 vs MELD 0.601, p < 0.001; and vs CTP 0.714, p = 0.003) and for calibration (Hosmer-Lemeshow: p = 0.91; R-square=0.911). Subdividing all patients (training plus validation sets) utilizing the values of HRR ensuring lowest false negative and false positive results and best positive-predicted-value and negative-predicted-value, the risk of waiting list drop-out significantly increased throughout the three HRR groups (HRR<15.2; HRR between 15.2 and 16.3; HRR>16.3). The analysis of survival benefit comparing the risk of waiting list drop-out with the mortality of the 170 transplanted patients with same HRR, showed an important benefit for LT in patients with HRR>16.3.

Conclusions: In patients with MELD<18, combination of ascites, sodium, albumin, bilirubin and renal function in a new score (HRR) is superior than MELD in identifying both patients at high risk of waitlist drop-out and patients in whom LT may be safely deferred.
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Methods: Using the UK Transplant Registry liver donors and their recipients in England (1990–2008) were identified and cases of cancer among these were identified by matching their details with the Cancer Registries. Classification of donor cancer transmission risk into ‘Standard/non-standard risk’ and ‘Unacceptable risk’ was performed using the Council of Europe guidelines, 2010. Confidence intervals (CI) at 95% level are presented.

Results: Of the 6743 liver transplants from 6571 donors, 6721 (99.7%) were from donors with standard/non-standard risk of cancer transmission (group 1) and 22 (0.3%) were from donors with unacceptable risk (group 2). There was no statistically significant difference between group 1 and group 2 in mean recipient age (42.2 years [CI 41.8, 42.7] and 40.0 years [CI 39.2, 40.8], p = 0.57), gender (males 54% and 73%, p = 0.08), mean MELD score (19 [CI18.8, 19.3] and 20 [CI 15.7, 25.0], p = 0.56), proportion of super-urgent transplants (16% and 14%, p = 0.77), five-year recipient survival (75% and 76%, p = 0.99) or risk adjusted hazard of death (HR 1.1 for group 2, CI 0.4, 3.4). None of the 599 recipients who developed post-transplant cancers had the same type of cancer as their donor, indicating that these were unlikely to be donor-transmitted. At 10 years from transplantation, additional survival benefit gained by transplanting livers from ‘unacceptable’ risk donors was 165 life-years (CI 163, 167) with an average survival of 7.5 years (CI 7.4, 7.6) per recipient.

Conclusions: Cancer transmission from donor is a rare complication of liver transplantation. The classification of donors with certain cancers as having an unacceptable risk of cancer transmission appears to be unduly cautious. Selected donors in this group can safely donate livers providing valuable additional survival for the recipients with low rates (zero in our study) of cancer transmission. With careful donor-recipient selection and informed consent, safe expansion of donor pool can be achieved.

151 RECURRENT OF PRE-EXISTING EXTRA-HEPATIC CANCERS FOLLOWING LIVER TRANSPLANTATION
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Background and Aims: A past history of cancer is a relative contraindication to undergoing liver transplantation because of the risk of recurrence of cancer. However, the extent of this risk is not fully established. The aim of this study was to assess the risk of recurrence of pre-existing extra-hepatic cancers following liver transplantation.

Methods: We used the data from the United Kingdom Transplant Registry to link all liver transplant recipients (1985–2010) in the West Midlands region with the regional Cancer Registry to identify recipients with a history of cancer diagnosed before organ transplantation (excluding recipients transplanted with liver cancer) and those who developed a recurrence of cancer following transplantation. Kaplan–Meier survival estimates were used to assess the risk of recurrence of cancer. Confidence intervals (CI) at 95% are presented.

Results: Of the 832 recipients, 16 (1.9%) had a history of cancer before transplantation, including cancers of breast (3), cervix (1), colon (1), leiomysarcoma (1), leukaemia (1), lymphoma (4), kidney (1), prostate (1), melanoma (1), thyroid (1) and uterus (1). Two recipients developed cancer recurrence (melanoma and leiomysarcoma) with a rate of recurrence within 5 years of transplantation of 19.8% (CI 0.4, 4.9). Both recipients with recurrence had been cancer-free for less than five years pre-transplant. In both cases, the recipients died as a direct consequence of recurrent cancer. There were no cases of recurrence of cancer in 14 recipients of whom 8 underwent transplantation more than five years after the diagnosis of cancer.

Conclusions: Our data suggest that a cancer-free period of 5 or more years is associated with a very low risk of recurrence of cancer in this selected cohort of patients. Because of the increasing age of recipients and higher incidence of cancer in patients with cirrhosis, more patients with previously treated cancer are considered for liver transplantation. Careful risk-benefit assessment should be adopted prior to offering transplantation to a patient who had a cancer treated within the previous five years. As the recurrence of cancer was fatal in both cases, informed consent will play an important role in clinical management and also has medico-legal implications.

152 HEPATITIS B PROPHYLAXIS POST LIVER TRANSPLANTATION WITH TENOFOVIR OR ENTECAVIR
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Background: The introduction of HBIG and lamivudine to prevent Hepatitis B (HBV) recurrence after liver transplantation (LT) has improved survival. However, both cost and disease recurrence primarily related to the development of lamivudine resistant strains remain a significant problem.

Aim: To assess the safety and efficacy of entecavir and tenofovir in preventing HBV recurrence following liver transplantation.

Method: Between January 1990 and December 2011 a total of 133 (106 males and 27 females) patients were transplanted at our center for HBV related cirrhosis. All patients received post transplant combination therapy with nucleos(t)ide analogue and anti-hepatitis B immunoglobulins. Breakthrough infection was defined as re-emergence of HBV-DNA or HBsAg while on treatment. Demographic, clinical and laboratory data including various viral markers were collected from all patients.

Results: The majority of patients received lamivudine and/or adefovir in combination with HBIG. Ten and five patients received tenofovir and entecavir monotherapy, respectively. Post LT survival and HBV recurrence during the follow up period were 89% and 11%, respectively. The 15 patients (10 male & 5 females) who received the newer nucleos(t)ide analogues were followed for a mean of 32 (range=12–114) months after transplantation. Their age ranged between 12–40. All 15 patients were positive for HBsAg and 13 were HBeAg negative. HBV-DNA was negative in 9 patients at the time of transplantation and positive in 6 patients (2 with high viremia). All cases of recurrence occurred on lamivudine or adefovir monotherapy and were controlled with switching or adding another agent. None of the 15 patients treated with tenofovir or entacavir developed resistance and all patients had normal renal function during the follow-up period, none of the patients who had entecavir or tenofovir before transplant had recurrence after transplant.

Conclusion: Tenofovir and Entecavir are safe and effective in prophylaxis of post liver transplant with high resistance barrier. No interaction with immunosuppression. No effect on renal function with tenofovir. It may provide an attractive alternative to long term HBIG use.
Background and Aims: Recurrence of hepatitis C virus (HCV) infection after orthotopic liver transplantation (OLT) is associated with reduced graft and patient survival. The protease inhibitor telaprevir may enhance virologic response rates in patients after OLT in combination with pegylated-interferon-alfa and ribavirin. Pharmacokinetic studies have shown significant drug–drug interactions between telaprevir and immunosuppression (IS), but telaprevir blood levels kinetics in OLT-patients with IS are unknown. Aim of the present study was to analyze telaprevir blood levels in patients with HCV genotype 1 infection after OLT in comparison to patients without OLT and IS.

Methods: Five patients with HCV genotype 1 infection after OLT were treated with telaprevir 2250mg daily, ribavirin 1000/1200 mg daily, and pegylated-interferon-alfa-2a 180μg once weekly (triple therapy) and compared to 37 HCV genotype 1 infected patients without OLT treated with triple therapy. Standard dose IS was maintained until day –1 before initiation of telaprevir. In patients with ciclosporin (n=3) based IS, dose was reduced 4-fold, and 35-fold in patients with tacrolimus based IS (n=2), and adjusted to blood trough levels as appropriate. Telaprevir blood levels were analyzed approximately 4 hours after intake by liquid chromatography electrospray-ionization-tandem mass spectrometry. HCV-RNA was assessed by Taqman reverse-transcription polymerase-chain-reaction.

Results: Mean ± SD telaprevir blood levels were 3920±733ng/mL and 2268±1157ng/mL in patients after OLT and ciclosporin or tacrolimus based IS, respectively, compared to 2686±1157ng/mL in non-OLT patients (p=0.01). Telaprevir blood levels were steady at treatment weeks 4, 8, and 12 in patients with and without IS (Figure 1). Mean ± SD ciclosporin and tacrolimus trough levels in patients after OLT were 77.92±34.39ng/mL and 733ng/mL respectively. No acute graft rejection was observed during triple-therapy. In OLT- and non-OLT-patients, HCV-RNA was undetectable in 1/5 and 15/37, 2/5 and 17/37, and 1/5 and 4/37 after 4, 8, and 12 weeks in patients with and without IS, respectively.

Conclusions: Telaprevir blood levels are unaltered in patients with ciclosporin or tacrolimus based IS in patients with HCV re-infection after OLT.
treat basis. Since prospective studies comparing both strategies will not be available, case-control analysis might become relevant.

**Aim:** To analyse the safety and the efficacy of standard antiviral treatment in a large cohort of cirrhotic patients awaiting LT.

**Methods:** 89 HIV-infected cirrhotic patients (Child A or B ≤ 7 points) who underwent antiviral therapy while on the waiting list for LT between 2000 and 2012 were included. Treatment started when estimated waiting time was < 4 months and was maintained until LT.

**Results:** 65 patients were Child A and 24 Child B. Mean age was 58 years (35–69). Mean duration of treatment was 18 weeks (1–52). Genotype 1b was the most frequent (71%). Baseline analytical values were: bilirubin 1.4 mg/dl (0.5–3.8 mg/dL), prothrombin time 78% (50–100%), albumin 36 g/L (27–55 g/L), MELD score 9 (6–16) and platelet count 107,000 (36,000–273,000). Treatment was discontinued in 17 patients (non-response in 7, removal from the waiting list in 3 and AE in 8 patients). Among 83 patients who underwent LT, 38 (46%) had undetectable HCV-RNA at the time of LT and 24 (29%) achieved post-LT sustained virological response. AE were anemia (<10 g/L, 50%), neutropenia (<750, 37%), thrombocytopenia (<30,000, 19%), sepsis (7%) and hemorrhage (2%). Forty-four percent received erythropoietin and 40% received filgastrim. Fifteen patients (17%) developed decompensation and 12 patients (14%) infections. Two treatment-related deaths occurred. Decompensation (50% vs. 4.8%; p < 0.001) and infection (37.5% vs. 4.8%; p < 0.001) rates were significantly more frequent in Child B patients. Indeed, severe AEs occurred almost exclusively in Child B patients.

**Conclusions:** Standard antiviral therapy prevents hepatitis C recurrence in only one third of treatment patients. Severe AE seem restricted to Child B patients. Future studies assessing triple therapy in compensated cirrhotics awaiting LT should compare both safety and efficacy with standard therapy in this population.

**156 HEPATITIS C RECURRENCE AFTER LIVER TRANSPLANTATION: MAINTENANCE THERAPY WITH PEGYLATED INTERFERON IN NONRESPONDERS TO STANDARD THERAPY SLOWS THE DISEASE PROGRESSION AND IMPROVES SURVIVAL**

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**Background and Aims:** Long-term maintenance therapy with low-dose pegylated interferon (PegIFN) in HIV-infected liver transplant (LT) recipients not responding to standard therapy has been scarcely evaluated, although it may improve prognosis in those patients who do not attain a virological response but achieve a significant improvement in liver enzymes. We aimed to evaluate the clinical effect of long-term PegIFN in recurrent hepatitis C patients without virological response but with biochemical response (BR) to standard antiviral therapy.

**Methods:** One-hundred and thirty-nine patients who presented a severe hepatitis C recurrence (cholestatic hepatitis or significant fibrosis/portal hypertension one year after LT) were considered. Among them, 89 patients received antiviral therapy with PegIFN and ribavirin and were divided into three groups:

1. patients who achieved a sustained virological response (SVR, n = 23);
2. non virological responders to therapy (NR, n = 47); and
3. non virological responders who achieved a BR and received maintenance therapy with Peg-IFN (NR-M, n = 19).

Patients in the NR-M group were treated with Peg-IFN alfa-2b 50 μg/week for a median time of 20 months (range 2–45). BR was defined as a decrease ≥ 75% or normalization in transaminas levels during standard therapy.

**Results:** As expected, in patients who achieved SVR the hepatic venous pressure gradient (HVPG) improved or remained stable in 95% of cases and graft survival 5 years after treatment was 100%. HVPG improved or remained stable three years after completing antiviral therapy in 82% of patients in the NR-M group compared to only 31% of patients in the NR group (p = 0.003). Importantly, graft survival 5 years after antiviral therapy was 59% in the NR-M group versus 40% in the NR group (p = 0.012). Regarding safety, only one patient developed a de novo autoimmune hepatitis during maintenance therapy; other adverse events included anemia and neutropenia.

**Conclusion:** Long-term maintenance therapy with PegIFN in HIV-infected LT recipients not responding to standard therapy who achieve a BR is associated with a stabilization in recurrent disease and a significant increase in survival. Considering the absence of severe side effects, it may be a bridging strategy for patients awaiting new direct antiviral agents.
RESPONSE PREDICTION IN CHRONIC HEPATITIS C AFTER LIVER TRANSPLANT BY ASSESSMENT ENT-1-RELATED SINGLE NUCLEOTIDE POLYMORPHISMS

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Background: Ribavirin antiviral activity is associated with its accumulation in hepatocytes and high levels of ENT-1 expression correlate with high Ribavirin uptake. The single nucleotide polymorphism (SNP) GG at rs 760370 of ENT-1 correlates with virological response to pegIFN-ribavirin therapy in no transplanted patients with HCV (Morello 2010). Aim of our study was to examine if donor hepatic polymorphism of ENT-1 may account for treatment outcome in LT patients.

Methods: We evaluated for enrolment patients undergoing standard treatment for HCV recurrence post-LT between 2009–2011. Genotyping of ENT-1 and IL-28-B rs 12979860 was performed by pyrosequencing technology from donor liver tissue sample collected at the time of transplantation (before reperfusion). Ribavirin plasma levels were evaluated by high pressure liquid chromatography. A multivariate logistic regression model was performed for independent predictors of SVR.

Results: Overall, 32 patients were treated with PEG-IFN and RBV for 48 weeks. ENT-1 donor hepatic allelic frequencies of the graft at rs 760370 were as follows: AA 44%; AG 28%; GG 28% (Table 1).

Table 1. Baseline characteristics of patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>AA (14 pts)</th>
<th>AG (9 pts)</th>
<th>GG (9 pts)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age, years, median (range)</td>
<td>52 (45–64)</td>
<td>62 (49–63)</td>
<td>53 (45–64)</td>
<td>0.32</td>
</tr>
<tr>
<td>Donor age, years, median (range)</td>
<td>47 (16–68)</td>
<td>46 (14–70)</td>
<td>42 (18–64)</td>
<td>0.10</td>
</tr>
<tr>
<td>Basal Fibrosis, Ishak stage, median (range)</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>0.85</td>
</tr>
<tr>
<td>Basal HCV RNA load, log10, median (range)</td>
<td>6.45 (5.5–7.6)</td>
<td>6.5 (5.6–7.3)</td>
<td>6.5 (5.7–7.1)</td>
<td>0.9</td>
</tr>
<tr>
<td>IL-28 B of donor liver</td>
<td>45%</td>
<td>64%</td>
<td>50%</td>
<td>0.19</td>
</tr>
<tr>
<td>TC/TT, %</td>
<td>55</td>
<td>36</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

ENT-1 hepatic genotypes were collapsed in a recessive model and comparisons were done as GG versus non GG polymorphism. Achievement of RVR and SVR was more frequent among GG carriers than in AA/AG (65% vs 27% and 66% vs 32%, respectively; p = 0.02 and p = 0.04). No difference in RBV plasma concentration was found between hepatic ENT-1 GG vs AA/AG (p = 0.8). RBV plasma concentration was significantly higher in SVR vs NR (p = 0.05). Independent predictors of SVR are shown in table 2.

Table 2. Independent predictors for SVR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95%CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENT-1 GG</td>
<td>2.8 (1.4–7.8)</td>
<td>0.05</td>
</tr>
<tr>
<td>Ribavirin concentration &gt;2ng/ml at week 12</td>
<td>16 (1.6–12)</td>
<td>0.04</td>
</tr>
<tr>
<td>Donor IL-28 B CC</td>
<td>1.8 (0.03–2.4)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Conclusions: Subjects with a donor hepatic polymorphism GG at rs760370 showed an higher SVR than the AA/AG carried, it seems reasonable that both the ENT-1 activity and Ribavirin plasma synergically contribute to virological response.
is still a matter of debate. The aim of our study therefore was to assess the clinical significance of anti-major histocompatibility complex (MHC) antibodies in LT recipients.

**Methods:** Data were retrieved from the medical records of 174 LT-patients. Univariate and multivariate Cox proportional regression analysis was performed. Kaplan Meyer method was used for survival analysis. Serum samples from each patient were analyzed for the presence of anti-MHC antibodies using LABScreen Single Antigen assay. Immunohistochemical C4d staining was performed on formalin-fixed, paraffin-embedded tissue.

**Results:** Overall 1, 5 and 10 years graft survival rates were 95.2%, 80.4% and 64.4%. Univariate Cox regression analysis identified the following risk factors for graft failure: presence of MHC class II donor specific antibodies (DSA), donor age >50 years, anti-cytomegalovirus (CMV) IgG-positive donor, recurrent episodes of cholangitis, ischemic type biliary lesions, diabetes mellitus, and hepatitis C virus (HCV) etiology of liver disease at LT. Multivariate Cox proportional analysis identified presence of MHC class II DSA and anti-CMV IgG-positive donor as independent risk factors for graft failure following LT. Patients with HCV infection had a significantly lower graft survival compared to non-HCV recipients. Within the subgroup of patients with HCV infection, patients with MHC class II DSA had a significantly lower graft survival in comparison to HCV recipients who were negative for DSA (median survival 5.35 years vs 11.2 years). C4d deposition was found more frequently in allograft biopsy specimens of patients with HCV recurrence and presence of MHC class II DSA compared to recipients who were DSA negative.

**Conclusion:** MHC class II DSA have a negative impact on allograft survival following LT, especially in HCV-infected recipients.

161 **EVEROLIMUS IN LIVER TRANSPLANTATION TO PREVENT HEPATOCELLULAR CARCINOMA RECURRENT IN THE PRESENCE OF EXTENDED MILAN CRITERIA**

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**Aim:** Hepatocellular carcinoma (HCC) is the second cause of liver transplantation (LT). Mammalian target of rapamycin (mTOR) inhibitor have immunosuppressant and antitumor properties. Sirolimus has been shown to improve survival after LT for HCC exceeding the Milan criteria, but few data concerning Everolimus (EVL) are available. The aim of this study was to compare patient survival and disease free survival according to the immunosuppressive protocol (EVL vs CNI) for patients with HCC exceeding Milan criteria at pathology.

**Methods:** We retrospectively examined patients who underwent LT between 01/01/2007 and 31/12/2011 with HCC exceeding Milan criteria at pathology. Histological features were reported for all patients (size and number of tumors, vascular invasion, histopathologic grading and nodes metastases). After LT, all patients received an immunosuppressive protocol with CNI, steroids and mycophenolate mofetil.

**Results:** In our center, 476 patients underwent LT from 2007 through 2011. 138 patients had HCC, with 36 exceeding Milan criteria at pathology. CNI were switched by EVL for 16 patients. The switch was performed between 1 and 24 months after LT and before 6 months for 11 patients. Forty patients remained on Tacrolimus regimen and 6 on Cyclosporine regimen. There was no significant difference between the two groups (EVL vs CNI) according to histological features. The 5 year overall survival was 80% and the 5 year disease free survival was 72%. There was no significant difference between EVL and CNI according to 1 and 3 year overall survival (93.7% vs 95%, (p=0.856) and 81.2% vs 85%, (p=0.713) respectively) and 1 year and 3 year disease free survival (87.5% vs 85%, (p=0.88) and 75% vs 75%, (p=0.952) respectively). Nine patients had HCC recurrence including 4 patients with EVL. Six of the seven deaths were related to HCC recurrence. Three patients who died had EVL.

**Conclusion:** In your population, the overall and disease free survivals were very good. EVL fails to improve survival. Our results need to be confirmed by prospective studies. Furthermore, the delay of EVL beginning after LT needs to be studied.
163 EVALUATION STUDY FOR THE INFLUENCE OF DIAGNOSTIC METHODS FOR BILIRUBIN, CREATININE AND PROTHROMBIN (INR) FOR LABORATORY MELD SCORE CALCULATIONS

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Background and Aims: According to current guidelines of the German Medical Association Organ transplantation, the allocation to the group of elective patients (≥16 years) is depending on the severity of liver diseases. These measurements based on the lab-MELD score (laboratory model of end-stage liver disease). The lab-MELD score provides a prognosis of three month mortality for most of the patients with end-stage liver disease. The lab-MELD score is calculated from laboratory values bilirubin (mg/dl), creatinine (mg/dl) and prothrombin time expressed as INR (International normalized ratio). Different detection methods are currently in praxis for these three parameters. Reference methods only exist for bilirubin and creatinine. Therefore both parameters can be detected with high precision and high accuracy. The aim of our study was to analyse the relevance of the different diagnostic methods for bilirubin, creatinine and INR using an external proficiency panel to calculate the lab MELD scores.

Methods: Clinical samples were tested within proficiency panels of the Reference institute for Bioanalytics with 2, 7 and 8 different methods for creatinine, bilirubin and INR, respectively. MELD-scores were calculated regarding the formula: 10·(0.378·LN(Bilirubin) + 1.12·LN(creatinine) + 1.12·LN(INR) + 0.643).

Results: 1154, 4087 and 3494 investigations were analysed for bilirubin, creatinine and INR, respectively. The influence on the different diagnostic methods on the final lab MELD score was 2–4 MELD-score-values for bilirubin, 4–5 MELD-score-values for creatinine and 6–8 MELD-score-values for INR.

Conclusion: Harmonization of laboratory methods is necessary to allow an equitable distribution of liver organs. Therefore, further clinical ring trials are planned to evaluate pre-analytical, analytical and post analytical procedures within calculations of lab-MELD scores.

164 RISK FACTORS FOR COMPLICATIONS OF ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY IN PATIENTS AFTER LIVER TRANSPLANTATION

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Aim: Endoscopic Retrograde Cholangiopancreatography (ERCP) plays a major role in the management of biliary complications after orthotopic liver transplantation (OLT). Our aim was to study the safety of ERCP in patients after OLT and to evaluate the risk factors for complications of ERCP in patients with OLT.

Methods: We identified 126 consecutive patients who underwent 503 ERCPs post-OLT at our center from 2006 to 2012. 356 control patients who underwent ERCP without undergoing OLT during the same time period were identified. Univariate and multivariate analysis were performed.

Results: One hundred twenty-six OLT patients had between 1 to 15 ERCPs done with a mean of 5.7±3.1 procedures per patient. Patients in the OLT group were significantly younger than the non-OLT group (55.2±8.9 vs. 58.9±16.5; p = 0.019). 14/126 (11.1%) of patients in the OLT group had at least one post-ERCP complications compared to 36/356 (10.1%) in the non-OLT group (p = 0.75). On multi-variate analysis, the presence of OLT status did not increase any complication risk (Table 1). The predominant indication for ERCP in the post-OLT group was anastomotic strictures (n = 247, 49.1%). Out of 503 ERCPs performed on 126 OLT patients, 7% (n = 35) had at least 1 complication (26 patients had 1, 3 had 2 and 1 had 3 procedures with at least 1 complication). Twelve procedures resulted in pancreatitis, 3 in bleeding, 12 in cholangitis and 9 deaths (1 procedure had both pancreatitis and bleeding). Sixty-six percent (23/35) of the complications resulted in hospitalization with a median length of stay of 5 days. On multivariable analysis, serum bilirubin (Odds Ratio [OR] 1.1; 95% Confidence interval [CI] 1.05, 1.12) and greater than 2 contrast injections into the pancreatic duct (OR 5.4; 95% CI, 1.1–27.4) increased the risk of developing any post-ERCP complications in OLT patients (Table 2).

Table 1. Multi-variable analysis for developing post-ERCP complications

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Transplant</td>
<td>1.07 (0.55, 2.1)</td>
<td>0.84</td>
</tr>
<tr>
<td>Age (5 year increase)</td>
<td>0.91 (0.82, 1.00)</td>
<td>0.053</td>
</tr>
<tr>
<td>Male</td>
<td>1.2 (0.68, 2.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>Caucasian</td>
<td>1.9 (0.78, 4.6)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 2. Multi-variable analysis for developing post-ERCP complications

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. bilirubin</td>
<td>1.1 (1.05, 1.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt;2 pancreatic contrast injections</td>
<td>5.4 (1.04, 27.4)</td>
<td>0.044</td>
</tr>
<tr>
<td>Balloon dilation</td>
<td>2.1 (0.93, 4.9)</td>
<td>0.088</td>
</tr>
<tr>
<td>No Mycophenolate</td>
<td>2.0 (0.90, 4.4)</td>
<td>0.072</td>
</tr>
</tbody>
</table>

Conclusion: In conclusion, ERCP is safe and effective in managing biliary complications in patients after OLT. The risk for developing any complication increases with pancreatic duct cannulation and contrast injections.

165 THE EFFECT OF OBESITY, RENAL DYSFUNCTION AND ADVANCED AGE ON LONG-TERM SURVIVAL IN PATIENTS UNDERGOING ORTHOTOPIC LIVER TRANSPLANTATION

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Introduction: Obesity, renal dysfunction and recipient age ≥60yrs are widely recognised as factors that increase the risk of liver transplantation.

Objectives: To determine the prognostic value of these factors (as a composite measure) in terms of long-term survival following orthotopic liver transplantation (LT).

Methods: Two hundred and nine consecutive patients who underwent primary, single organ orthotopic LT at Flinders Medical Centre between 1992 and 2011 were studied; Male: 134 (64.1%), Age: 49.3±11.6yrs, MELD: 18.8±8.9, CREAT: 940±499μmol/L; median follow-up: 5.1yrs (minimum 1yr). Patients were categorized into groups via a composite risk score: (BMI: <30 [0], ≥30 [1]). Creatinine: <90 (F) or <110 (M) [0], ≥90 (F) or ≥110 (M) [1] and Age: <60 [0], ≥60 [1]. Cox regression models were fitted to determine the association between the composite risk score (at time of organ offer) and patient survival; adjusted for gender, etiology and MELD.
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Results: The overall 1-, 5- and 10-year posttransplant survival rates, stratified by the composite risk score are presented in Table 1. More than half (53.6%) of patients had at least one of the risk factors (obesity, renal dysfunction or advanced age). Patients with two or more risk factors (score ≥2) experienced a significantly higher covariate-adjusted risk of death compared to patients with zero (HR: 5.9, 95% CI: 2.3–14.3; p < 0.001) or one (HR: 2.6, 95% CI: 1.3–5.6; p = 0.009) risk factor.

Table: Patient survival, stratified by risk score

<table>
<thead>
<tr>
<th>Risk score</th>
<th>Posttransplant Survival, Years</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>97 (46.4)</td>
<td>0.17</td>
<td>0.07</td>
<td>0.43</td>
</tr>
<tr>
<td>1</td>
<td>86 (41.1)</td>
<td>0.38</td>
<td>0.18</td>
<td>0.78</td>
</tr>
<tr>
<td>≥2</td>
<td>26 (12.4)</td>
<td>0.38</td>
<td>0.18</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Conclusions: Patients who underwent transplantation in the presence of two or more risk factors (composite risk score ≥2 points) experienced significantly reduced long-term survival rates. Therefore, these findings identify patients at increased risk of postoperative mortality who may benefit from preoperative medical optimization.

166 HCV DIVERSITY AND FIBROSIS PROGRESSION: NSSA AND CORE VARIANTS CORRELATE WITH SEVERITY OF HCV RECURRENT AFTER LIVER TRANSPLANTATION

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Introduction: Recurrence of HCV-disease after liver transplantation (LT) impairs patient survival, with up to 30% of recurrent HCV-infections progressing to cirrhosis within 5 years. We evaluated the role of host (IL-28b genotype) and viral (NS3, NSSA and core polymorphisms) factors in determining worse clinical-outcomes and faster disease progression in patients with HCV-recurrence.

Methods: Nineteen patients (57.9% males, median (IQR) age: 65 (60–69) years with HCV-recurrence after LT were studied. All were transplanted between 1995 and 2009 and were previous non-responders to peg-interferon+ribavirin treatment performed after LT.

Patients were classified as slow-fibrosis or fast-fibrosis progressors according to histological staging at year 1 after LT (cut-off: ≥1 points of Ishak-score). NS3- protease (181 amino-acids) and full length NSSA and core sequences were obtained by population-sequencing. Detected mutations were correlated with fibrosis progression by the Fisher exact-test.

Results: Eight patients were classified as slow-fibrosis progressors (42.1%; 1a=1; 1b=7) and eleven as fast-fibrosis progressors (57.9%; 1a=4; 1b=7). This classification was confirmed also at year 3 after LT (cut-off: ≥3 points of Ishak-score). Patients with IL28B C/C genotype were only slow-progressors (N=3), while patients with T/T were only fast-progressors (N=3), differently patients with the T/C genotype were both fast (N=8) and slow (N=5).

NSSA, NS5A and core sequences were obtained in 16 (100%), 18 (94.7%) and 17 (89.5%) patients, respectively. One NS5A-mutation (D402N), located in the V3-domain of the interferon-resistance-determining region (IRRDR), was significantly correlated with a slower fibrosis progression of HCV-related disease after LT, being detected in 1/11 (9%) fast-progressors and in 4/7 (57.1%) slow-progressors, respectively (p = 0.047).

No other mutations in either NS3, NS5A or core proteins were significantly associated with fibrosis progression. The already-described M91I core mutation showed only a trend of association with faster fibrosis-progression, since it was found in 3/10 (30%) fast-progressors and 0/7 slow-progressors (p = 0.22).

Conclusions: The present study suggests that a novel mutation within the IRRDR region of NS5A protein is associated with slower progression of HCV-related liver fibrosis of LT-recipients. A trend was found for a specific core mutation previously associated with HCV-pathogenesis. These preliminary results could be relevant to individualize treatment schedules in this clinical scenario, although need to be confirmed in larger studies.

167 DONOR/RECIPIENT SEVEN GENE SIGNATURE (CIRRHOSIS RISK SCORE) AND DONOR AGE AS PREDICTORS OF LIVER FIBROSIS PROGRESSION DUE TO HEPATITIS C RECURRENT AFTER LIVER TRANSPLANTATION

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Background and Aim: A seven gene signature of the recipient was recently investigated to calculate a cirrhosis risk score (CRS) that predicts liver fibrosis progression and the probability of developing cirrhosis in transplanted patients with recurrent hepatitis C. The aim of this study was to evaluate the utility of both donor and recipient CRS calculation in predicting the severity of liver fibrosis progression due to recurrent hepatitis C after liver transplantation (LT).

Methods: Fifty-four consecutive HCV positive liver transplanted recipients recruited in our Centre (41 males, median age 53 years), with at least one year of follow-up, were included in the study. Thirty-one were treated for recurrent hepatitis C with peginterferon and ribavirin and 11 of them achieved sustained viral response. In each patient, the amount of liver fibrosis, calculated by the Ishak staging score, was assessed in annual per protocol or on demand liver biopsies. Donor and recipient CRS was calculated using the Naive-Bayes formula. The seven polymorphisms contributing to the CRS signature were: AZIN1, TLR4, TRMPS, APO2, AP3S2, STXB5PL and DEGS1.

Results: During the median follow-up of 83 months (range 18–172), 22 (40.7%) patients reached an Ishak staging ≥3 and 11 (20.4%) developed cirrhosis (Ishak staging ≥5). Patients with donor age ≥45 years were found to reach more frequently an Ishak staging ≥3 (18/29 Vs 4/25, p<0.001) and ≥5 (10/29 Vs 1/25, p=0.006). Three groups were identified:

a. those with donor age <45 years and one or both CRS scores ≤0.55 (N=14),
b. those with donor age <45 years and both CRS scores >0.55 (N=11),
c. those with donor age ≥45 years independently from CRS scores (N=29).

Patients reached staging scores ≥3 and ≥5 with increasing frequencies from groups a) to b) to c): score ≥3 (14.3%, 18.2%, 62.1%, p = 0.001), score ≥5 (0.0%, 9.1%, 34.5%, p = 0.006). These findings were confirmed at time to event analysis: Ishak score ≥3 p = 0.002; Ishak score ≥5 p = 0.011.

Conclusions: Calculation of CRS score, in conjunction with the donor age, could be a useful tool in predicting less severe graft fibrosis progression due to recurrent hepatitis C after LT.
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### 168
**HIGH RATES OF UNDETECTABLE HCV RNA IN LIVER TRANSPLANT RECIPIENTS TREATED WITH PROTEASE INHIBITOR-BASED ANTIVIRAL THERAPY FOR RECURRENT HEPATITIS C**

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**Introduction and Aim:** Hepatitis C (HCV) related liver disease and hepatoma continue as the leading indication for liver transplantation (LT). Recurrent HCV is universal; response to PEG-IFN/Ribavirin (P/R) therapy in Genotype 1 patients has been disappointing. Experience with triple therapy using protease inhibitors (PI; Boceprevir (BOC), Telaprevir (TPV)) in LT recipients is limited. This report summarizes the results in 20 patients treated for recurrent HCV using triple therapy.

**Patients:** 20 patients (18 male, mean age 59y) were treated for G1 HCV (13 G1a) with either BOC (14 pts) or TPV (6 pts) at a mean of 45mo. post LT. 5 pts were treatment naïve; 15 were non responders or relapsers to treatment with P/R either prior to (8) or after (7). 16 pts (12 BOC, 4 TPV) had a P/R lead-in of 4–20wks. 17 patients were on cyclosporine, 2 patients on tacrolimus, and one on mycophenolate only.

**Results:** Of the 16 pts with a lead-in phase, 4 had undetectable HCV RNA at time of BOC or TPV treatment. One patient has not yet had RNA measured while receiving PI; 15 of 15 with HCV RNA available on PI are undetectable, along with all 4 pts who received TPV with no lead-in. Mean cyclosporine reduction was 2/3, mean ribavirin dose reduction was 50%. 15/20 patients received erythropoietin; half have required transfusions. TPV treatment was ended early in 3 pts: one for intractable vomiting, one for infection, and one for acute pancreatitis; P/R were also stopped in the latter 2. BOC was stopped early in one patient due to intolerance and in another (along with P/R) for acute pancreatitis. No patient has completed treatment to date. One patient developed acute rejection while on treatment; this was treated with steroids without treatment interruption.

**Summary:** Triple therapy achieved undetectable HCV RNA in all 19 evaluable patients; the major side effect has been anemia. Two patients (both on cyclosporine, one on TPV, one on BOC) developed severe acute pancreatitis requiring treatment cessation.

**Conclusions:** Antiviral therapy including PI appears significantly more effective than dual therapy in reducing HCV RNA; however, significant ribavirin dose reductions and anemia are observed.

### 169
**EARLY EVALUATION OF HISTOLOGICAL MARKERS OF FIBROSIS AND CELLULAR PROLIFERATION IN LIVER TRANSPLANT PATIENTS WITH HCV RECURRENCE**

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**Background and Aims:** Liver fibrosis is the pathophysiological consequence of a chronic liver injury (HCV) and it is accelerated in immunosuppressed patients. There are two main patterns of fibrosis progression after liver transplantation (LT): rapid fibrosers (RF, fibrosis ≥F2 and hepatic venous pressure gradient (HVPG)≥6 mmHg 1 year after LT) and slow fibrosers (SF, F0–F1 and/or HVPG<6 mmHg). HCV recurrence after LT is characterized by inflammation, hepatocellular necrosis and hepatic stellate cell (HSC) activation. No biomarker has been able to identify patients with a rapid fibrosis rate at a very early stage (3 months) after LT. Our objective is to evaluate whether immunohistochemical markers of liver fibrosis in 3-month liver biopsies are able to identify RF.

**Methods:** Forty-seven patients with HCV recurrence after LT with an early liver biopsy (3 months) were included. Collagen-III (fibrosis marker), alpha-SMA (HSC activation marker) and Ki67 (cellular proliferation marker) were evaluated by immunohistochemistry and by two independent observers. Collagen-III and alpha-SMA were evaluated semi-quantitatively in the portal tract and lobule, respectively. Hepatocyte proliferation was evaluated by the number of Ki67-positive nuclear in the sample.

**Results:** Collagen-III was tested in 47 liver biopsies (32 RF and 15 SF). Agreement between observers was good (kappa=0.61). Of the 16 pts with a lead-in phase, 4 had undetectable HCV RNA at time of BOC or TPV treatment. One patient has not yet had RNA measured while receiving PI; 15 of 15 with HCV RNA available on PI are undetectable, along with all 4 pts who received TPV with no lead-in. Mean cyclosporine reduction was 2/3, mean ribavirin dose reduction was 50%. 15/20 patients received erythropoietin; half have required transfusions. TPV treatment was ended early in 3 pts: one for intractable vomiting, one for infection, and one for acute pancreatitis; P/R were also stopped in the latter 2. BOC was stopped early in one patient due to intolerance and in another (along with P/R) for acute pancreatitis. No patient has completed treatment to date. One patient developed acute rejection while on treatment; this was treated with steroids without treatment interruption.

**Conclusion:** The evaluation of immunohistochemical markers in liver samples can be useful for the early identification of patients with an accelerated course of hepatitis C after LT, by reflecting the initial fibrogenic and tissue repair processes in the graft.

### 170
**IMPACT OF ADJUVANT CHEMOTHERAPY FOR HEPATOCELLULAR CARCINOMA BEYOND MILAN ON HEPATITIS C RECURRENT AFTER LIVER TRANSPLANTATION**

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**Background and Aim:** Hepatitis C virus (HCV) recurrence (HCV-R) is the most frequent cause of graft failure when HCV is active before liver transplantation (LT). HCV-patients, transplanted for a hepatocellular carcinoma (HCC), are also exposed to HCC recurrence (HCC-R), mainly when the native liver shows bad prognosis criteria (tumour size>50 mm, tumour number>3, microvascular or macrovascular invasion). Adjuvant-chemotherapy (ACT) is sometimes used in this context to prevent HCC-R but this impact on HCV-R is currently unknown.

**Patients and Methods:** This monocentric retrospective study compared the prognosis of patients with (ACT-group, n=34) or without (No-ACT-group, n=66) ACT in terms of HCV-R, defined by a METAVIR score A≥1 and F≥1 on graft biopsy. Between 1987 to 2011, 100 LT-patients for HCV and HCC were included (men: 81%; age: 7.1 years). The meantime follow-up was 64.7 months.

**Results:** Overall survival was 98%, 77% and 50% at 1, 2 and 5 years (p=ns). ACT-patients experienced a higher rate of HCC-R, 33% in ACT-group vs. 2.9% in the No-ACT-group (p<0.0001). It was the most frequent cause of death in ACT-group (62.9% vs. 11.7%, p=0.011). Both groups were comparable for known predictors of HCV-R. The HCV-R-free survival at 1 year was 62% in ACT-group vs. 56% in No-ACT (p=ns). The mean fibrosis was 1.2±0.4 vs. 1.3±0.5 (p=0.56) at 1 year and 1.2±0.4 vs. 1.3±0.5 (p=0.48) at 2 years in ACT-group and in No-ACT-group, respectively. In multivariate analysis, predictive factors of HCV-R were the female gender (OR=5.2; p<0.01) and the use of mycophenolate.
mofetil (OR=7.22; \(p<0.01\)), at 1 and 2 years post-LT. Domino-LT was the only protective factor of HCV-R at 2 years post-LT (OR=6.23; \(p<0.01\)). Premature ACT-discontinuation due to side effects occurred in 78.4% of patients (abnormal liver-tests: 38.7%, hematological toxicity: 6.5%, severe infections: 12.9%). Biopsy proven acute-rejection occurred in 52.9% patients in the ACT-group vs. 21.2% in the No-ACT-group (\(p=0.002\)).

**In conclusion**, ACT does not seem to impact on HCV-R in LT-patients. Tolerance is a limiting factor of ACT. Despite poor prognosis criteria of HCC-R in the ACT-group, OS rates are similar compared to the No-ACT-group.

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**AN ASSESSMENT OF CORONARY ARTERY CALCIFICATION (CAC) IN ORTHOTOPIC LIVER TRANSPLANT (OLT) PATIENTS**

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**Background:** Cardiovascular disease is increasing worldwide. For patients being assessed for OLT, it is imperative to accurately stratify cardiovascular (CV) risk, to allow them to receive the maximal benefit from transplantation. Coronary artery calcification (CAC) is a novel and independent predictor of cardiovascular risk, the prevalence of which has been previously described in patients with end stage liver disease (ESLD). We have recently demonstrated CAC as an independent risk factor for cardiac events post OLT. The correlation of CAC with classical and novel CV risk factors in the OLT group is currently unclear.

**Aim:** To determine the relationship of CAC with classical and novel CV markers in patients with ESLD undergoing OLT assessment.

**Methods:** Single centre, prospective observation study. CAC scores were derived by the Agatson method from thoracic CT scans with patients followed up from time of assessment.

**Results:** 199 patients recruited (125M: 74F) with mean age 55.12 years (range 23–70yrs). 121 patients were listed for OLT with 97 undergoing OLT. Median follow up of 41.28 months. CAC was performed in 177 patients with mean CAC of 381.84 (range 0–1017). A significant correlation was identified between the CAC score and age (\(p=0.000\)), gender (\(p=0.001\)), fasting gluc (\(p=0.018\)), Cystatin C 0.029, Framingham risk score (\(p=0.000\)), PR interval (\(p=0.005\)) and number of vessels involved (\(p=0.000\)). The mean CAC scores in ALD appeared higher than in non-ALD aetiology; at 608 vs 238 respectively.

**Conclusion:** This study confirms the high prevalence of occult coronary artery disease in OLT assessment patients and identifies a relationship between CAC scores and age, gender, fasting glucose, Cystatin C, Framingham risk score and number of vessels involved but no relationship seen with other classical CV risk factors.

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**RISK FACTORS AND MANAGEMENT OF BILIARY TRACT COMPLICATIONS POST LIVER TRANSPLANTATION**

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**Background and Aim:** Biliary complications (BCs) after liver transplantation (LT) remain a major source of morbidity and mortality. Our aim was to focus on this complication in a tertiary referral academic LT center.

**Methods:** Between 2005 and 2006, all LT recipients in the Hannover Medical School were prospectively followed up till end of 2009 (pediatric and re-LT were excluded). Comparison between patients with and without BCs in the collected pre-, peri- and post-LT parameters was achieved. The management modalities and outcome of BCs in addition to the microbiological analysis of the collected bile during intervention were documented.

**Results:** The cohort included 179 LT recipients, mainly due to viral hepatitis (30%), alcoholic liver disease (20%), HCC (20%) and PSC (14%). From all patients, 32% developed 89 different BCs, mainly biliary strictures (57%), biliary stones/casts (25%) and biliary leak (17%).

**Risk factors and consequences of BCs:** Multi-variate analysis revealed that LT due to AIH, ascites pre-LT, renal dialysis pre-LT, and longer CIT and operation time are independent risk factors for BCs. Older recipient or donor and CMV hepatitis post LT were special risk factors for ITBL. The development of BCs was associated with significantly shorter survival (\(p=0.029\)) and higher mortality (\(p=0.012\)).

**Cholangiographic intervention:** ERCP (in 84%) or PTC (in 9%) was the main management tool especially in anastomotic strictures, choledocholithiasis and biliary leak, whereas the surgical repair (in 7%) was needed in severe complications. ERCP presentations (\(n=243\)) were associated with minor post-procedural complications mainly cholangitis (5.8%), pancreatitis (2.5%) and bleeding (1.6%).

**Microbiological analysis of bile:** 97% of samples showed positive bacterial growth with total of 229 organisms representing 53 different species, mainly Gram positive organisms (48%), gram negative organisms (29%), fungal growths (14%) and anaerobes (8%).

**Conclusion:** BCs affect more than 1/3 of the LT recipients and associated with shorter survival and higher mortality. The advanced the hepato-renal condition prior LT and the longer CIT and operation time are risks for BCs. ERCP is successful in the management with minor post interventional complications. Positive bacterial growth in bile was detected in almost all patients presented for the management of the BCs.

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**ATTITUDES OF PATIENTS, CARERS, CLINICIANS AND THE PUBLIC TO ACCESS TO LIVER TRANSPLANTATION IN THE UK**

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The number of patients who need liver transplantation greatly exceeds the number of donor organs available. As a consequence available organs must be rationed. In the UK, patients must be referred to a transplant centre, and once referred, undergo an assessment process prior to be added to the waiting list. This system limits access to the waiting list but still lists more people than there are organs available. There are no data to describe preferences of patients, carers, clinicians or the public regarding access to liver transplant. This study was designed to assess the opinions of these groups.

A questionnaire was designed explaining the current system in the UK and two theoretical extremes of position:

- listing all patients who would benefit from a transplant
- listing only as many people as there are organs available

Potential advantages and disadvantages of each position were detailed. This questionnaire was circulated to patient groups, clinicians and members of the public. Participants were asked to state their preference for either of these approaches, or the current system. Ethics approval was obtained.
A total of 307 people completed the questionnaire: 47 patients, 17 carers, 62 clinicians and 181 public respondents. Overall there was a preference for maintaining the status quo: 52.1% stated a preference for the current system, 38.4% for an unrestricted listing policy and 9.4% for a highly restricted policy. However, preferences differed between groups: an less restrictive listing policy was preferred by the majority of carers and also public respondents, whereas a majority of patients and clinicians preferred the current listing policy (Figure 1). However, there were differences between patient subgroups, with those who had been listed being more inclined to favour the current process.

The results show for the first time that there is general support amongst relevant groups for a process of selection for liver transplantation. There are some differences in opinion between groups. It is important that the views of these groups are considered when formulating transplant policy. The general support for selection of liver transplant candidates mandates a transparent and rational selection policy.

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**SYSTEMATIC REVIEW AND META-ANALYSIS OF THE EFFECT OF PRE-TRANSPLANT DIABETES MELLITUS ON 5-YEAR MORTALITY AFTER LIVER TRANSPLANTATION**

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Selection of candidates for liver transplantation depends on identification and assessment of risk factors that may affect post-transplant survival. Diabetes has been identified as a risk factor for selection of candidates for liver transplantation mandates a transparent and rational selection policy.

**Methods:** PubMed, Embase and Google Scholar were searched with the terms: Diabetes Liver Transplant (Outcome or Mortality or Survival). Papers that appeared suitable were screened further. We also examined references cited in relevant papers. Papers were included if they reported the effect of pre-transplant diabetes on long-term survival post-liver transplant.

**Results:** Initial searches yielded 368 titles. Of these, 18 appeared relevant and 9 were included. Meta-analysis of these papers showed that diabetes increased risk of 5-year mortality after liver transplant: RR 1.34 (95% CI 1.10–1.63, p = 0.003) (Figure 1). There was considerable heterogeneity between studies (Chi² 39.12, I² = 80%). Most studies did not report differences between subgroups, e.g. type 1/type 2 diabetes, insulin treated/non-insulin treated, and therefore the outcome of these subgroups could not be evaluated.

**Conclusion:** Presence of pre-transplant diabetes slightly increases risk of long term mortality after liver transplant. There are insufficient data to confidently assess effects of disease subtypes or treatment of diabetes on transplant outcomes.

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**CEREBRAL MYO-INOSITOL DECREASE BEFORE LIVER TRANSPLANTATION INDICATES HIGHER RISK FOR POST-TRANSPLANT ENCEPHALOPATHY**

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**Background and Aims:** About 30% of liver transplant patients develop post-transplant-encephalopathy (PTE) after surgery. Symptoms comprise disorientation, hallucinations, cognitive deficits and seizures. The cause of PTE is unclear, however, cerebral osmolyte changes in patients with liver cirrhosis are known. We hypothesise that patients with liver cirrhosis and cerebral osmolyte changes have a higher risk to develop PTE after liver transplantation (LTx).

**Methods:** 63 patients with liver cirrhosis underwent neurological examination, magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). The examination included diffusion sequences and MRS of glutamine/glutamate (Glx)-, myo-inositol-, choline-, creatine- and N-acetyl-aspartate concentrations. The spectroscopy was carried out in the thalamus, nucleus lentiformis and white matter, the diffusion sequences additionally in the rostrum and splenium of the corpus callosum. 20 patients received liver transplantation in the meantime and were neurologically evaluated at defined time points (day 1, 7 and 90). The control group consists of 20 healthy age matched controls.

**Results:** After LTx 8 of 20 patients (40%) developed PTE and 8 patients (40%) showed no neurological symptoms. 4 (20%) patients were excluded because 2 patients died directly after LTx and 2 patients had incomplete MRS. MRS before LTx showed significantly higher Glx concentrations (p < 0.05) in the white matter and nucleus lentiformis as well as significantly decreased myo-inositol concentrations (p < 0.05) in the thalamus and white matter in patients with PTE than in patients without PTE and controls. Additionally before LTx patients with PTE had significantly higher diffusion values (p < 0.05) in the nucleus lentiformis compared to patients without PTE and controls and significantly lower diffusion values (p < 0.05) in the splenium of the corpus callosum compared to patients without PTE. Choline, creatine and N-acetyl-aspartate showed no significant group differences.

**Conclusions:** Alterations of cerebral osmolytes and water homeostasis in patients with liver cirrhosis are an indicator for a higher risk to develop PTE after LTx. Causal factors could be accentuation of osmolyte changes under steroid or calcineurin-inhibitor therapy, extreme electrolyte shifting or disorder of the cerebral water balance during or after surgery in pre-existing cerebral damage. The exact pathomechanism remains to be clarified.

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**Figure 1. Forrest plot.**
176 PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PILOT STUDY EVALUATING EFFICACY AND SAFETY OF INTRAVENOUS SILIBININ IN PATIENTS WITH HCV RECURRENCE ON THE GRAFT AFTER LIVER TRANSPLANTATION

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Background: The recurrence of HCV hepatitis after LT leads to graft loss and death and the response to the currently standard antiviral treatment is still unsatisfactory. Preliminary data on triple therapy with HCV protease inhibitors (PI) in these patients (pts) showed an improved-on-treatment virological response. However, major safety concerns aroused in clinical practice with triple therapy in transplant setting.

Aims: To assess safety and efficacy of 14 days intravenous silibinin (ivSIL) monotherapy in pts with HCV recurrence after liver transplantation (LT), non-responders to standard therapy (NR).

Methods: Twenty NR pts were randomly assigned 3:1 to receive 20 mg/Kg/day of ivSIL (4-hr daily infusion) or placebo for 14 days. Efficacy analyses were performed in intention-to-treat on all randomized pts. Mean age of pts: 59.1yrs, 82% genotype 1 and 18/20 men. IL-28b polymorphisms: C/C, C/T and T/T in 14%, 57% and 28% respectively. Mean (±SD) interval between LT and therapy was 89±72.2 months (range13–195). Pt were comparable in fibrosis stage and type of immunosuppressant used. The average baseline viral load (VL, as HCV-RNA log IU/ml) was 6.34±0.58 and 6.32±0.47 in patients treated with ivSIL and placebo, respectively.

Results: On day 14 of treatment VL decreased by 2.30±1.31 vs 0.6±0.6 log10 (p=0.0002 ANOVA) in the ivSIL and placebo group respectively. At the end of treatment VL was ≥2 log lower than baseline in 60% (9/15) pts of ivSIL group versus 0% in the placebo group (p=0.03, Fisher exact test). VL returned to levels similar to baseline in both groups (6.11±0.72 vs 6.23±0.40), in the 2 weeks following drug withdrawal. Treatment resulted well tolerated apart from transient feeling hot and diarrhoea. Notably, nor changes in immunosuppressant through levels neither dosage adjustments were necessary Treatment discontinuation was necessary in only 1 patients at day 11 for biliary gallstones not related to study drug.

Conclusions: This proof-of-concept randomized, double blind, placebo-controlled study shows that ivSIL monotherapy in patients with stable HCV recurrent hepatitis after liver transplantation is able in inducing a clinically relevant decrease in viral load and is safe. Thus, further studies focusing on prolonged and/or combined treatment are warranted.

177 EVALUATION OF PHYSICIAN AGREEMENT FOR DEFINING “CLINICAL SUSPICION OF REJECTION” IN LIVER TRANSPLANTATION

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Background and Aims: Biopsy-proven acute rejection (ACR) is currently used as primary endpoint in most randomized trials evaluating immunosuppression after liver transplantation (LT). However there is no published consensus regarding selection of patients to liver biopsy, nor concerning the definition of clinical ACR. We aimed to determine the clinical agreement in selecting patients to liver biopsy early after LT to diagnose ACR.

Methods: 100 LT patients at the Royal Free Hospital (1997–2007) were randomly selected from a protocol biopsy population, in which the biopsy was taken at day 7–10 post-LT to diagnose ACR. The clinical information between LT and protocol biopsy (demographic features, liver disease, MELD, daily liver function tests, and immunosuppression regimen, including dosage and trough concentrations), was given to 9 clinicians from 3 European transplant centres who had to decide between: liver biopsy, treat empirically with boluses of steroids or “wait and see”. Clinical agreement among clinicians and with histology was evaluated by kappa coefficient.

Results: There was no histological ACR in 21 patients (21%), mild ACR in 37 patients (37%) and moderate-severe histological ACR in 42 patients (42%). The agreement among clinicians to select candidates for liver biopsy was poor: k=0.06–0.62, with k<0.40 in 76% of comparisons (table). The concordance between indication for liver biopsy or empirical treatment, and moderate-severe ACR in protocol biopsy was β<0.30 in all cases (figure, last row). According to this, 28%–71% of patients with moderate-severe ACR would have not been diagnosed. Conversely, 15%–43% of patients with no or mild ACR would have undergone a liver biopsy, and up to 24% would have received empirical treatment with boluses of steroids.

Conclusion: The agreement concerning parameters which indicate a clinical suspicion of ACR is very poor between clinicians. This impacts on clinical practice and on the design of randomized controlled trials, especially multicentre and open label trials in which indication to biopsy is never defined. Thus there is a need to have clinical consensus for a definition of ACR after liver transplantation, and to search for new biomarkers to substitute biopsy.

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Histology

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Notice: each hepatologist is named with his/her hospital’s initial and a number ranging from 1 to 3.

RFH: Royal Free Hospital. London (UK).
PUH: Padova University Hospital. Padova (Italy).
HURS: Reina Sofia University Hospital. Córdoba (Spain).

178 IMMUNOSUPPRESSION AND HEPATOCELLULAR CARCINOMA RECURRENCE AFTER LIVER TRANSPLANTATION

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Background and Aims: Evidence from animal models suggests that immunosuppression after liver transplantation (LT) facilitates
hepatocellular carcinoma (HCC) recurrence. We aimed to identify aspects of immunosuppression impacting on HCC recurrence in a multicentre cohort of LT patients.

**Methods:** 219 consecutive patients with HCC under Milan criteria receiving a LT in two European centres (Royal Free Hospital, n = 103; Reina Sofia University Hospital, n = 116) between 2000 and 2010 were included. Exclusion criteria were: HIV positive, multiorgan transplantation, or surviving less than 1 month after LT. Immunosuppression protocol within the first month was evaluated with respect to HCC recurrence using Kaplan–Meier curves and multiple Cox regression.

**Results:** Major causes of cirrhosis were HCV (n = 106; 48.4%), alcohol (n = 81; 37%) and HBV (n = 52; 23.7%). In explanted liver the number of nodules was 2.5 ± 3.6, and size of the biggest nodule was 3 ± 2.1 cm. Macrovascular invasion was detected in 11 patients (5%), and microvascular invasion in 41 patients (18.7%). After a median follow-up post-LT of 51 months (IQR 26–93), HCC recurrence rates were 13.3% at 3 years and 17.6% at 5 years. The use/non-use of steroids and antimetabolites did not influence HCC recurrence (p = 0.69 and p = 0.70 respectively). There were similar rates of HCC recurrence with tacrolimus or cyclosporine (p = 0.24). Patients with higher exposure to calcineurin inhibitors (mean tacrolimus trough concentrations >10 ng/mL or cyclosporine trough concentrations >300 ng/mL within the first month after LT) had increased risk of HCC recurrence (27.7% vs 14.7% at 5 years; p = 0.007). In the multivariate analysis (table), high exposure to calcineurin inhibitors defined as above, was an independent predictive factor for HCC recurrence (RR=3.1, p = 0.002). Total tumour volume, microvascular invasion and macrovascular invasion in the explanted liver were also independent factors of HCC recurrence (p < 0.001, p = 0.004 and p = 0.03 respectively) (see table).

**Conclusion:** The exposure to calcineurin inhibitors within the first month after LT is an independent predictive factor for HCC recurrence. Immunosuppression protocols with reduced tacrolimus/cyclosporine should be prefered for LT patients with HCC.

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<td>Total tumour volume in the explanted liver</td>
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<td>Microvascular invasion</td>
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<td>Macrovascular invasion</td>
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†Mean trough levels of tacrolimus >10 ng/mL or cyclosporin >300 ng/mL within the first month after liver transplantation.

Variables eliminated from the model: viral cirrhosis (p = 0.96); local treatment of HCC in waiting list (p = 0.52); AFP (0.91); histological tumour differentiation (p = 0.22); type of calcineurin inhibitor used (p = 0.58).

179 RECURRENT HEPATOCELLULAR CARCINOMA IS THE PRIMARY CAUSE OF GRAFT YEARS LOST AFTER LIVER TRANSPLANTATION

**Background and Aims:** Due to a shortage of donor organs patients are selected for liver transplantation based on patient need and the likelihood lasting benefit. Hepatocellular carcinoma (HCC) is a frequent indication for liver transplantation and patients are highly selected to limit the risk of cancer recurrence. However, recurrence remains a significant issue and the aim of this study was to determine the impact of recurrent HCC on graft years lost after transplantation.

**Methods:** The causes and timing of graft loss in all adult first graft recipients surviving at least one year after transplantation were collected prospectively. Graft loss was defined as patient death or liver retransplantation. Published estimates of median survival after liver transplantation and data on the causes of graft loss after transplantation were used to calculate graft years lost in hypothetical 1000 patient cohorts surviving at least one year after transplantation.

**Results:** The estimated median survival after transplantation in the UK is 22 years (Barber et al Gut 2007). The most frequent causes of graft loss were: cardiovascular disease (17% of all grafts lost), recurrent non-malignant disease (16%), recurrent HCC (15%), de novo cancer (11%), and renal failure (7%). The median time from transplantation to graft loss was shortest for recurrent HCC (3.6 years), and recurrent non-malignant disease (5.3 years), and longest for cardiovascular disease (10.4 years). Using these data we calculated graft years lost for each of these causes. The greatest number of graft years lost was in patients with recurrent HCC (1380 graft years lost per 1000 patients), and recurrent non-malignant disease (1340). The number of graft years lost was significantly less in those with cardiovascular disease (985), and renal failure (487).

**Conclusions:** Recurrent HCC is the greatest single contributor to graft years lost after liver transplantation. HCC as an indication for liver transplantation is increasing in frequency in Europe and these data indicate that using current selection criteria the number of graft years lost to recurrent HCC will also increase. There is a need for more accurate selection systems to maximise the benefit of transplantation in this group.

180 EVEROLIMUS-BASED IMMUNOSUPPRESSION IN HCV POSITIVE DE NOVO LIVER TRANSPLANT RECIPIENTS: 24-MONTH RESULTS OF A RANDOMIZED CONTROLLED TRIAL

**Background and Aims:** HCV recurrence in liver transplant recipients (LTxR) often leads to an accelerated fibrosis progression under current immunosuppressive regimens. The 12-month (M) results of the H2304 study (NCT00622869) showed a tendency of lower fibrosis progression in HCV+ LTxR with everolimus-facilitated tacrolimus reduction (EVR+rTAC) as compared to standard TAC (TAC-C) regimen. We present here the M24 results for the HCV+ population.

**Methods:** In this 24M, multicenter, open-label study, de novo LTxR were randomized (1:1:1) on day 30 to receive EVR (C0 6–10 ng/mL; EVR+rTAC, n = 245) or EVR with TAC withdrawal (TAC-WD, n = 231) at M4 or TAC-C (C0 3–8 ng/mL; EVR+rTAC, n = 245) or EVR with TAC withdrawal (TAC-WD, n = 243) all with steroids. Composite efficacy failure (treated BPAR, graft loss, or death) and renal function (eGFR, MDRD4) were assessed at M24 for the overall and HCV+ population. Fibrosis progression (Ishak-Knodell score; IKS) and changes in HCV viral load were evaluated in the HCV+ patients. Comparison of EVR+rTAC vs. TAC-C is presented as enrollment into TAC-WD arm was stopped early due to efficacy failure.

**Results:** As for the overall population, the HCV+ population, EVR+rTAC (n = 79) had a comparable composite efficacy failure rate (10.6% vs. 11.6%; difference: −1.0% [95% CI: −11.3%, 9.3%]), and higher eGFR (adjusted mean difference: 5.11 ml/min/1.73m² [97.5% CI: 4.59, 14.82]; p = 0.236) vs. TAC-C (n = 76) at M24. The mean change...
in fibrosis from baseline to M24 was 0.2 (±1.86) for EVR+rTAC vs. 0.6 (±2.04) for TAC-C. Fewer patients experienced fibrosis progression of ≥1 IKS stage with EVR+rTAC vs. TAC-C (14/29 (48.3%) vs. 22/35 (62.9%), OR 0.37, 95% CI 0.12, 1.16, p = 0.0866). The mean viral load (log10 transformed) was comparable with EVR+rTAC vs. TAC-C at randomization and at M24 (6.1 vs. 5.8 and 6.1 vs. 6.0, respectively).

Safety profile in HCV+ patients was comparable to the overall population. Eleven deaths were reported in HCV+ patients, four in the EVR+rTAC and seven in the TAC-C group.

Conclusions: The M24 results confirm that early EVR-facilitated TAC minimization in HCV+ patients provides similar efficacy and safety to the overall population. Viral load was comparable in both groups; whereas proportion of HCV+ patients with fibrosis progression tended to be lower with EVR+rTAC.

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MMP-2 CT/TT GENOTYPE IS A RISK FACTOR FOR PATIENT MORTALITY OR OVERTHE ORTHOTOPIC LIVER TRANSPLANTATION IN PRIMARY SCLEROSING CHOLANGITIS
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Background: Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of the bile ducts, resulting in fibrotic strictures frequently necessitating orthotopic liver transplantation (OLT), often accompanied by ulcerative colitis (UC). Matrix metalloproteinases (MMPs) play a role in many fibrotic diseases due to their involvement in connective tissue remodeling related to cancer, inflammatory diseases and complications after OLT.

Aim: Evaluate MMP-2 and MMP-9 gene polymorphisms within a group of PSC patients in relation to disease severity as evaluated by death or need for OLT.

Methods: For this study, 132 PSC patients were included from two liver transplantation centers. Follow-up was from initial onset of PSC until OLT, death or end of follow-up. From these patients genomic DNA was extracted routinely from peripheral blood and/or tissue samples. MMP-2 (−1306 C/T) and MMP-9 (−1562 C/T) gene promoter polymorphisms were analyzed using high-resolution DNA melting analysis (HRMA) or by PCR followed by restriction length polymorphisms (RFLP) respectively. Demographical and clinicopathological variables such as OLT, time of OLT, age, gender, type of IBD and survival were analyzed.

Results: Of the PSC patients 88 (66.7%) were male. Sixty (45.5%) PSC patients underwent OLT with a mean follow-up from PSC onset to OLT of 7.2 years (range 0.4-21 years). Mean age at OLT was 46 years (range 18.3-67.7 years). Twenty-years cumulative incidence of death or OLT in the CT/TT group was 92.7% compared to 53.0% in the wild-type group (CC) (p = 0.02) and reached 93.2% when MMP-2 CT/TT genotype was accompanied by ulcerative colitis (UC) compared to 51.2% with UC and wild-type MMP-2 (p<0.01). Age at onset of PSC (aHR=1.03; 95%CI 1.01–1.05) and MMP-2 CT/TT genotype were independent risk factors for OLT or death (aHR=1.97; 95%CI 1.0–3.32), both p<0.001. In contrast, no significant association was found between MMP-9 genotype and the risk of OLT in PSC patients.

Conclusion: MMP-2 CT/TT genotype in PSC is a significant independent risk factor for disease severity as reflected by patient mortality or need for OLT and identifies, therefore, this polymorphism as a disease modifying gene.

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SEVERE ISCHEMIA–REPERFUSION INJURY DEFINED BY PEAK ALANINE AMINOTRANSFERASE IS A RISK FACTOR FOR NONANASTOMOTIC BILIARY STRICTURES AFTER ORTHOTOPIC LIVER TRANSPLANTATION WITH DONATION AFTER CARDIAC DEATH
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Introduction: Nonanastomotic biliary strictures (NAS) frequently develop after orthotopic liver transplantation (OLT) with donation after cardiac death (DCD). DCD livers are more prone to ischemia-reperfusion injury (IRI) and biliary complications such as NAS than livers from donation after brain death (DBD).

Aim: Evaluation of peak aspartate aminotransferase (AST) and peak alanine aminotransferase (ALT) as parameters of IRI post-OLT as potential predictors for the development of NAS in livers from DCD and DBD donors.

Methods: All first OLTs from a single center were included. IRI was defined using peak AST or peak ALT evaluated at day 0 to day 7 post-OLT. A peak AST and ALT of >1500 IU/L was defined as mild IRI whereas a peak AST or ALT >1500 IU/L was considered as severe IRI. NAS was considered as any treated stricture of the intra- or extrahepatic bile ducts at least 1 cm above the anastomosis occurring within four years post-OLT.

Results: A total 299 OLTs were performed using 42 DCD donors and 257 DBD donors. Median recipient age at OLT was 58 years with a mean MELD score of 18. After DCD-OLT, NAS developed in 38.1% versus 17.7% in DBD-OLT (p <0.01). Four-year cumulative incidence of NAS after DCD-OLT with severe IRI based on peak ALT >1500 IU/L was 72.2% compared to 11.8% with mild IRI (p <0.01); after DBD-OLT this was 15.3% versus 12.6% (p = 0.45). Multivariate analysis showed severe IRI, as evaluated by peak ALT >1500 IU/L, to be an independent significant risk factor for development of NAS post DCD-OLT adjusted for cold ischemic time, recipient warm ischemic time, donor warm ischemic time, primary sclerosing cholangitis as indication for OLT, recipient gender and recipient age (peak ALT >1500 IU/L aHR=8.82, 95% CI 1.99–38.97, p = 0.004). No association was found for peak AST and NAS in DCD-OLT or DBD-OLT.

Conclusion: Severe IR-injury as evaluated by peak ALT >1500 IU/L is a significant independent risk factor for the development of NAS after DCD-OLT. Peak ALT could thus be used as a predictive marker for NAS in DCD-OLT.

Figure: Peak AST and NAS in DCD donors.
Conclusion: rPSC is common after LT for PSC, and is associated with worse graft survival. The risk is modified by immunosuppressive agents used, but unrelated to pre-transplant colectomy.

Risk factors RR 95%CI P
Steroid-resistant ACR 1.94 0.50–6.26 0.31
Tac-based (v. CsA-based) 0.61 0.08–3.24 0.58
Mycophenolate (Y:N) 3.25 1.31–9.10 0.010
Intact Colon at LT (Y:N) 1.43 0.64–3.36 0.39
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THE PROTECT STUDY: EVEROLIMUS MONOTHERAPY vs.
CALCINEURIN INHIBITOR-BASED THERAPY RESULTS IN
PROGRESSIVE RENAL FUNCTION BENEFIT OVER 35 MONTHS
IN LIVER TRANSPLANT RECIPIENTS
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Post-transplant immunosuppression with calcineurin inhibitors (CNIs) is associated with impaired renal function. The PROTECT study showed that conversion from CNI to the mTOR-inhibitor everolimus (EVR) at 4 weeks after liver transplantation (LTx) achieves better renal function at 11 months (M) without compromising efficacy. Results of the PROTECT extension period up to 35 M are presented. In this multicenter, prospective, open label study LTx patients with initial good renal function (calculated GFR ≥50mL/min) 4–8 weeks after liver transplantation (LTx) were randomized to either continued CNI treatment (n=96; standard CNI dose tacrolimus or cyclosporine ± steroids) or switch to EVR ± steroids (n=98). EVR was adjusted to target trough level of 5–12ng/mL and CNI was withdrawn stepwise until week 16 post randomization. Patients who completed the 11 month core study were followed up to month 35 in the extension phase.

A total of 81 patients (EVR, n=41; CNI, n=40) continued in the follow-up phase. From M12 to M35 further renal function deterioration was observed in the CNI-arm while renal function remained stable in patients receiving EVR. Difference in eGFR between EVR and CNI: Cockcroft-Gault M11: −6.8mL/min [p=0.240]; M23: −9.8mL/min [p=0.104]; M35: −10.5mL/min [p=0.086] and Nankivell formula (M11: −6.6mL/min [p=0.084]; M23: −8.8 mL/min [p=0.039]; M35: −10.5mL/min [p=0.015]). At M35 there were no significant differences in rates of mortality (EVR: 4.3% vs. CNI: 10.0%, p=0.535), biopsy-proven acute rejection (24.4% vs. 15.8%, p=0.434), and efficacy failure (29.8% vs. 28.2%, p=0.903) were similar. Discontinuation of study treatment due to an AE during the follow-up period was observed in 5 (12.2%) patients on EVR vs. 6 (15.0%) in the CNI group. Conversion from CNI-based to EVR-based immunosuppression proved to be a safe alternative post-LTx and potentially reduces the risk of end-stage renal disease.

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PRETRANSPLANT SERUM ALPHA-FETOPROTEIN HAS NO PROGNOSTIC ROLE IN PATIENTS WITH HBV-ASSOCIATED HEPATOCELLULAR CARCINOMA WITHOUT VASCULAR INVASION
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Background and Aims: Alpha-fetoprotein (AFP) has been proposed to correlate with vascular invasion of hepatocellular carcinoma (HCC) and predict tumor recurrence after liver transplantation (LT). However, the prognostic value of AFP in patients with HCC without vascular invasion during the waiting list for LT has not been clearly defined. In this study, we determined the prognostic role of preoperative AFP in patients who underwent LT for HBV-associated HCC without vascular invasion.

Methods: We analyzed the outcome of 80 patients who underwent LT for HBV-associated HCC without vascular invasion. Vascular invasion was defined as the presence of tumor emboli within the lobar or segmental branches of the portal or hepatic veins, which was diagnosed or highly suspected by preoperative imaging examination. Patients were divided into two groups according to different AFP cut-off level (20 ng/mL, 100 ng/mL, 200 ng/mL, and 400 ng/mL).

Results: The 1-, 3- and 5-year disease-free and overall survivals were 97.1%, 89.1%, and 79.9%, and 92.1%, 81.5%, and 72.7%, respectively. Ten patients developed tumor recurrence and 13 patients died during 6 years of follow-up. Univariate analysis revealed that multiple tumor number was the only preoperative predictor of disease-free survival (DFS). Surprisingly, there was no significant difference in DFS with regard to the tumor size, AFP level, preoperative tumor therapy, histologic grade, Milan criteria, and UCSF criteria. All four patients with tumor size greater than 8 cm had no tumor recurrence during 3 years of follow-up. The 3- and 5-year DFS for patients with AFP ≤400 ng/mL were 86.8%, 82.4%, and 86.8%, 72.4%, respectively (P>0.05). The disease-free and overall survivals were not significantly different among the five AFP classes (≤20 ng/mL; 21–100 ng/mL; 101–200 ng/mL; 201–400 ng/mL; >400 ng/mL).

Conclusions: Preoperative serum AFP level has no prognostic role in patients who underwent liver transplantation for HBV-associated HCC without vascular invasion. Although the accuracy and objectivity of the radiological imaging remains a problem, carefully studying the radiologic imaging is still regarded as a first-line test for selecting appropriate candidates for liver transplantation and predicting tumor recurrence following liver transplantation in patients with HCC.

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PREDICTIVE MODELS AND RISK FACTORS OF KIDNEY FAILURE AFTER LIVER TRANSPLANTATION
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Background and Aims: Kidney failure is one of the most serious complications after liver transplantation (LT) impacting patient survival. So far no established concepts exist to predict glomerular filtration rate (GFR) development in liver recipients. We performed a retrospective analysis in 320 patients after LT and followed their kidney function for 3 years.

Methods: We developed models for predicting chronic kidney disease (CKD) stages at 1 and 3 years after liver transplantation. Models were fitted for covariables available before LT, for peri- and postoperative factors and for immunosuppression. We checked discrimination, calibration and internal validity of the models.

Results: Within the overall patient cohort we detected three subgroups with a distinct pattern of their GFR development. The first group started with a mean GFR of 73 mL/min, developed a significant loss of GFR within the first year (mean GFR 36 mL/min) and did not improve over the next 3 years following LT. A second group started with a mean GFR of 69 mL/min and developed a significant loss of kidney function at year 1 (mean GFR 51 mL/min) but showed a significant improvement of kidney function at year 3 with a mean GFR of 63 mL/min. The third group started with a mean
GFR of 61 ml/min and showed an improvement of kidney function at year 1 (mean GFR 69 ml/min), which was maintained over the 3 years. Comparing baseline characteristics of the respective groups, short cold ischemia time, lower age and short ICU stay were associated with a better development of kidney function. In a multivariable clinical prediction model we found predicted probabilities for GFR very close to observed probabilities for development of CKD 3 or worse. However, prediction of CKD 4 or 5 was less accurate.

Conclusions: Imputing the classical risk factors in a prediction model leads to a good prediction of loss of kidney function, but cannot predict whether a patient will later require dialysis. The recognized patterns further underline that liver recipients are a very heterogeneous group and that even a deterioration of kidney function in the first post transplant year does not predict if it later recovers or not.

188 THE MODE OF DEATH OF THE LIVER DONOR IMPRINTS DISTINCT IMMUNE ADAPTIVE RESPONSES ON THE HEPATIC RESIDENT LYMPHOCYTES

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Background: Experimental liver transplantation studies have shown that brain death in the donor induces a systemic inflammatory response which affects allograft’s quality and increases their immunogenicity. It is currently unclear which systemic events preceding the retrieval of the organ have damaging effects to the graft. We hypothesise that brain death in otherwise hemodynamically stable donors influence the differentiation of immune cells resident in the liver hence, predisposing their response to post-transplantation events.

Aim: To test whether the mode of death of the donor alters the phenotype and function of the adaptive immune cells of liver allografts prior to transplantation.

Methods: Liver resident lymphocytes (LRL) were isolated from liver perfusates obtained before implantation, to assess their phenotype and function after ex vivo polyclonal stimulation by anti-CD3 and anti-CD28 antibodies. We compared the LRL from brain dead donors (DBD n=22), from donors after cardiac death (DCD n=10) and from living donors (LD n=10) as control.

Results: We found that LRL from DCD livers preferentially produce IL-17 (IL-17 positive cells being 4.5% in DCD, 0.9% in DBD and 0.8% in LD, p<0.01). This IL-17 secretion is attributed to CD4 T (mean at 3.3% in DCD, 1.6% in DBD and 1.2% in LD p<0.05) and CD8 T cells (mean at 1.07% in DBD, 2.7% in DCD and 1.2% in LD p<0.05).

In contrast to DCD, LRL from DBD livers were enriched in CD8 T cells which exhibit an activated phenotype (mean of CD8+CD69+ cells at 60% in DBD, 38% in DCD and 37% in LD, p<0.05). Assessment of cytokine production shows a significant increase in IFN-gamma production by CD8 T cells of DBD grafts (mean at 32% in DBD, 15% in DCD, p<0.01 and 18% in LD livers, p<0.05).

Conclusion: Our data show that the inflammatory environment of DBD promotes activation of potentially cytotoxic IFN-gamma producing CD8 T cells, whereas DCD livers stimulate IL-17 secretion from both CD4 and CD8 T cells. They suggest that the mode of death of the donor leads to distinct adaptive immune responses in the graft, which may influence the transplant outcome.

Figure 1. Probability of survival in patients with and without spur cells.

Results: Patients with compared to those without HSCA had more advanced liver disease (higher MELD and MELD-Na, both P<0.001), lower hemoglobin (P=0.024), higher total bilirubin (P<0.0001), INR (P<0.0001) and ferritin levels (P=0.015). There was no difference in age, causes of liver disease, total cholesterol and albumin. Patients with HSCA had a worse survival (log rank P<0.0001). In particular, at the first, second and third year of follow-up, the survival of patients with compared to those without HSCA was 23%, 23% and 19% in the former and 52%, 42% and 41% in the latter, respectively. It
is noteworthy that survival of patients with HSCA at the first, second and third month was 77%, 45% and 33%, respectively. A multivariate analysis showed that age ($P<0.001$), MELD-Na ($P<0.0001$) and presence of HSCA ($P=0.009$) were independent predictive factors of death.

**Conclusions:** HSCA is prevalent in advanced cirrhosis. Mortality in liver cirrhosis seems to be strongly affected by the presence of HSCA.

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**AST-120 (SPHERICAL CARBON ADSORBENT) IN COVERT HEPATIC ENCEPHALOPATHY: RESULTS OF THE ASTUTE TRIAL**

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Covert hepatic encephalopathy (CHE = minimal and/or ≤ grade 1 HE) adversely affects cirrhotic patients, but no standard-of-care is yet established. AST-120 has demonstrated efficient binding capacity for NH$_3$ and other gut-based toxins.

**Aim:** To provide proof-of-concept and safety/tolerability for AST-120 treatment of CHE.

**Methods:** A multi-center, double-blind, randomized, placebo-controlled, dose-ranging study of AST-120 was conducted over 8 weeks. Compensated cirrhotic patients with MELD Score ≤25 were eligible. CHE was defined as a global summary score on Repeatability Battery for the Assessment of Neuropsychological Status (RBANS) below 10th percentile at screening and/or ≤1 HE by West-Haven criteria. The primary endpoint was neurocognitive improvement, defined as change in global RBANS at 8-weeks compared to baseline. Secondary endpoints included Psychometric HE-Score (PHEx), clinical global assessment of HE (CGA-HE), and frequency of occurrences of overt HE and hospitalization. RBANS testing was performed at screening, baseline (+1 week), and 4 and 8-weeks after assigned intervention.

**Results:** 148 patients (mean 55 yrs, MELD 10, 53% HCV) were randomized to AST-120 12 g ($n=50$), AST-120 6 g ($n=50$), or placebo ($n=48$). No significant changes were noted in the RBANS global-summary scores at week 8 (3.27±0.797 vs. 0.2584, 4.51±0.772 vs. 0.7812, and 4.57±5.90, Δ vs. baseline; AST-120 12 g, AST-120 6 g, and placebo respectively). A strong learning effect on RBANS (p < 0.0001) was apparent between screening and baseline visits in all groups. No differences in PHEx, CGA-HE or overt HE/hospitalization events between groups were observed. Over 8 weeks, venous NH$_3$ significantly decreased from baseline in both treatment groups but increased in the placebo group: Δ ammonia: −17, −14 and +5 μg/dL (AST-120 12 g, AST-120 6 g, and placebo, respectively). The frequencies of treatment-emergent adverse events were similar for all groups (32%, 26% and 37.5%; AST-120 12 g, AST-120 6 g, and placebo, respectively, p = N.S.).

**Conclusion:** This was the largest controlled trial yet conducted in CHE or Minimal HE. AST-120 was well tolerated but did not achieve its primary endpoint of RBANS improvement. Results were confounded by study design that allowed for an improvement in neurocognitive measures before drug randomization. NH$_3$ improved significantly but independently of neurocognitive change.

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**EIGHT QUESTIONS OF THE PATIENT-REPORTED SICKNESS IMPACT PROFILE CAN EFFECTIVELY SCREEN FOR MINIMAL HEPATIC ENCEPHALOPATHY**

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**Background:** Minimal hepatic encephalopathy (MHE), which impairs quality of life (QOL), is difficult to diagnose using current cognitive tests by non-specialists. The detection rates could potentially improve with easier, patient-administered methods that do not require specialized testing or equipment.

**Aim:** To detect MHE using a validated QOL questionnaire, Sickness Impact Profile (SIP).

**Methods:** 170 cirrhotics (55yrs, 13 yr education, MELD 9, 50%HCV, 11%alcohol) without prior overt HE were administered a standard cognitive battery (NCT-A/B, Digit Symbol and Blocks) as the gold standard for MHE diagnosis along with SIP. SIP consists of 136 questions across 12 QOL domains (body care and movements, mobility, ambulation, emotional behavior, social interactions, alertness, communication, work, sleep and rest, eating, home management and recreation/pastime) that requires a yes/no answer over the past day. Proportion of patients that responded ‘yes’ to each question was compared between MHE and no-MHE groups. Variables independent of cognitive testing; demographics (age, education, gender, alcoholic etiology) and SIP questions differentiating between groups were analyzed using logistic regression and ROC analysis for MHE diagnosis.

**Results:** 93 (55%) patients had MHE on standard tests. On SIP, a ‘yes’ response was found in a higher proportion of MHE patients on 54 questions across all QOL domains. On regression age, male gender and eight questions ‘I stay away from home only for brief periods of time’, ‘I do not maintain balance’, ‘I react slowly to things said or done’, ‘I do not keep my attention on any activity for long’, ‘I act irritable or impatient with myself’, ‘I am not doing any of the shopping that I would usually do’, ‘I am not doing any of my usual physical recreation or activities’ and ‘I am eating much less than usual’ differentiated between MHE/no-MHE groups. These questions spanned domains of alertness, eating, recreation/pastimes, emotional behaviour, body care, mobility and home management. The AUC on ROC for MHE diagnosis was 0.90 with 81% sensitivity and 78% specificity with all 54 statements, age and male gender.

**Conclusions:** Eight patient-reported questions on SIP can effectively screen for MHE in outpatient cirrhotic patients. MHE screening strategies that do not include specialized testing could increase detection rates and therapy.

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**RIFAXIMIN IMPROVES COGNITION AND ENDOTOXEMIA IN MINIMAL HEPATIC ENCEPHALOPATHY BY SHIFTING GUT MICROBIAL FUNCTIONALITY WITHOUT ALTERING THEIR ABUNDANCE**


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Minimal hepatic encephalopathy (MHE) has a presumed gut-based pathophysiology. Rifaximin, a gut-specific antibiotic, is effective in
MHE but its mechanism of action is unclear. We hypothesized that modulation of gut microbiota and their end-products by rifaximin would improve cognition in MHE.

**Aim:** To perform a systems biology analysis of the microbiome, metabolome and cognitive change after rifaximin in MHE.

**Methods:** Cirrhotics with MHE underwent cognitive testing (seven recommended tests), endotoxin analysis, urine/serum metabolomics (GC-Mass Spectrometry) and fecal microbiome assessment (multi-tagged pyrosequencing) at baseline and 8 weeks post-rifaximin 550mg BID. Diet was constant during the trial. Changes in cognition, endotoxin, serum/urine metabolites (supervised/unsupervised techniques) and microbiome (metastats, QIIME and principal-component analysis) were analyzed. Correlation networks between cognition, microbiota and metabolome were analyzed and compared pre/post-rifaximin.

**Results:** Twenty cirrhotics (60 yrs, MELD 9, 55%HCV) were included and all patients completed the trial with >92% adherence and stable diet. There was a significant improvement in cognition (six of seven tests improved, p < 0.01) and endotoxemia (0.55 to 0.48 Eu/ml, p = 0.02) without MLD score change after rifaximin. Metabolomics showed a significant increase in serum saturated (myristic, caprylic, palmitic, palmitoleic, oleic and eicosanoic) and unsaturated (linoleic, linolenic, gamma-linolenic and arachidonoid) acids post-rifaximin without urinary changes. No significant microbial abundance change at the phylum/order level apart from a modest decrease in Veillonellaceae (2.5 to 1%) and increase in Eubacteriaceae (0% to 1%) was seen.

On network analysis, post-rifaximin networks showed that while features of microbiome and metabolome remained same, their interaction significantly shifted compared to baseline resulting in a significant reduction in network connectivity and clustering. Specifically, the networks centered on potentially pathogenic and HE-associated taxa; Enterobacteriaceae, Porphyromonadaceae and Bacteroidaceae indicated a shift from pathogenic to beneficial metabolite linkages. Pre-rifaximin, these taxa were linked with poor cognition, aromatic amino-acids, ammonia, glutamate and endotoxin while post-rifaximin, these correlations significantly weakened or disappeared. Networks centered on autochthonous taxa (Lachnospiraceae, Ruminococcaceae and Clostridium-ClusterXIV) however, remained similarly linked to beneficial metabolites, fatty acids and good cognition pre/post-rifaximin.

**Conclusions:** Rifaximin therapy changes gut bacterial linkages with metabolites without significant change in microbial abundance that is associated with improved cognitive function and endotoxinemia in MHE.

**193 RELIABILITY OF THE ESTIMATION OF HEPATIC BLOOD FLOW (HBF) BY DOPPLER ULTRASOUND IN PATIENTS WITH CIRRHOTIC PORTAL HYPERTENSION: COMPARISON WITH HBF BY INDOCYANINE GREEN**

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**Background and Aim:** The best method to assess clinically the hepatic blood flow (HBF) of cirrhosis is its estimation at hepatic vein catheterization by Fick’s method during indocyanine green constant infusion (ICG-HBF). Alternative non-invasive methods are needed. We investigated the consistency and agreement of HBF measured by Doppler ultrasound (US-HBF) as compared with ICG-HBF in portal hypertensive patients with cirrhosis.

**Methods:** We studied 50 portal hypertensive cirrhotics undergoing HVPG measurement (56% compensated; Child score 7±2; HVPG 16.6±6.0 mmHg; varices in 75%). US-HBF and ICG-HBF were measured the same day in fasting conditions. ICG (Pulsion Medical Systems, Munich, Germany) was infused intravenously at a constant rate of 0.2 mg/min, preceded by a priming dose of 5 mg. ICG-HBF was measured according to the Fick’s method after an equilibration period of at least 45 minutes. A fractional clearance over 0.1 was required for the calculation of ICG-HBF.

Diameter and mean flow velocity were measured in the portal vein and in the hepatic artery by Doppler-ultrasound (Sequoia-512-Acuson; 4.5–7 MHz convex probe) following international recommendations. Portal vein blood flow (PVBF) and hepatic artery blood flow (HABF) were calculated as: Blood flow (ml/min) = (vessel diameter² (cm/4) × π × mean blood flow velocity (cm/s)) × 60. Hepatic blood flow (US-HBF) was calculated as PVBF + HABF. Intraclass correlation coefficient (ICC) for consistency and for absolute agreement between US-HBF and ICG-HBF were calculated.

**Results:** Mean ICG-HBF and US-HBF were similar, being respectively 1004±543 ml/min, and 994±494 ml/min (p = 0.661 vs. ICG-HBF). However, results in individual patients disclosed marked differences between the two methods (386±415 ml/min), and showed only moderate consistency (ICC 0.456; p < 0.0001), absolute agreement (ICC 0.461; p < 0.0001) and linear correlation (R = 0.464; p < 0.0001). Bland-Altman plots showed that the discrepancy increased with worsening liver function and if measured HBF (by any of the two methods) was >1600 ml/min.

**Conclusions:** HBF estimations by Doppler-ultrasound and ICG are significantly, but moderately correlated. The discrepancy between the two methods is maximal in patients with poor liver function and high HBF. HBF assessment can be considered reliable by both ICG and Doppler-ultrasound if values do not exceed 1600 ml/min.
vs 60.5±11.9), INR values (2.14±0.69 vs 2.13±0.35) or platelets level (54±26 vs 53.3±34.7x10³/ mmc). No post-procedural bleeding or complications occurred in both groups. As expected, all subjects in the PPG received transfusions as compared to 4 in the TEG-G (100% vs 21%, p <0.000). In the PPG 9 patients (75%) required FFP, 2 (16.7%) PLTs, and 1 (8.3%) both PLTs and FFP; in the TEG-G 1 needed PLTs (5.5%), 3 both PLTs and FFP (16.7%). A transfusion-related reaction to FFP was recorded in the PPG, none in the TEG. Mean transfusion cost for the TEG-G was 129.3 €/patient (including TEG cost), 234.8 €/patient for the PPG.

Conclusion: TEG is safe and effective in guiding hemoderivates infusion before invasive procedures in cirrhotics with coagulation disorders. The use of TEG as a guide for transfusion before invasive procedure can reduce transfusion requirement, risk of transfusion-related side effects, and medical expenses.

195 HEPATIC VEINS DOPPLER PROFILE MODIFICATIONS DURING ACUTE PORTAL PRESSURE INCREASE INDUCED BY PARAUMBILICAL VEIN COMPRESSION

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**Background:** Portal hypertension of liver cirrhosis is characterized by abnormalities of hepatic veins waveform. The pathophysiology of these changes is still not clear.

**Aim:** We evaluated the effect of acute portal pressure elevation by compression of paraumbilical vein on hepatic veins flow profile.

**Patients and Methods:** We studied 63 cirrhotic patients (42 male and 21 female) with a mean age of 54.51±13 years, 24 with a patent paraumbilical vein, 9 with spleno-renal shunt, 8 with complete portal thrombosis. Abdominal echo color Doppler examination was performed in each subject, associated with ECG registration. In 8 patients the hepatic venous pressure gradient (HVPVG) was also determined. Hepatic veins A and S waves velocity, A/S ratio, mean velocity (MV) were measured by echo-color-Doppler; in patients with paraumbilical vein patency these parameters were recorded before and during 60sec manual vein obliteration along its superficial course in the abdomen.

**Results:** Hepatic veins flow profile was triphasic in 51%, biphasic in 30% and monophasic in 19% of cirrhotics. Hepatic veins A and S waves and A/S ratio were not different between patients with and without portal-systemic shunts. During paraumbilical vein compression, hepatic veins A wave velocity was reduced in 21 patients (15.2±15.80 vs -11.20±18.08, p <0.001), S wave was not different (-35.61±18.31 vs -30.83±15.58 cm/s), A/S ratio was increased in 23 patients (-0.07±0.46 vs 0.33±0.50, p <0.001); MV was not different (-20.43±13.0 vs -22.40±15.10 cm/s). Considering hepatic veins catheterization, triphasic Doppler profile of hepatic veins was found in 66% of patients with HVPVG <12 mmHg. All patients with HVPVG >12 mmHg had biphasic or monophasic profile. Subjects with portal vein thrombosis had a higher prevalence (88%) of triphasic pattern.

**Conclusions:** Portal hypertension plays an important role in determining the phasicity of hepatic veins Doppler flow profile as shown by the effect of acute portal pressure elevation and portal thrombosis. Hepatic veins Doppler profile alterations can be evaluated by A wave velocity and A/S ratio. Biphasic or monophasic profiles can predict a >12 mmHg HVPVG. The presence or different types of portal-systemic shunts don't change hepatic veins haemodynamics.

196 COEPTIN AS BIOMARKER OF SYSTEMIC HEMODYNAMIC DERANGEMENTS IN TAA CIRRHOTIC RATS

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**Background and Aims:** Portal hypertension is characterized by splanchnic and systemic hemodynamic disturbances which affect outcome. Serum levels of vasopressin reflect the degree of hemodynamic impairment. Copeptin is a stable fragment of the C-terminal part of the vasopressin AVP precursor. The aim of this study was to prove that copeptin is a potential biomarker of hemodynamic derangements in cirrhosis.

**Materials and Methods:** Thirty age-matched male Wistar rats were used, 25 were intoxicated with thioacetamide through their drinking water and 5 animals served as controls. At 18 weeks measurements of portal pressure (PP), mean arterial pressure (MAP) and mesenteric blood flow (MBF) were performed and blood samples collected. Heparin-plasma copeptin concentration was determined using a double-antibody sandwich rat copeptin ELISA. Approval has been obtained from the ethical committee on animal research.

**Results:** Cirrhotic rats had systemic hypotension (MAP 70±17 mmHg vs. 137±4 mmHg, p <0.01) and portal hypertension (PP 10.5±2.2 mmHg vs. 5.6±0.5 mmHg, p <0.01) as compared to controls and MBF was also higher (5.1±1.1 ml/min/100 g b.w. vs. 2.5±2.1 ml/min/100 g b.w., p <0.05). Body weight of cirrhotic rats was lower as compared to control rats (337.49±52.47 g vs. 524±7 g, p <0.001). CIRRHOTIC rats had a significantly higher plasma copeptin concentration (1.49±0.51 pmol/l) than controls (0.87±0.09 pmol/l; p =0.012). Plasma copeptin was negatively correlated to MAP (R =-0.574, p =0.013) and body weight (R =-0.565, p =0.015). Correlation between copeptin and PP (R =0.318, p =0.199) or mesenteric blood flow (R =0.03, p =0.907) was not statistically significant.

**Conclusions:** Plasma copeptin level is significantly elevated in cirrhotic rats in association with systemic hypotension. Copeptin warrants further study as a potential stable biomarker for hemodynamic disturbances in cirrhosis.

*Dr. Coenraad and Dr. Verbeke contributed equally to this work.*

197 FACTORS RELATED TO HOSPITALIZATION BUT NOT THE SEVERITY OF THE LIVER DISEASE INCREASE THE RISK OF NOSOCOMIAL INFECTION IN CIRRHOTIC PATIENTS


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**Background and Aim:** Nosocomial infections are 2-times more frequent in cirrhotics than in other group of hospitalized patients. The aim of the present study was to assess predisposing factors for nosocomial infections specific for these patients.

**Patients and Methods:** Consecutive cirrhotic patients, hospitalized at our University Hospital in the last 24 months, without a diagnosis of infection at the admission were enrolled. Patients assuming immunosuppressive drugs or with a diagnosis of immunopression were excluded. During the hospitalization, infections were actively searched and classified as nosocomial. Severity of liver disease, variceal bleeding, bearing a TIPS, hospitalization in a room with additional bed, days of hospitalization, invasive procedures were recorded as possible risk factors.

**Results:** A nosocomial infection was diagnosed in 43 patients (68% males; median age 62 years; 70% Child B-C, MELD score
199 PERIPHERAL GLAND DILATATION: MORPHOMETRIC AND CLINICAL CORRELATION IN 74 PATIENTS
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Background and Aims: Peribiliary glands (PBG) are found adjacent to large intra and extra-hepatic ducts, however their function remains uncertain. Recently, multipotent stem/progenitor cells have been described in these glands suggesting a possible role in regeneration. PBG cystic dilatation has previously been described in association with cirrhosis however its natural history, clinical associations and pathophysiology remain unclear. Our aim was to better characterise the prevalence, historical and clinical findings of PBG dilatation in a retrospective cohort of patients undergoing liver transplantation (LT) for end-stage liver disease.

Methods: Explants of patients undergoing LT between October 2006 and October 2011 were included; exclusion criteria included paediatric patients, biliary pathology, acute liver failure (ALF) or insufficient clinical data. We performed a morphometric analysis of the PBG by measuring their maximal diameter in the hilar region. PBGs were judged to be dilated when their luminal diameter exceeded 1000 μm. We correlated this data to clinical and biological information.

Results: 74 patients were included in this cohort (exclusion (n): paediatric=20, ALF=9, biliary=11, other=8, insufficient data=46). Clinical characteristics were as follows: average age: 52 years, mean MELD: 14, men: 52%, alcoholic cirrhosis: 48%, viral cirrhosis: 54%, HCC: 53%, ascites: 52%. 30% of patients had histologically proven PBG dilatation. In univariate analysis PBG dilatation was associated with a higher MELD (20.6 vs 12.5, p=0.007), higher Child–Pugh score (10.3 vs 8.01, p=0.003) higher bilirubin (212 vs 84.3, p=0.035) higher INR (2.1 vs 1.4, p=0.024) and less frequent viral aetiology to the cirrhosis (29% vs 61%, p=0.063). Sex, age, presence of ascites, alkaline phosphatase level and alcoholic aetiology were not associated with PBG dilatation. In multivariate analysis, MELD (OR=1.11 per unit increase in MELD score, 95% CI 1.03–1.17, p=0.005) was the only significant factor associated with PBG dilatation.

Conclusions: PBG dilatation is a frequent finding in cirrhotic patients undergoing liver transplantation. In our cohort of patients transplanted for advanced liver disease it was significantly correlated to the degree of liver failure. Further studies should attempt to characterise this finding more fully and assess whether PBG dilatation is associated with proliferation of multipotent stem cells found in PBGs.

200 EFFECT OF DIFFERENT SEDATION ON CRITICAL FLICKER FREQUENCY, A DIAGNOSTIC TOOL FOR MINIMAL ENCEPHALOPATHY
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Introduction: Critical flicker frequency measurements are a valid tool for assessing early stages of cerebral dysfunction in patients with acute and chronic liver diseases. However, it may also serve as a tool for assessing the fitness of these patients after specific diagnostic or therapeutic interventions. Driving after sedation for endoscopic procedures is a matter of debate, in particular since recent results from a large study (Horiuchi et al. Am J Gastroenterol 2009) have suggested that driving home after propofol sedation might be safe. We used CFF analysis to assess the time-dependent effect of different sedation methods on brain function in the endoscopy unit of a tertiary referral centre.
cirrhosis is usually treated with non-selective 

Background and Aims: Portal hypertension in patients with E-mail: saraheeboll@dadlnet.dk identify markers of response/non-response. we aimed to investigate the response rate at our institution and side effects to obtain the hemodynamic effect and these patients are at risk of bleeding. However, a significant fraction of the patients do not propranolol as primary or secondary prophylaxis against variceal bleeding. Therefore we aimed to evaluate the role of vWF-Ag in patients with advanced portal hypertension.

Discussion/Conclusion: Our study clearly shows that the use of CFF assessment may be beyond testing for hepatic encephalopathy. We here show that the combination of propofol with midazolam leads to long lasting effects on CFF, which may affect driving capability, whereas sedation with propofol wares off within one hour. CFF analysis may be an easy to use tool to asses driving ability in this setting.

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LONG-TERM EFFECT OF PROPRANOLOL ON PORTAL HYPERTENSION IN PATIENTS WITH CIRRHOSIS: A SINGLE CENTRE PROSPECTIVE EXPERIENCE
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Background and Aims: Portal hypertension in patients with cirrhosis is usually treated with non-selective β-blockade using propranolol as primary or secondary prophylaxis against variceal bleeding. However, a significant fraction of the patients do not obtain the hemodynamic effect and these patients are at risk of side effects to β-blockade without the targeted risk reduction. Here, we aimed to investigate the response rate at our institution and identify markers of response/non-response.

Methods: In total, 83 patients were included in the study. All patients received a CFF analysis before, 30, 60, 90 and 120 min after the endoscopy. CFF results were correlated to sedation methods. Differences in the CFF between groups and within groups were tested by non-parametric Mann-Whitney U or paired t-tests comparisons as appropriate.

Results: Overall, 33.7% of patients received no sedation, 26.5% were sedated with propofol (P) alone, and 38.6% of patients received a combination of propofol with midazolam (P/M). While in the control group no changes in CFF results were detected, patients with sedation experienced a clear drop in CFF results at 30 min. This drop was more pronounced in patients P/M-sedation as compared to P-mono sedation (30 min CFF: 37.6 Hz vs. 42.2 Hz). In addition the effect of sedation was detectable for >120min in P/M sedated patients, while CFF results in patients with P-mono sedation recovered to baseline values after 60min.

Discussion/Conclusion: Our study clearly shows that the use of CFF assessment may be beyond testing for hepatic encephalopathy. We here show that the combination of propofol with midazolam leads to long lasting effects on CFF, which may affect driving capability, whereas sedation with propofol wares off within one hour. CFF analysis may be an easy to use tool to asses driving ability in this setting.

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VON WILLEBRAND FACTOR – A MARKER FOR HEPATOPULMONARY SYNDROME
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Background: Hepatopulmonary syndrome (HPS) occurs in 20–30% of patients with liver cirrhosis and is associated with a >2fold increased mortality. Pulmonary angiogenesis and endothelial dysfunction seem to play a central role in its pathogenesis, von Willebrand factor antigen (vWF-Ag), a marker of endothelial dysfunction, is significantly elevated in patients with liver cirrhosis and portal hypertension. vWF levels are associated with increased pulmonary angiogenesis in a rat model of HPS. Single nucleotide polymorphisms (SNPs) in the vWF-gene are associated with HPS. Therefore we aimed to evaluate the role of vWF-Ag in patients with HPS.

Methods: 128 patients (94 male, 34 female; mean age: 56 years) with liver cirrhosis were included in this prospective study. vWF-Ag was assessed by ELISA. All patients were screened for presence of clinically significant HPS according to established consensus guidelines (presence of cirrhosis, AaDO2 >15mmHg & PaO2 <80 mmHg, intrapulmonary vasodilatation in contrast enhanced echocardiography).

Results: Criteria of HPS were fulfilled in 27 patients. Liver cirrhosis was caused mainly by alcoholic liver disease (63%), chronic hepatitis C (24%) and others (13%). vWF-Ag level was significantly higher in patients with HPS compared to patients without HPS (483±123% versus 331±100%; p <0.05). vWF-Ag correlated significantly with gas exchange abnormalities in the total cohort by means of AaDO2 (r=0.47; p <0.05) and PaO2 (r=0.4; p <0.05). ROC AUC of vWF-Ag for detection of HPS was 0.825. The best cut off with maximal sensitivity was 327% (sensitivity of 100% and specificity of 51.5%; positive predictive value: 34.2%, 95% CI: 23.9–45.7%; negative predictive value: 100%, 95% CI: 92.7–100%). vWF-Ag levels were significantly associated with HPS (OR: 1.016, 95%CI: 1.009–1.023, p <0.05) and remained significantly associated with HPS after correction for sex, age, MELD score and hepatic venous pressure gradient (OR: 1.019, 95% CI: 1.002–1.036, p <0.05).

Conclusion: vWF-Ag is a significant predictor for presence of HPS, independently of severity of cirrhosis. vWF-Ag using a cut-off level >327% may help to identify HPS in patients with cirrhosis.
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ORAL GLUTAMINE CHALLENGE – DOES IT IMPROVE REVEALING MINIMAL HEPATIC ENCEPHALOPATHY IN CIRRHOSIS?
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Background and Aims: Minimal hepatic encephalopathy (MHE) is a prevalent asymptomatic condition, whose diagnosis is difficult due to the lack of a gold standard test. Oral glutamine challenge (OGC) has been found to increase blood ammonia in patients with cirrhosis which could lead to significant cognitive impairment. The aim of our study was to evaluate the value of OGC in improving the performance of psychometric tests and arterial blood ammonia for the diagnosis of MHE. Secondary we followed the risk for development of overt hepatic encephalopathy (OHE).

Methods: Fifty-four patients (male/female: 34/20; mean age 59.5yrs) with cirrhosis and 13 healthy controls matched by sex and age were included. Follow-up lasted 12 months. MHE was assessed using the psychometric hepatic encephalopathy score (PHES). Arterial ammonia blood concentrations and PHES were evaluated pre- and post-60 minutes of a 20g oral glutamine load. Statistical analysis used paired t-test, relative operating characteristics (ROC) and multivariate logistic regression analysis.

Results: At baseline, 29 (53.7%) out of 54 patients met the criteria for MHE, while post glutamine challenge, 43 patients (79.63%) had MHE (p < 0.0001). Arterial blood ammonia levels had significantly raised after glutamine challenge in the cirrhotic patients (baseline vs post glutamine: 85.2±20.8μg/dl vs 159.82±66.01μg/dl, p < 0.0001) as compared to controls values which remained unchanged. ROC analysis showed a pathological glutamine tolerance cut-off value of 124μg/dl. For baseline arterial blood ammonia the AUROC was 0.54 (CI: 0.402–0.680, p = 0.58), with no significant change post glutamine challenge (AUROC = 0.53, CI: 0.389–0.667, p = 0.77). In the follow-up 16 patients (29.62%) developed OHE. In multivariate model, MELD score (OR = 1.5187, 95% CI: 1.0690–2.1574, P = 0.0197), but not Child Pugh class, arterial blood ammonia, esophageal varices grading, was a strong independent predictor of OHE.

Conclusions: In cirrhotic patients, OGC appears to improve the performance of psychometric tests, but not arterial blood ammonia for the diagnosis of MHE. MELD score has proven to be independently related with OHE in the follow up. Acknowledgements: This work was made possible by the project ‘Doctoral Scholarships for increasing competitiveness in the medical and pharmaceutical field’ POSDRU/88/1.5/S/58965.

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ACUTE KIDNEY INJURY (AKI) AT ADMISSION AND ITS RESPONSE TO TERLIPRESSIN AS A PREDICTOR OF MORTALITY IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)
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Background and Aims: Patients with ACLF have high mortality without liver transplantation. While cirrhotics with AKI are known to have poor clinical outcomes; the prevalence of AKI at admission and its response with terlipressin as a predictor of associated complications and mortality in ACLF is not known.

Methods: Consecutive ACLF patients with AKI [Serum creatinine (Scr) >1.5 mg/dl] at admission were compared with controls (no AKI at admission) in terms of short term (7 day) as well as long term (6 month) mortality and for the presence of complications such as hepatic encephalopathy (HE), spontaneous bacterial peritonitis (SBP) and acute variceal bleed (AVB). Patients with ACLF were further classified based on the severity of AKI (mild AKI; Scr 1.5–3 mg/dl and moderate to severe AKI; Scr >3 mg/dl).

Results: Out of 241 consecutive ACLF patients (Mean age: 46.12 years, M:F 6:1), 55 (22.8%) had AKI at admission (mean age: 45.72 years, M:F 7:1). The probable etiology of AKI were related to sepsis (45.5%), volume depletion (30.9%) and hepatorenal syndrome (HRS) (27.3%). ACLF patients with AKI at admission had higher 6 month mortality [21/55 (38.2%) vs. 32/186 (17.2%); p < 0.05]. Both the presence of AKI [6/21 (28.6%) vs. 3/34 (8.8%); p = 0.05] as well as the severity of AKI [51.7% vs. 14.3%; p < 0.05] at admission predicted lower 7 day survival. The degree of liver failure manifested by high serum bilirubin (>25 mg/dl; OR2.13) and INR (>2.5; OR1.71) had significantly lower survival. Presence of AKI at admission was more often associated with HE [54.1% vs. 30.6%; p < 0.05], SBP [9.1% vs. 5.9%; p < 0.05] and AVB [16.4% vs. 12.9%; p < 0.05]. Of the 29 (52.7%) patients treated with terlipressin (initial dose 2–4 mg/d), 13 (44.8%) showed treatment response (Scr <1.5 mg/dl). Responders to terlipressin had lower mortality (2/13; 15.38%) compared to non-responders (7/16; 43.75%) (p < 0.05).

Conclusions: Almost one fourth of ACLF patients have AKI at admission and the presence and degree of AKI predicts a higher 7 days mortality. Nearly 45% of ACLF patients with AKI respond to terlipressin therapy which helps in improved survival.

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LONG TERM OUTCOMES OF PORTOPULMONARY HYPERTENSION (POPH) AFTER ORTHOTOPIC LIVER TRANSPLANTATION (OLT)
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Background and Aims: POPH occurs in 2–12.5% of cirrhotic patients. Survival in the absence of OLT is reportedly 38% at 3 yrs and 28% at 5yrs. Although mild disease poses little perioperative risk, moderate-to-severe POPH (mean PA pressure ≥35 mmHg) is associated with 50–80% mortality after OLT. Consequently, POPH may be considered a contraindication for OLT. Although UNOS allows MELD exception points, only 79 patients were transplanted for POPH in the USA from 2007–2011. Single-center series have demonstrated the feasibility and short-term efficacy of OLT following medical control of POPH, but long-term outcomes have not been reported. Here we report the long-term outcomes of our patients who were transplanted for POPH.

Methods: Of 488 adult patients transplanted between January 2004 and January 2011, seven were transplanted for POPH after responding to vasodilators.

Results: The 7 patients (1.4% of transplanted adults) included 3 men and 4 women, ages 38 to 53 yrs at the time of OLT. The etiologies of cirrhosis were hepatitis C (n = 3), alcohol (n = 2), and cryptogenic (n = 2). All patients were transplanted with MELD exception points of 25–34 (calculated MELD 6–14). Six patients required pretransplant IV epoprostenol (EPO), and one was managed with oral sildenafil. All patients received IV or inhaled EPO in the perioperative period, and EPO was weaned over 3 days to 8 months. No patient required long-term EPO. Graft and patient survivals are 100% after median follow-up of 7.5 years (1.7 to 8.3 years). One patient has recurrent cirrhosis secondary to hepatitis C, and has recently started sildenafil for late recurrent POPH. Five patients (71.4%) (including the one with cirrhosis) require oral vasodilator therapy for persistent or recurrent pulmonary hypertension (PHT). New York Heart Association Functional Class in the patients with PHT is I-II. Two patients (3.3 and 7.5 years post-OLT) do not have PHT.
Conclusions: POPH that responds to vasodilator therapy is an appropriate indication for OLT with excellent potential for long-term graft and patient survival – functional class after OLT appears similar to the outcome for other indications. The majority of patients require oral vasodilators for recurrent PHT.

206 RIFAXIMIN IN THE TREATMENT AND PROPHYLAXIS OF HEPATIC ENCEPHALOPATHY IN CHRONIC LIVER DISEASE: A META-ANALYSIS

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Background and Aims: Hepatic encephalopathy (HE) is a severe complication to liver cirrhosis and associated with a high mortality. The exact mechanism behind HE is still unsettled, but ammonia from bacterial fermentation in the gut may be important. Rifaximin exhibit broad-spectrum antimicrobial activity and may reduce ammonia production. We aimed to evaluate the clinical efficacy and safety of rifaximin in the treatment and prophylaxis of overt and minimal HE.

Methods: A systematic review including randomised clinical trials comparing rifaximin to disaccharides, placebo and antibiotics was conducted. Eligible trials were identified through electronic and manual searches (last update October 2012). The primary outcome measure was mortality. Secondary outcome measures were remission and relapse of HE and serious adverse events. Random effects meta-analyses were performed with results expressed as risk ratio (RR) or risk difference (RD) and I² as a marker of heterogeneity.

Results: Seventeen trials comprising 1112 patients were included, 578 received rifaximin, 534 controls received other antibiotics (n=99), disaccharides (n=190) or placebo (n=270). Rifaximin had no effect on mortality, RD 0.00 (−0.03; 0.03), p=1.00 I² 0%, 8 trails. Rifaximin did not improve HE, RD 0.06 (−0.01; 0.13) p=0.09, I² 59% compared to all controls. Subgroup analyses on overt HE, RD 0.03 (−0.03; 0.03), p=0.31 I² 0%, covert HE, RD 0.01 (−0.04; 0.07) p=0.61 I² 0%, and HE prophylaxis (one trial) RD 0.24 (0.13; 0.34) p=0.0001 NNT 5, was performed. Treatment of overt HE showed a RD 0.04 (−0.02; 0.10) p=0.22 I² 0%, compared to disaccharides. The risk of serious adverse events was similar in the two groups RR 1.11 (0.80; 1.54) p=0.52 I² 0%. Six studies had high risk of bias.

Conclusion: Rifaximin had no effect on mortality. In the treatment of overt and covert HE, rifaximin was not superior to disaccharides, antibiotics or placebo. Rifaximin may have a protective effect against relapse of HE. Randomised trials are still needed to determine the role of rifaximin in hepatic encephalopathy.

207 DIAGNOSTIC PERFORMANCE OF C-REACTIVE PROTEIN AND NEUTROPHIL-TO-LYMPHOCYTE RATIO FOR INFECTION IN PATIENTS WITH DECOMPENSATED CIRRHOSIS

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Background: Infection is associated with poor prognosis, but often difficult to diagnose in cirrhotic patients. The role of clinical parameters such as systemic inflammatory response syndrome (SIRS) criteria in diagnosing infection remains unclear in the cirrhotic population. The aim of this study was to determine the prevalence of infection in decompensated cirrhotic patients and evaluate the usefulness of inflammatory markers including C-reactive protein (CRP) and neutrophil-to-lymphocyte ratio (NLR) in the diagnosis of infection.

Methods: The study population consisted of 263 consecutive events in a cohort of 183 decompensated cirrhotic patients admitted to a tertiary centre from September 2011 to September 2012. The presence of SIRS and overt infection were evaluated. CRP and NLR were measured.

Results: Male sex was 70% and the alcoholic cirrhosis was 64.6%. The main cause of admission was uncontrolled ascites (30.4%), followed by varix bleeding (28.1%), overt infection (20.2%) and hepatic encephalopathy (15.2%). Ninety-nine patients (37.6%) met the SIRS criteria. Both baseline CPR (≥12mg/L) and NLR (>3.2) levels were significant factors for the diagnosis of infection (Odds ratio 4.191 (2.023–7.971), P=0.000; odds ratio 2.078 (1.099–7.437), P=0.024, respectively). Particularly in patients of the Child–Pugh C group, combined CPR (≥12mg/L) and NLR (>3.2) enhanced diagnostic accuracy of infection. However, there were no differences in the presence of SIRS, WBC counts, Child–Pugh and MELD scores at baseline between the patients with or without infection.

Conclusions: The present study suggests that NLR as well as CRP are significant indicators of the diagnosis of infection among decompensated cirrhotic patients. Combined CPR and NLR increase diagnostic ability of infection in the Child–Pugh C group. Decompensated cirrhotic patients with elevated CPR level and NLR should be carefully checked the presence of infection and considered for antibiotic therapy.

208 FREE 25(OH) VITAMIN D IS HIGHER IN CIRRHOTICS WITH SYNTHETIC DYSFUNCTION COMPARED WITH INDIVIDUALS WITHOUT LIVER DISEASE DESPITE TOTAL 25(OH)D DEFICIENCY

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Background: Vitamin D (D) deficiency is thought to be highly prevalent in patients with cirrhosis. Current clinical assays measure circulating total 25(OH)D that reflects D bound to D binding protein and albumin as well as unbound (free) D that is available for conversion to active 1.25(OH)2D. We hypothesized that cirrhotics with synthetic dysfunction would have lower levels of D binding protein and thus, higher free 25(OH)D levels for any given level of D. Cirrhosis may reduce D binding protein and albumin with synthetic dysfunction defined as serum albumin <2.9g/dL and compared to measurements in similarly-aged community-dwelling adults with total 25(OH)D ≤25ng/mL without a history of liver disease. Total 25(OH)D was measured by mass spectrometry (Mayo Laboratories). Free 25(OH)D was measured by immunoassay (Future Diagnostics).

Results: Median age among the 14 cirrhotics was 58y [interquartile range(IQR) 56–64y], 36% women, 86% Caucasian; median (IQR) albumin was 2.6g/dL (2.5–2.6). Patients without liver disease (n=29) were aged 63y (54–66y), 59% women, 69% Caucasian. The range of total 25(OH)D was similar between cirrhotics vs. individuals without liver disease, but both free 25(OH)D >5.4 (5.0–7.1) vs. 3.0 (2.2–3.7) pg/mL; p<0.001 and %free 25(OH)D >0.044 (0.039–0.048) vs. 0.018 (0.016–0.022); p<0.001 were significantly higher at any total 25(OH)D level in cirrhotics (Figure).
ONLINE ANALYSIS OF BREATH BY PROTON TRANSFER REACTION TIME OF FLIGHT MASS SPECTROMETRY IN CIRRHOTIC PATIENTS

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Background and Aim: Being rapid and non-invasive, breath analysis is a promising diagnostic tool although difficulties related to data interpretation, reproducibility and sensitivity have limited its application. The aim of the present work was to investigate whether a recently realized direct injection mass spectrometric technique (Proton Transfer Reaction Time of Flight Mass Spectrometry, PTR-ToF-MS) allows the direct and non-invasive compound identification in breath of cirrhotic patients. A comparison between cirrhotics and healthy subjects was performed to assess the capability of the technique to distinguish cirrhotic from healthy subjects and well compensated cirrhosis from Child–Pugh A cirrhosis.

Methods: Twelve patients (M/F 8/4, mean age 70.5, range 42–80 years) with liver cirrhosis of different etiologies and status were enrolled in the study. The etiology of cirrhosis was viral in 9 patients, metabolic in 2 and hereditary in 1. Real time breath analysis was performed using a buffered end-tidal (BET) online sampler coupled to a PTR-ToF-MS. Spectra were acquired using the data acquisition software TOF-DAQ (Tofwerk AG, Switzerland) with a mass/charge range of 10–400 Th. The data were analyzed by non-parametric ANOVA (Kruskal-Wallis test) using the Statistica 9.1 (StatSoft, USA) software.

Results: Eight peaks resulted significantly different in cirrhotic patients compared to healthy controls: two related to ketones, (2-pentanone, C8-ketone), two to terpenes and four to sulfur compounds. Three peaks resulted significantly different between Child–Pugh A cirrhotic patients and Child–Pugh B+C cirrhosis patients and precisely: C8-ketone, a monoterpene and a NS-compound.

Conclusion: In conclusion, real time analysis of breath allows to distinguish cirrhotic from healthy subjects and well compensated liver disease from more advanced liver stage. The proposed method can be used to identify the stage and severity of liver disease in real time with a safe and non-invasive procedure.

210 SUSTAINED VIROLOGICAL RESPONSE WITH PEG-INTERFERON alfa-2a AND RIBAVIRIN DECREASES PORTAL PRESSURE GRADIENT AND PREVENTS CLINICAL DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C RELATED CIRRHOSIS

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Background and Aims: In patients with chronic hepatitis C (CHC) related cirrhosis, clinically significant portal hypertension (hepatic venous pressure gradient HVPG ≥10 mmHg) is associated with greater risk of decompensation and worse prognosis. Antiviral treatment with peg-interferon and ribavirin has been shown to decrease HVPG. The objective of our study was to assess the effect of antiviral therapy in HVPG in patients with CHC-cirrhosis and its effect on sustained virological response (SVR), hepatic decompensation and long term survival.

Methods: We assessed HVPG in 61 patients with CHC-cirrhosis before antiviral therapy with peg-interferon and ribavirin. 55 patients underwent a second HVPG measurement 6 months after the end of treatment (final HVPG). Clinical events were recorded during an average follow up of 7 years (range 1.8–8.6).

Results: 61 patients with CHC-cirrhosis received antiviral therapy, 70% males, mean age 52 years (range 35–68), 92% genotypes 1 or 4 and 67% were treatment-naïve. Median baseline HVPG was 12 mmHg (4.5–24), 46 patients had HVPG≥10 mmHg. The overall SVR rate was 34.4% (21 patients), baseline HVPG was lower in SVR than in non-SVR although the difference did not reach statistical significance; 11.3 ± 4.2 vs 12.7 ± 4.6 mmHg (p = 0.28). By multivariate analysis, viral genotype was the only factor predicting SVR (p = 0.03), a non significant trend was observed with baseline HVPG (p = 0.07). HVPG was significantly reduced in the SVR group (11.3 ± 4.2 to 9.8 ± 4.6 mmHg; p = 0.014), but not in non-SVR (12.7 ± 4.6 to 12.4 ± 5.7 mmHg; p = 0.46). During follow up, 17 patients (28%) presented hepatic decompensation (9 hepatocellular carcinoma, 3 ascites, 2 portal hypertension related hemorrhage, 2 spontaneous bacterial peritonitis and 1 hepatic encephalopathy), 6 patients died and 5 underwent transplantation. Actuarial probability of decomposition free survival at 5 years was significantly higher among SVR patients than in non-SVR (90% vs 70%, p < 0.03). Transplant free-survival was also higher in SVR group (95% vs 80% non-SVR; p = 0.07). By multivariate analysis final HVPG was the only predictor of decomposition and of transplant free survival (p = 0.001 and p = 0.004 respectively).

Conclusion: In patients with CHC-cirrhosis and antiviral therapy, HVPG after treatment is the only significant factor predicting the risk of hepatic decompensation and of transplant free survival.
Background and Aims: Indocyanine Green retention test is a liver function test reflecting total blood flow and functional reserve: it is able to predict clinical decompensation and survival in compensated patients undergoing liver surgery and in patients with advanced liver cirrhosis. The aim of our study is to evaluate ICG clearance as a predictor of clinical decompensation and mortality in patients with compensated cirrhosis.

Material and Methods: From Jan-2010 to Jan-2011 we prospectively enrolled compensated cirrhotic patients (MELD <15; Bilirubin <2 mg/dL; INR <1.5); present or previous decompensation and HCC were excluded from the study. All underwent lab-tests, gastroscopy, HVPG and ICG clearance measurement and completed at least 18 months of follow up during which we recorded all liver-related events, liver transplant and death. Patients who underwent liver transplantation were censored. Cumulative incidence and predictors of clinical outcomes were determined by Kaplan–Meier analysis and Cox regression.

Results: 110 patients (75 male; 35 female; age 60.6±11.6 yrs) were followed up for 29 (19–34) months. 46 out of 110 (41.8%) developed decompensation, 6 (5.5%) underwent liver transplant and 9 (8.2%) died during follow-up. Together with MELD values [P 0.041; HR 1.137 (1.004–1.287)] multivariate analysis identified ICG-r15 as an independent predictor of decompensation [P 0.0001; HR 9.140 (4.812–17.371)]. Time-dependent ROC curve identified a cutoff value of 23% of ICG-r15 for the development of decompensation (Sensitivity 87.0%; Specificity 65.6%; +LR 2.53; −LR 0.20; AUROC 0.75). 18 patients (16.3%) died during follow-up. Together with calculated PVR not meeting the full ERS taskforce criteria, there is a spectrum of haemodynamic changes across the continuum of the disease, with vascular remodelling occurring despite calculated PVR not meeting the full ERS taskforce diagnostic criteria for PoPHTN. Herein, we sought to assess the haemodynamic subsets and the accuracy of CT in the diagnosis of PoPHTN – identifying such patients early has important therapeutic implications.

Methods: Patients between 2006 and 2011, with suspicion of PoPHTN, who underwent right heart catheterisation, had diagnostic data collated. In those with contemporaneous CT, radiology was correlated with mPAP, TPG and PVR in a blinded fashion. Haemodynamics were grouped according to mPAP and transpulmonary gradient (TPG) was used to subdivide groups into those with a TPG r240 as well as those who had a TPG r12 suggestive of vascular remodelling and incipient PoPHTN despite PVR r240.

Results: 62 patients were identified – median age 56 [23–72] and mean MELD 18 [6–40]. 35 had PH (mPAP >25 mmHg) and 19 demonstrated an elevation in TPG (>12). However, only 9 of these had a PVR >240 and met the full ERS criteria for PoPHTN – the remainder had a calculated PVR <240 due to their high cardiac index (CI) despite a raised TPG. Main mPAP subset (PVR <120, 120–240, >240) measured 32, 39 and 47 mmHg and mean CI fell from 6.5 to 4.6 and 3.81/min/m² respectively. 49/62 underwent CT in whom 28 had mPAP >25 mmHg. Main pulmonary artery/ascending aorta diameter ratio (PA/AA) predicted PH (AUC=0.73, p <0.005) whilst main left and right PA diameter predicted raised TPG (AUC=0.82, p <0.0001) and PVR (AUC=0.91, p <0.0001). Segmentary artery-bronchus ratio (ABR) also predicted raised PVR (AUC=0.89, p <0.0001).

Conclusions: 1. High CI may conceal the true incidence of vascular remodelling which can be discerned in groups with a PVR <240 but a TPG >12 who lie outside of the full ERS taskforce diagnostic criteria for PoPHTN. 2. CT imaging may predict PH in CLD as well as PoPHTN.

Background and Aim: Bacterial infections are frequent in cirrhosis. Among the factors involved in the pathogenesis, intestinal bacterial translocation (BT) plays a crucial role. Therapies commonly adopted in cirrhotic patients may influence BT. This study was aimed to investigate the correlation between the chronic assumption of drugs with a possible influence on BT, commonly used in cirrhosis, and the prevalence of infections.

Patients and Methods: Three hundred and seventeen cirrhotic patients (67% males; age 62±13yrs; 26% Child C) consecutively admitted at our University hospital from October 2008 to May 2012 were enrolled in the study. Clinical, biochemical data and the outpatient therapy with a possible effect on BT were recorded. Episodes of infection were actively searched. During hospitalization 106 patients had a diagnosis of bacterial infection. The drugs more often assumed were proton pump inhibitors (PPIs) (65%), non absorbable disaccharides (41%), beta blockers (BBs) (33%) and biliary salts (16%). The rate of infections in patients assuming or not assuming all type of drugs was similar, except as concern PPIs and BBs: the incidence of bacterial infections was lower in cirrhotic patients assuming BBs (p =0.006) while was increased in those under chronic PPIs therapy (p =0.046). Demographic data, severity of liver disease and sites of infection were similar either when patients were divided according to the assumption of PPIs or when they were divided according to the assumption of BBs. A multivariate analysis selected
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STAGED MANAGEMENT OF BUDD–CHIARI SYNDROME CAUSED BY CO-OBSTRUCTION OF THE INFERIOR VENA CAVA AND MAIN HEPATIC VEINS

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Background: Collateralized intra- and extraneoplastic routes in patients with Budd–Chiari syndrome (B-CS) were important. The aim of this study was to investigate the feasibility and clinical outcomes of the staged management of Budd–Chiari syndrome (B-CS) based on the degree of compensation provided by intra- or extraneoplastic collaterals.

Methods: A total of 103 adult patients with B-CS caused by co-obstruction of the inferior vena cava (IVC) and main hepatic veins (MHVs) between March 2001 and October 2009 were enrolled in this study. Based on the pathological classification and degree of hemodynamic compensation by collaterals, treatment priority for IVC hypertension (IVCHT) was determined in the first-stage treatment. Patients were deemed eligible for second-stage treatment when the first treatment failed to relieve their presentations.

Results: Imaging results revealed that most patients had collaterals to different extents. Based on the degree of compensation provided by these collaterals, 74 patients underwent single-stage treatment for IVCHT, i.e., radiologic intervention (RI) for 61 patients and surgical procedures (SPs) for 13. One patient was treated for portal hypertension. There were 29 patients who underwent two-stage treatment; 25 underwent RI and SP, and 4 patients underwent only SP. The general morbidity and mortality after all procedures were 8.3% and 1.5%, respectively. After a median follow-up of 35 months, 8 patients underwent second-stage treatment and 7 underwent recanalization of the IVC/MHVs. There were 2 patients who died of HCC and 1 who died of graft obstruction.

Conclusions: Staged management produces excellent outcomes for patients with B-CS caused by co-obstruction of the IVC and MHVs.

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IS FLOW-MEDIATED DILATATION (FMD) ASSESSMENT A RELIABLE MARKER OF ENDOTHELIAL DYSFUNCTION IN LIVER CIRRHOSIS?

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Background and Aims: Portal hypertension (PH) complications are leading causes of death in patients with liver cirrhosis (LC). PH development in chronic liver disease depends on increased vascular intra-hepatic resistance and on hyperdynamic splanchnic circulation. Disturbances in 'endothelium-dependent' vasodilation, a condition known as 'endothelial dysfunction' (ED), has been claimed as an important factor responsible for increased vascular hepatic resistance and PH development in LC. Aims of this study were to assess in LC patients: 1. the presence of ED and its correlation with disease stage and 2. the existence of a correlation between of ED serum markers(MED) and flow-mediated dilatation (FMD), the gold standard test for the study of ED.

Material and Methods: 60 consecutive LC patients (mean age 65±10 years, 17 female) without portal thrombosis (40 with compensated and 20 with decompensated disease) underwent a complete clinical, radiological and biochemical evaluation in order to assess the stage of disease (Child–Pugh-Turcotte) and drug history; all subjects were assessed for MED [P-selectin, von Willebrand factor (vWF), endothelin-1 (ET-1), thromboxane (TM) and nitric oxide (NO)] serum levels and FMD (measured by ultrasound at brachial artery according to guidelines). MED and FMD were also assessed in 11 healthy subjects (mean age 26±6, 6 female) (controls).

Results: MED plasma levels increased with the degree of liver dysfunction (p for trend p<0.001 in all cases); accordingly, FMD values decreased with worsening of the stage of liver cirrhosis [controls (9.9±1.1%), compensated cirrhosis (6.1±1.8%), decompensated cirrhosis (5±1.3%), p for trend <0.01]. In LC patients a statistically significant correlation between MED markers and FMD was observed for ET-1: r = −0.4427 (p = 0.0004) and P-selectin: r = −0.477 (p = 0.0001), vWF (r = −0.166, p = 0.05), but not for TM (r = −0.245, p = 0.05951) and NO (p = 0.961). At multivariate analysis, ET-1 and P-selectin remained significantly associated to FMD.

Conclusions: Our data confirm the presence of ED in LC patients, as indicated by the significant increase in serum MED and by FMD reduction observed in LC patients. All these parameters show also a significant correlation with the severity of liver disease. Significant correlation and association of FMD with serum MED values also suggest that FMD may be a reliable marker of ED in patients with LC.

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A GENETIC VARIANT IN THE PROMOTER OF PHOSPHATE ACTIVATED GLUTAMINASE (GLS) GENE PREDICTS THE RISK OF DEVELOPING HEPATIC ENCEPHALOPATHY

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Background: Hepatic encephalopathy (HE) is one of the major complications of liver cirrhosis and impairs patients’ survival. The exact pathogenesis of HE is unknown. Recently an association between a microsatellite in the promoter region of the phosphate activated glutaminase (GLS) gene and the risk of developing HE has been detected by Romero-Gomez et al. (Ann Int Med 2010). Here, we aim to assess whether the described GLS variant increases the risk of developing HE in cirrhotic patients.

Patients and Methods: We recruited 158 patients (104 males, 54 females; mean age 59 years) with liver cirrhosis mainly due to alcoholic liver disease (59%) or chronic viral hepatitis (19%). The mean MELD score at the time of admission was 14 (range 6–35); 61% of the patients presented with Child–Pugh scores B or C. In all patients, HE was quantified by critical flicker frequency (CPF), and individuals with CPF ≤39Hz were regarded as cases. The GLS variants were genotyped by PCR-based assays with 5-nucleotide and fluorescence detection.

Results: (significant, p<0.05): Mean CPF value in our cohort was 38.98 Hz (range 26–58), and 53% of the patients displayed abnormal CPF results. GLS genotype distributions were consistent with Hardy-Weinberg equilibrium. Genetic variants of the GLS microsatellite classified in homozygous minor, homozygous major and heterozygous alleles were carried by 32 (20%), 51 (32%) and 75 (48%) individuals, respectively. CPF values significantly differed between the three groups (ANOVA). Genotype distribution of
patients with minimal HE or grade I HE in comparison to patients without HE provided evidence for an association between the homozygous major GLS variant and the development of HE. In multivariate analyses homozygous carriers of the major GLS variant had a significantly higher risk than heterozygous patients to develop HE independent of age and presence of transjugular intrahepatic portosystemic shunt.

Conclusions: The genetic analyses demonstrate that homozygous carriers of the major GLS variant display significantly lower CFF results. Our findings support a potential role of variant GLS in the development of HE. This could be used for the identification of susceptible patients and for prevention of complications.

217 TIPS CREATION IS TOLERATED BY PATIENTS WITH PORTAL VEIN THROMBOSIS WITH HIGH MELD SCORES
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Background and Aims: Non-occlusive and total portal vein thrombosis (PVT) develops in patients with cirrhosis due to impaired and stagnated flow [1]. Transjugular intrahepatic portosystemic shunt (TIPS) has been proposed as a treatment due to its ability to restore portal vein (PV) flow through a low resistance shunt. TIPS is generally contraindicated in patients with high MELD scores due to concern for further deterioration in liver function [2]. The purpose of this study is to compare clinical outcomes in patients with non-occlusive and total PVT receiving TIPS with low (<15) and high (≥15) MELD scores.

Methods: A single-center, retrospective chart review of 28 patients undergoing TIPS with non-occlusive and total PVT was conducted. The primary endpoint was 30- and 90-day mortality. Secondary outcomes included change in MELD score, number of hospitalizations for overt hepatic encephalopathy (HE), and change in PV patency. Patients with non-occlusive and total PVT with MELD scores <15 and ≥15 were compared using the Student’s t-test and the Kaplan–Meier method.

Results: Baseline MELD scores were 15.3±5.0 and 16.5±7.1 among non-occlusive and total PVT cohorts, respectively (p=0.63). Survival was 100% and 100% for those with MELD score <15 versus 100% and 86% for those with MELD scores ≥15 at 30 and 90 days, respectively (p=1.0, p=0.35). Change in MELD score was 3.0±5.1 and 0.3±4.6 for those with MELD scores <15 versus 0.3±5.9 and 0.5±11.5 for those with MELD scores ≥15 at 30 and 90 days, respectively (p=0.21, p=0.95). Patients with MELD <15 had an average of 0.7 as compared to 1.2 hospitalizations/person/year for overt HE among those with MELD ≥15 (p=0.20). Of patients with non-occlusive PVT, 30% (n=7) achieved complete resolution of thrombus on follow-up imaging at 6 months.

Conclusions: Patients with non-occlusive and total PVT with high MELD scores tolerated TIPS better than expected. This may be a consequence of decreased dependence of the liver on portal blood circulation in these patients.

Reference(s)

218 THE MATERNAL AND INFANTILE PROGNOSIS OF PREGNANT WOMEN COMPLICATING CIRRHOSIS DUE TO HEPATITIS B
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Background and Aims: Pregnant women complicating cirrhosis were difficult to fecundate because of abnormal metabolism of estrogen. Cirrhosis and pregnancy may affect each other. This study aimed to analyse the maternal and infantile prognosis of women with cirrhosis due to Hepatitis B virus (HBV).

Methods: Analyse the clinical symptoms, complications, laboratory examinations, outcome of pregnancy of 54 cases with cirrhosis since 1998–2011 in the Second Affiliated Hospital of Southeast University, Nanjing, China.

Results: The average gestational age was 28.3±7.4 weeks. All of them had some fatigue and gastrointestinal symptoms and accompanied with jaundice (n=16), ascites (n=22), esophageal varices bleeding (n=4), hepatic encephalopathy (n=4), splenectomy and portosystemic shunt progenation. Their serum hepatitis B surface antigen were all positive and 42 of them were positive in HBV deoxyribonucleic acid too. The average of alanine aminotransferase was 85±19U/L, total bilirubin was 57.9±22.6 μmol, hemoglobin was 8.3±2.6 g/L, prothrombin time was 18.5±6.6 seconds. They encountered abortion (n=16), prematurity (n=8), full-term births (n=30) including 8 cases of vaginal delivery, 30 cases of cesarean section. Occurrence of pregnancy-induced hypertension in 12 cases, postpartum hemorrhage in 22 cases with an average of 743±365 ml bleeding and the maximum of 2800 ml, 3 of which eventually died of hepatic encephalopathy due to bleeding and infection. Meanwhile, stillbirth occurs in 2 cases, fetal distress in 26 cases, neonatal asphyxia in 11 cases, low birth weight in 12 cases with the average weight of 2060±410g.

Conclusion: Cirrhosis and pregnancy affect each other adversely. Cirrhosis not only increased the complications, but also increased the mortalities of mothers and their babies. Before delivery, clotting disorders and hypoproteinemia must be corrected and puerperium hemorrhage and infection should be prevented to reduce the occurrence of hepatic encephalopathy and even death.

219 PA SYSTOLIC PRESSURE BY RIGHT HEART CATHETERIZATION OFFERS ADDITIVE VALUE TO THE MELD SCORING SYSTEM TO PREDICT OUTCOMES IN PATIENTS WITH END-STAGE LIVER DISEASE
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Background: End stage liver disease patients often have concomitant pulmonary hypertension. The current MELD scoring system does not take into account hemodynamic parameters. The aim of this study was to assess the value of hemodynamic parameters in predicting outcomes.

Methods: A total of 215 charts of patients with end stage liver disease undergoing evaluation for liver transplant were retrospectively reviewed. Cardiovascular profile including right and left heart catheterization at evaluation were retrieved. Primary outcome was death at 1 year and censored for transplant, total 195 patients included. ROC curves were constructed and optimal cutoff values determined, p values <0.05 considered significant.

Results: Mean age at evaluation was 55±10, with 63% being male, and 47% Caucasian, 17% with DM and 6% with stable CAD. The primary diagnosis in this patient population was Hepatitis C (25%). Mean MELD score 213±8, mean Ejection Fraction was 66±1, mean PA systolic pressure (PASP) was 33±12 mmHg, mean Cardiac Output 8.2±2.5 L/min. PASP had an AUC of 0.83 (p < 0.0001) with an optimal cut-off >37 mmHg, sensitivity 84%, specificity 76% with a likelihood ratio of 4.56.

Conclusions: The hemodynamic assessment in a patient with end-stage liver disease has independent and additive value to the MELD scoring system.
PROGNOSTIC BENEFIT OF THE ADDITION OF AN ELECTROENCEPHALOGRAPHIC (EEG) INDEX TO THE MELD SCORE: THE MELD-EEG

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Background and Aims: A reduced EEG mean dominant frequency (MDF <7.3 Hz) is indicative of hepatic encephalopathy (HE). Since HE is not reflected in the MELD score and is an important prognostic parameter, the aim of this study was to assess the prognostic benefit of the addition of an EEG-based index to the MELD score.

Methods: 392 consecutive patients with decompensated cirrhosis underwent an EEG with automated MDF determination. MELD was calculated at the time of EEG. Patients were excluded if they had advanced hepatocellular carcinoma, HE ≥ grade III or significant comorbidity. They were monitored for up to 18 months (median 12 months) in relation to the occurrence of death/liver transplantation. The prognostic value of the stand-alone/combined MELD and MDF indices was calculated using standard survival analysis techniques (patients transplanted for hepatic decompensation were considered dead on the day of transplantation, those transplanted for hepatocellular carcinoma were censored). The findings were validated using a split sample technique: the Cox regression curve was re-calculated in a random sample of 259 patients, and the remaining 133 served as a test group.

Results: During the follow-up period, 107 patients died/were transplanted for hepatic decompensation. Both the MELD and the MDF predicted mortality on Kaplan–Meier analysis, and both were independent predictors of mortality on a Cox model.

<table>
<thead>
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<th>Variable</th>
<th>beta</th>
<th>SE (beta)</th>
<th>Wald T</th>
<th>P</th>
<th>O.R.</th>
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<td>MDF</td>
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<td>0.068</td>
<td>20.2</td>
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Based on Cox regression parameters, a novel prognostic index was devised: MELD-EEG=0.087*MELD-0.306*MDF. On ROC-curve analysis, the MELD-EEG had a higher prognostic accuracy in predicting 12- and 18-month mortality compared to MELD (AUC12m=0.69±0.03 vs. 0.62±0.04, p=0.016; AUC18m=0.71±0.03 vs. 0.64±0.03, p=0.018) and had higher Youden index (12 months: 0.31 vs. 0.18; 18 months: 0.35 vs. 0.20). On validation, no significant differences were observed between the reference and test groups.

Conclusion: The addition of an automatically obtained EEG-based index improves the prognostic accuracy of MELD. Confirmation of these findings is underway.

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DIAGNOSTIC VALUE OF PRESEPSIN IN CIRRHOTIC PATIENTS

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Introduction: The natural course of chronic liver disease is often complicated by acute episodes of potentially reversible decompensation, triggered by a precipitating event such as an infection. Presepsin (sCD14) has been identified as a protein whose levels increase specifically in the blood of patients with bacterial infection. Pathfast Assay System (PAS) is able to detect the levels of sCD14. In this study, we evaluated the clinical performance of PAS and its usefulness in the early diagnosis of bacterial infection in cirrhotic patients.

Materials: Twenty-five patients with were enrolled in this study. Mean age of patients was 49.5 years, 12 female and 13 male. The heparinized whole blood for PAS was used in the evaluation of bacterial infection [T0]. The PAS was repeated after 48h [T1]; at 96h [T2]; at 144h [T3] than at 15 days [T4] for monitoring the clinical responses to therapeutic interventions. Blood cultures were performed in all patients at moment that PAS test was performed. The assay time was 15 min using a sample volume of 100µL. A value >377 pg/mL was considered positive as indicated by manufacturers.

Results: Sixteen patients resulted positive to PAS. The mean sCD14 level was 1854±1744 pg/mL. Microbiological findings confirmed the presence of bacterial infections within 72±4.8h from enrolment in all 16 positive patients. Presepsin level at [T1], [T2] remained stable in five patients. These 5 patients (31%) did not respond to empiric antibiotic treatment and after antibiogramme results, the antibiotic therapy was modified. When the PAS was performed, 47% of patients no showed signs or symptoms of bacterial infection.

Conclusions: Early diagnosis is essential to improving the results of treatment of infections in particular in cirrhosis where infection represents one of the primary complications that lead
to decompensated form. PAS test highlighted a complete sensitivity (100%) in showing the presence of infection in a very short time (15 min), confirmed by the results of positive blood cultures. A greater number of patients is necessary to confirm these data.

223 COST-EFFECTIVENESS OF A NEW MODEL OF SPECIALISTIC CAREGIVING FOR OUTPATIENTS WITH CIRRHOSIS AND ASCITES; A PROSPECTIVE STUDY

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Background and Aims: The development of ascites in patients with cirrhosis is associated with a high rate of health care utilization. New models of specialist caregiving support are necessary to optimize its management. The aim of the study was to evaluate the “Day management check-up” as a new model of specialist caregiving support based on a series of diagnostic facilities performed in real time and on the integrated activity of hepatologists, dedicated nurses and primary physicians, opposed to standard care in outpatients with cirrhosis and ascites.

Methods: 100 cirrhotic patients recovered in our hospital for complication of their liver disease were allocated, after dimission, to the “Day management check-up” group (40 patients, group 1), or to the “Standard outpatient care” group (60 patients, group 2), and followed prospectively as outpatients up to death or for at least 12 months. Patients of both groups could access to “Day hospital” when an invasive procedure was required. In group 1 the “Day management check-up” and the “Day hospital” taken together defined the “Day management program”.

Results: Twelve-month mortality was higher in group 2 than in group 1 (45.7% vs 23.1%, p < 0.025). The rates of 30-day readmission and 12-month readmission were also higher in group 2 (42.4% vs 15.4%, p < 0.01 and 71.2% vs 46.2%, p < 0.025 respectively). The global cost attributable to the management per patient-month of life was lower (1479.19±2184.43 Euros) in group 1 than (2816.13±3893.03 Euros) in group 2 (p < 0.05).

Conclusions: The study suggests that this new model of specialist caregiving, based on real time diagnostic facilities and on an integrated team activity reduces 12-month mortality in patients with cirrhosis and ascites. The favorable cost profile, due to a more rational use of hospital services, provides further evidence of the potential value of this model.

224 NON-INVASIVE PREDICTION OF UPPER GASTROINTESTINAL BLEEDING BY RUPTURE OF ESOPHAGEAL VARICES (ROV) USING LIVER AND SPLEEN STIFFNESS (AIXPLORER), LIVER STIFFNESS BY FIBROSCAN AND FIBROTEST

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Background: Cirrhotics frequently undergo endoscopic screening for oesophageal varices (OV) to establish primary prevention for ROV. FibroTest (BioPredictive) and the liver stiffness measure (LSM) by Fibroscan (Echosens) have a good NPV for excluding the diagnosis of OV grade II-III (LiverInternational-2006, JHepatol-2009). The spleen stiffness by Fibroscan could predict the OV presence but not the grade (JGastroenterolHepatol-2011).

Aims: To evaluate the diagnostic values for OV presence and for bleeding by ROV of the liver-stiffness (SWE) and spleen-stiffness (SWE-S) by Aixplorer (Supersonic Imagine) compared to Fibrotest and LSM.

Methods: Chronic liver disease patients have undergone prospectively LSM (M and XL-probes), Fibrotest, SWE, SWE-S and LSM. Applicability (App) was determined: for LSM, excluding failures and unreliable LSM (success rate >80%, <10LSM, ratio IQR/median-LSM>30%); for Fibrotest, according to the manufacturer’s recommendations. Non-App minimum, maximum and mean SWE and SWE-S by Aixplorer, were considered in case of doubt, failures and minimum stiffness<0kPa. Diagnostic values were expressed by AUROCs.

Results: 71-patients had OV-screening in a time interval of 0.1 months (0–1.2 years) from the non-invasive assessment of fibrosis. Non-App were excluded: Fibrotest 3/71 (4.2%), LSM (M/XL) 7/71 (9.9%), SWE-S 4/71 (5.6%) and SWE 10/71 (14%). 57-patients all App were included: age 56 years, 81%men, cirrhosis as per Fibrotest 38/57 (67%) and LSM 40/57 (70%), 26-patients had OV grade-III or grade-II with red signs (II-RS) and 16-patients were admitted for ROV. Median (range) stiffness by Aixplorer were: SWE 12.4kPa (1.5–146), SWE-Max 23.3kPa (4–300), SWE-S 24.9kPa (0.4–149) and SWE-S-Max 76kPa (1.2–179). Only LSM (p = 0.0003) and Fibrotest (p = 0.04) excluded OV (absence vs OV grade-I or higher), AUROCs: 0.76 vs 0.65 (p = 0.17) with PPV 0.97 for LSM (cut-off 20kPa) and Fibrotest 0.94 (cut-off 0.80). For excluding OV, both LSM and Fibrotest had higher AUROCs than SWE (0.62), SWE-Max (0.62), SWE-S (0.54) and SWE-S-Max (0.53), all p < 0.05. For OV grade-III and grade-II-LS AUROCs were: SWE-Max 0.77, SWE 0.76 and LSM 0.73, all higher than the SWE-S-Max, SWE-S and FT (all p < 0.05). In 10 cirrhotics with OV grade-III, only SWE-S-Max was predictive for ROV (AUROC 0.84, p = 0.01).

Conclusion: Previously validated LSM and Fibrotest had the best PPV to exclude OV. For OV grade-III and grade-II-LS liver-stiffness was better than the spleen-stiffness. Maximum spleen stiffness by Aixplorer was associated with ROV in cirrhotics with OV grade-III.

225 THE PROTEIN FINGERPRINT TECHNOLOGY REFLECTS LIVER FUNCTION AND DETECTS CLINICALLY SIGNIFICANT PORTAL HYPERTENSION IN PATIENTS WITH ALCOHOLIC LIVER CIRRHOSIS

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Introduction: Liver fibrosis increases intrahepatic resistance, a crucial step in development of portal hypertension. Fibrosis is a dynamic process, which requires permanent remodeling of extracellular matrix (ECM). Small fragments of ECM, the Protein Fingerprint markers, generated during this remodeling and released into the circulation might reflect portal hypertension. This study investigates the relationship between a panel of these markers and clinical data, and its ability to predict the hepatic venous pressure gradient (HVPG).

Methods: In plasma from 94 cirrhotic patients and 20 controls without liver disease ECM degradation markers (C1M (type I collagen), C3M (type III collagen), C4M (type IV collagen), C5M...
(type V collagen), C6M (type VI collagen), BGM (Biglycan), ELM (Elastin), and CRPM (CRP), and ECM formation markers (P3NP (type III collagen) and P4NP7S (type IV collagen)) were measured.

Results: The markers measured in hepatic venous blood correlated to the level measured in arterial blood (R=0.89–0.98; p<0.0001). In hepatic venous blood, all markers correlated directly to Child Score and MELD, and inversely to ICG clearance and serum albumin, strongest with P3NP (R=0.46, 0.48, -0.53, and -0.46; respectively; p<0.0001). All markers except ELM were inversely correlated to hematocrit and to hemoglobin, especially C4M and C1M showed strongest correlations (R= -0.31; -0.40; respectively; p=0.001- 0.0001). In both arterial and hepatic venous blood all markers except CRP correlated to HVPG (e.g. P3NP for both sites R=0.47, p<0.0001). A multiple regression analysis including P3NP, C6M and MELD improved the correlation (R=0.62, p<0.0001). P3NP could clearly separate controls from HVPG levels <10 mmHg (p<0.01) and those with HVPG <10 mmHg from those with HVPG levels ≥10 mmHg (p<0.0001). C4M, C5M, and ELM were all significantly higher in patients with HVPG levels ≥12 mmHg compared to lower HVPG levels (p<0.01–0.0001).

Conclusion: The Protein Fingerprint markers reflect liver function and stage of disease. A strong correlation between the markers measured in hepatic venous and arterial blood was observed. A biomarker model in combination with clinical scores predicted HVPG and separated clinical relevant HVPG thresholds non-invasively. Therefore these markers and their models are suitable for non-invasive evaluation of portal hypertension in cirrhosis.

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LONG-TERM ANTIBIOTIC AND PROTON-PUMP INHIBITOR USE ARE STRONG PREDICTORS OF RECURRENT INFECTIONS IN CIRRHOSIS: A PROSPECTIVE MULTI-CENTER STUDY FROM NACSELD


Methods: Infections in cirrhosis negatively impact prognosis and transplant candidacy. However, in survivors, factors associated with risk of recurrent infections remain unclear.

Aim: To determine risk factors for recurrent infections in cirrhotic patients following resolution of initial infection.

Methods: NACSELD comprises North-American tertiary-care hepatology centers that prospectively enter data on hospitalized cirrhotics into a centralized database. Data collected include demographics, co-morbidities, infections, and medications. This report focuses on 6-month post hospital discharge outcomes of infected cirrhotic patients. Descriptive statistics and logistic regression modeling using recurrent infection as the outcome were performed.

Results: Thirty-eight patients were men and age was ranged 29–89 years. Etiology of cirrhosis was alcohol/viral hepatitis/both/others in 36/14/9/6 patients. AI was present in 22 patients (44%). No significant difference was observed regarding age, gender, mean arterial pressures, and heart rates between patients with and without AI. The etiology of cirrhosis and degree of alcohol consumption did not affect presence of AI or basal and peak serum cortisol levels either. Total bilirubin and prothrombin time were higher, while albumin and cholesterol levels were lower in patients with AI than those without AI. However, in multivariate analysis, there was no independent predictor of AI. The prevalence outcomes reported. In a median follow-up of 4.5 months, 82 (47%) had recurrent infections. 67% of the 175 cirrhotics were on proton-pump inhibitors (PPI), 47% on rifaximin and 45% on SBP prophylaxis. Patients with recurrent infections were older (58 vs. 55 yrs, p=0.04) with similar Child (9.6 vs. 10.4, p=0.10) and MELD scores (19 vs. 19, p=0.12). There were significantly higher rates of PPI and antibiotic use in patients with recurrent infections (PPI: 84% of patients with recurrent infection vs. 52% uninfected, p=0.0001, rifaximin: 67% recurrent infection vs. 30% uninfected, p<0.0001 and SBP prophylaxis: 61% recurrent infection vs. 31% uninfected, p<0.0001). There were no significant differences in MELD/Child scores between patients on PPI, rifaximin or SBP prophylaxis and the rest.

In conclusion, even after resolution of the index infection, cirrhotic patients remain at high risk for recurrent infections (47%). Patients at highest risk for recurrent infections are previously infected older patients, and those on rifaximin, PPI, and SBP prophylaxis. Primary and secondary infection prevention strategies are needed to improve patient outcomes.
of AI increased according to severity of liver disease (25, 44, and 73% in Child–Pugh class A, B, and C respectively; p < 0.047). There were negative correlations between Child–Pugh score and both basal cortisol (γ = –0.377, p = 0.007) and peak cortisol levels (γ = –0.373, p < 0.005) (Figure).

Conclusions: In this study, AI was frequent in stable cirrhotic patients without infections or hemodynamic instability. AI was not related to the etiology of cirrhosis or alcohol consumption, either. AI tended to be associated with only severity of liver disease.

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THE TRIANGULAR RELATIONSHIP BETWEEN LEAKY-GUT, MICROBIOTA AND SPONTANEOUS BACTERIAL PERITONITIS IN LIVER CIRRHOTIC PATIENTS
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Introduction: The impairment of gut barrier and microbial translocation are frequent in liver cirrhotic patients, predisposing to infectious complications such as spontaneous bacterial peritonitis (SBP). However, not all cirrhotics who present a leaky-gut develop SBP. Little is known about the role of small intestinal bacterial overgrowth (SIBO) in SBP development.

Aim: To assess if there is a cut-off of intestinal permeability (IP) necessary to develop SBP in liver cirrhotic patients and to investigate role of SIBO in this process.

Material and Methods: 28 patients were enrolled in this study (8 Child-A/9 Child-B/11 Child-C; mean age 58yrs, all viral etiology). SIBO was diagnosed by glucose-hydrogen breath test, IP by the (51)Cr-EDTA test (sum of Cr excreted in urine and ascites, UACr).

Results: UACr values ranged between 1.8% and 12% (mean 4±2.3%) and were linearly associated to Child score (p=0.026). We identified 3 subgroups within the population with a similar UACr (Cluster and mean UACr: C1= 11%, C2= 6%, C3= 3%); mean UACr was higher in Child-C than in Child-A patients (Bonferroni post-hoc test p=0.037; Fig 1). 12/28 (42.9%) patients had at least one episode of SBP at the time of enrollment, 4/9 (44.5%) Child B and 8/11 (72.8%) Child C. 81% of patients with no episodes of PBS belonged to the C3, and 66.7% of those with at least an episode of SBP belonged to the C2 and C3 (p=0.028; chi-square 7.116). SIBO was diagnosed in 9/28 (32.2%) patients, 3/8 (37.5%) Child A, 7/9 (77.8%) Child-B, 9/11 (81.9%) Child-C. All patients with at least an episode of SBP except one (11/12, 91.6%; Fig 2) were diagnosed with SIBO, but the association did not reach the statistical significance.

Conclusions: Little is known about the role of small intestinal bacterial overgrowth (SIBO) in SBP development. Further data are needed to establish the indication to a preemptive decontaminating approach in cirrhotic patients with ascites.

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ASSESSMENT OF LIPOPROTEIN SUBFRACTIONS IN LIVER CIRRHOSIS: RELATIONSHIP WITH BASAL AND ACTH INDUCED CORTISOL
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Background and Aims: In liver cirrhosis, hepatoadrenal syndrome has been described as a progressive impairment in the adrenocortical reserve, together with deficient production of glucocorticoids resulting in adrenal insufficiency (AI). A lack of substrates has been suggested as one of the pathogenetic mechanisms causing adrenal insufficiency. We studied lipoprotein levels in cirrhotic patients and in control group in order to evaluate the role of lipoprotein for steroidogenesis in cirrhosis and its correlation to adrenal function.

Methods: A total of 82 patients with cirrhosis and 37 normal volunteers matched by sex and age were enrolled. The severity of liver disease was graded by the Child–Pugh score. We measured in all subjects total serum cortisol (TC), basal and ACTH induced cortisol (Cortisol 0′, Cortisol 30′), delta cortisol, defined as the difference between peak and basal cortisol (Cortisol 30′ – Cortisol 0′), plasma total cholesterol, HDL, LDL triglycerides and apolipoprotein A1. Moreover, HDL subfractions were measured by gradient gel electrophoresis. Adrenal function was assessed by performing the Low Dose Short Synacthen Test (LDSST). AI was defined by a total serum cortisol (TC) <18 mg/dl at 20 or 30 min after injection of 1 mg of tetracosactrin.

Results: Cirrhotic patients showed a significant reduction of total cholesterol (p<0.0001), total HDL (p<0.001), LDL (p<0.001), triglycerides (p<0.001) and Apo lipoprotein A1 (p<0.0001) levels compared to the control group. Among the components of HDL subfractions, HDL3 showed a significant reduction (p<0.0001), while HDL2 was higher in the cirrhotic patients. Adrenal insufficiency (AI) was noticed in 26 patients. Total cholesterol (108.1±30.3 vs 126.3±36.7), triglycerides (67.1±17.9 vs 95.1±32.6) and Apo A1 (59.9±26.6 vs 83.3±33.5) were significantly reduced in AI group compared to the cirrhotics with normal adrenal function. In contrast, total HDL (21.5±10.3 vs 25±15, p=ns), HDL 2 (0.80±0.15 vs 0.82±0.12, p=ns) HDL3 (0.19±0.15 vs 0.18±0.12 p=ns) did not differ in these two groups. Delta cortisol, defined as the difference between peak and basal cortisol (Cortisol 30′ – Cortisol 0′), was closely related to total cholesterol (r=0.31, p=0.005), and Apolipoprotein A1 (r=0.38, p=0.0007).

Conclusions: Cirrhotics patients showed a significant reduction of plasmatic lipoprotein levels that worsen in adrenal insufficient patients, directly related to adrenal depletion. In this setting a lack of Apolipoprotein A1 appears to play a primary role in cortisol deficiency.
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COMPARISON OF SIX SERUM TESTS, LIVER STIFFNESS AND HVPG IN THE PREDICTION OF CLINICAL DECOMPENSATION IN CHRONIC LIVER DISEASES
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Background and Aims: The prognosis of chronic liver diseases (CLD) is determined by the presence of portal hypertension (PHT). The standard method for PHT diagnosis is hepatic venous pressure gradient (HVPG). Recently, non-invasive methods were proposed to evaluate patients with CLD. The aim of this study is to compare non-invasive tests with HVPG in terms of diagnosis of clinical significant portal hypertension (CSPHT) and esophageal varices and, for their capacity of clinical decompensation prediction.

Methods: Two hundred thirty-eight patients underwent HVPG measurement along with serological test (AST/ALT index, APRI, Lok, FIB-4, GUCI, Risk score) and liver stiffness (LS) measurement on the same day. A subgroup of 100 patients was follow-up for 2 year or until decompensation. Decompensation was defined as variceal haemorrhage, ascites, hepatic encephalopathy, hepatocellular carcinoma and/or sepsis. The patients were censored at 2 years or at the time of the first decompensation, liver transplantation, or death. PHT related complications (variceal bleeding and/or ascites) were also studied separately.

Results: In the whole studied population, cirrhosis was found in 142 patients, from which 93 (65%) had EV and 103 (72%) had CSPH. At the time of inclusion all cirrhotic patients were compensated. For CSPHT and esophageal varices the Lok score was the best serum test, AUROC=0.86 and 0.83, respectively. However, LS is the best non-invasive methods for these end-points (AUROC=0.95 for CSPH and 0.90 for esophageal varices). The best correlation with CSPH was for LS (r=0.769, p<0.0001), followed by the Lok score (r=0.616, p<0.0001). Worse correlation was with APRI (r=0.377, p<0.0001).

During the follow-up 41 patients suffered a clinical complication within a mean period of 491±282 [8–730] days. FIB-4 is the best serological test, which predicts clinical decompensation (AUROC=0.84), but Lok score predicts slightly better the occurrence of PHT related complications (AUROC=0.77). The worse performance had the AST/ALT index in predicting either clinical decompensation and PHT related complications.

Conclusion: LS is the most efficient non-invasive method in diagnosis and prognosis of CLD. The Lok and FIB-4 scores are good non-invasive alternatives for diagnosis and prediction of clinical decompensation.

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NOVEL NONINVASIVE MEASUREMENT OF HEPATIC VENOUS PRESSURE GRADIENT AND PORTAL PRESSURE FROM ANATOMIC CT ANGIOGRAPHY
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Background and Aims: Assessment of hepatic venous pressure gradient (HVPG) and portal pressure (PP) is significant in diagnosing portal hypertension (PHT) and determining the treatment for cirrhotic patients. Invasive HVPG and PP measurements can not be performed routinely and repeatedly. This study intends to develop a novel noninvasive assessment of HVPG (HVPGni) and PP (PPni) computed from three dimensional (3D) hepatic portal venous models.

Methods: HVPGni and PPni were calculated through 3D hepatic portal venous models reconstructed from CT angiography. Finite element and computational fluid dynamics in ANSYS software were applied to analyze the pressure distribution in vitro. Clinical data of a compensated cirrhotic patient were calculated to test the feasibility and accuracy of the novel measurement with diagnostic results of Doppler ultrasound (DUS) and CT angiography as reference standards.

Results: CT images showed that the diameter of portal venous was around 1.6cm and the average velocity measured by DUS was 12.5cm/s, both of which indicated a severe PHT. According to calculation through the novel assessment, HVPGni and PPni of the decompensated cirrhotic patient were 65.2Pa (Figure 1) and 3535.0Pa (Figure 2), which were generally consistent with the previous diagnosis of PHT. Besides, the pressure (Figure 3.I) and velocity (Figure 3.II) distribution of different cross-sections of portal venous were simulated in vitro.

Figure 1.

Figure 2.
**Conclusions:** HVPCni and PPNi were in vitro quantified successfully. Although comparisons of noninvasive index with invasive ones are still needed, the novel evaluation of PHT from anatomic hepatic portal venous models has made up deficiencies and provides a potentially noninvasive and repeatable approach for the diagnosis of PHT.

**232 PARALLEL TIPSS FOR THE MANAGEMENT OF SHUNT INSUFFICIENCY IN PATIENTS WITH COMPLICATIONS OF PORTAL HYPERTENSION: A TERTIARY LIVER UNIT 19 YEAR EXPERIENCE**

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**Background:** Transjugular Intrahepatic Portosystemic Shunts (TIPSS) insufficiency can be addressed with a side placement of another TIPSS beside the original (“parallel” technique) thus improving portosystemic pressure gradient (PPG). There is a paucity of data assessing the efficacy of this technique.

**Aim:** To assess the efficacy of parallel TIPSS in a large UK tertiary referral centre.

**Methods:** A retrospective study was performed from patient electronic radiology and laboratory databases. Parallel TIPSS were performed over a 19 year period in a single centre.

**Results:** 11 patients (8M:3F) were identified (2% of all TIPSS procedures). Mean age at time of parallel TIPSS was 48.6 (±13.7). Background aetiology of portal hypertension included: 5 ALD, 2 PSC, 2 PBC, 1 liver graft failure, 1 non-cirrhotic portal hypertension (NCPH). Indications for index TIPSS (5 covered stents) were: 4 Oesophageal variceal (OV) haemorrhage, 3 gastric variceal (GV) haemorrhage, 1 stomal variceal haemorrhage and 3 for refractory ascites. At time of 1st TIPSS, documented mean PPG was 16.6 (±7.71) and post TIPSS 10.8 (±7.35) mmHg. Median time between index TIPSS and parallel TIPSS insertion was 72 (IQR 4–1122) days. Prior to parallel stent placement, 7 patients had dilatation of the index TIPSS. At parallel TIPSS, the mean initial PPG was 16.0 (±7.40) post procedure 6 (±2.28) mmHg. 63% had covered stent as the parallel TIPSS. One patient had transient encephalopathy, but no other complications were encountered. Nine patients had a resolution in symptoms. One patient had ongoing GV bleeding requiring Thrombin injection and 1 patient had ascites with no flow in parallel TIPSS 4 days post-procedure. Secondary patency was 82% by the end of follow-up period with a median number of interventions of 1.5 (IQR 1–3).

**Conclusions:** Parallel TIPSS is a safe and effective method to treat TIPSS insufficiency. The majority of patients not only had a good haemodynamic result, but also resolution of symptoms.

**233 SOLUBLE VEGFR-2 SERUM MAY BE A PREDICTIVE FACTOR FOR PROGRESSION OF CHRONIC LIVER DISEASE AND HEPATOCELLULAR CARCINOMA**

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**Background:** Angiogenesis process in chronic liver disease (CLD) is occurred as result from hypoxia of necro-inflammation hepatocyte. The alteration serum VEGF and sVEGFR-2 reflected intrahepatic neo-vascularization and induced hepatocellular carcinoma (HCC). The aim of study is to identify sVEGFR-2 serum as a predictor of severity diseases in CLD and HCC patients.

**Methods:** A prospective study was conducted in LCD and HCC patients with un-matching consecutive sampling. Liver Cirrhotic (LC) and HCC patients were case subjects, and Chronic Hepatitis (CH) patients as control subject. Healthy subjects were participated for assessing normal range. All subjects were enrolled during 2 years study (2010–2012) at Dr. Sardjito General Hospital, Yogyakarta, Indonesia. Physical examination, liver function test, α-fetoprotein, viral sero-marker, ultrasound/CT imaging, and fine needle biopsy for HCC were performed to define the diagnosis. Serum of sVEGFR-2 was examined using Quantikine® HS kit human immunoassay (R & D System, Minneapolis, MN, USA). Data were analyzed by computer. ANOVA test, spearman correlation, ROC curve and OR were calculated with significant value p < 0.05 and 95% CI.

**Results:** 113 subjects were enrolled (33 HCC; 32 LC; 20 CH; and 28 healthy), 70 (61.9%) male and 46 (38.1%) female, and no difference mean of age (52±11 yr.). By ANOVA and posthoc tests, there were significant difference between group for sVEGFR-2 level (HCC = 8016.51±1785.55 pg/mL; LC = 7117.84±1554.40 pg/mL; CH = 9225.67±2231.33 pg/mL; p < 0.001; Healthy = 10038.95±1827.12 pg/mL). There was significant correlation between sVEGFR-2 serum with platelet count (r = 0.34; r = 0.39) and CPT score (r = −0.31; r = 0.38) in HCC and LC subjects. Significant correlation was showed in FIB4 score (r = −0.29) and albumin level (r = −0.30) in LC subject. Based on AUCROC the cutoff sVEGFR-2 level in HCC-LC was 7301.40 pg/mL; HCC-CH was 8177.45 pg/mL. Cutoff in LC-CH was used median level in CH (9192.15 pg/mL). The OR LC-HCC was 3.26, OR CH-HCC was 2.31, and OR CH-LC was 9.67.

**Conclusions:** The sVEGFR-2 serum can be used as predictor factor of HCC and LC progression in chronic liver diseases. There were good correlation between level of sVEGFR-2 with severity diseases (albumin, platelet, CP-score and FIB4-score).
FUNCTIONAL MAGNETIC RESONANCE IMAGING AND STROOP TEST TO STUDY FRONTAL CORTICAL FUNCTION IN PATIENTS WITH CIRRHOSIS AND MINIMAL HEPATIC ENCEPHALOPATHY

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Background: Minimal hepatic encephalopathy (MHE) is a neurocognitive dysfunction without clinical symptoms. The impairment of attention, executive function and psychomotor skills may impair complex activities. These patients show attention deficit and mild cognitive impairment probably related to altered metabolism in different brain regions mainly in the prefrontal and cingulate areas.

Aim: To assess the degree of activation in the frontal brain region in cirrhotic patients with MHE using the Stroop colour word test.

Patients and Methods: We included cirrhotic patients without overt hepatic encephalopathy and healthy subjects (control group) to validate the paradigm of the Stroop test in patients with cirrhosis. MHE was diagnosed using the psychometric hepatic encephalopathy score (PHES). We employed the Stroop task as the target stimulus to observe task-state brain activation using functional Magnetic Resonance Image (fMRI). The Stroop test was used to explore the area involved in the selective attention and the ability to suppress the automatic response in the anterior cingulate cortex (ACC).

Results: Nineteen cirrhotic patients (9 patients with MHE/10 without MHE) and 10 healthy subjects matched by sex, age and level of education (control group). Patients with and without MHE were similar in age, sex, Child-Pugh and MELD score and serum sodium. Patients with cirrhosis with and without MHE showed no differences in the ability to perform the Stroop task (P=NS). PHES values and values of the Stroop test showed a positive correlation in the limit of statistical significance (r=0.5, P=0.052). Both healthy controls and cirrhotic patients showed fMRI activation in the frontal brain area during the Stroop test. Patients with MHE showed a higher activation in the anterior cingulate cortex compared to patients without MHE (P>0.001). In the group of patients with cirrhosis the activation of these areas showed a negative correlation with serum sodium and a positive correlation with MELD score (P>0.001).

Conclusions: MHE is associated with increased activation of the anterior cingulate cortex during a Stroop test. This increment in activity can be explained as a mechanism to compensate for a higher difficulty in performing the same task (greater task-related effort).

PREDICTORS OF RESPONSE TO ANTICOAGULANT THERAPY IN CIRRHOSIS PATIENTS WITH PORTAL VEIN THROMBOSIS (PVT)

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Background and Aims: Anticoagulation has been demonstrated to be effective in the treatment of PVT; however, it is not known which factors predict the therapeutic response. The purpose of this study was to assess hemostatic status (pro- and anti-coagulant factors), and thrombus and patient characteristics as predictors of therapeutic efficacy of anticoagulation.

Patients and Methods: 46 cirrhotics with PVT who received anticoagulation therapy with LMWH were retrospectively evaluated. Nadroparin 95 IU/Kg was administered to all patients (40% dose reduction if <50.000×10⁹/L platelets). Interval between PVT onset and start of anticoagulation was estimated. All patients underwent thrombophilia screening and dosing of plasmatic pro- and anti-coagulation factors. Coagulation imbalance was further evaluated using the FactorVIII/Protein C ratio. Vessel recanalization was evaluated monthly using abdominal ultrasound and every 3 months by CT scan.

Results: 34 patients were male and mean age was 58±11 years. Etiology of cirrhosis was viral in 47.8% and alcohol-related in 32.6% of cases. Partial PVT was found in 36/46 patients. Estimated interval from appearance of PVT and start of anticoagulation was ≥6 months in 35/46, and >6 months in the remaining 11 cases. Thrombophilic mutations were found in 4 patients. Recanalization of the portal vein was obtained in 30 patients (24 complete recanalization) after a mean time of 4.5±3.1 months of therapy. No correlation was found between standard coagulation parameters, plasmatic activity of factors VII, IX, XI, AT, PS, PC, fibrinogen, or factor VIII/PC ratio, and thrombus disappearance. Likewise, repermeation did not correlate with the extension of PVT, presence of thrombophilic mutations, severity of liver disease, or etiology of cirrhosis. An interval between development of PVT and start of anticoagulation therapy >6 months was the only significant predictor of anticoagulation efficacy (93% versus 15.2%, p<0.001) with only 2 patients with older thrombus achieving recanalization after 6 months of therapy.

Conclusions: The interval between PVT occurrence and start of anticoagulation therapy is the only predictor of recanalization; on the contrary, hemostatic imbalance does not correlate with anticoagulant response. For patients with recent thrombus, continuation of anticoagulant therapy beyond 6 months could increase the possibility of repermeation.
present in most men. Blood levels of sexual hormones were similar in the alcoholic liver disease group compared to those of other etiology. In addition, low levels of DHEA-S were found in 97% of men. Total cholesterol and fractional cholesterol, precursors of sexual hormones correlated significantly with the level of total and free testosterone, free androgen index, SHBG and DHEA-Sulfate.

Conclusion: SD, an infra-estimated condition, is extremely common in cirrhotic patients awaiting LT. SD is likely a key factor in the impaired QOL typical of these patients. Factors associated with worsening of sexual function include advanced age, chronic spironolactone use and presence of anxiety disorders. Besides central hypogonadism, the reduced levels of DHEA, possibly due to adrenal dysfunction, is an aspect that deserves further investigation. Sexual dysfunction could, in part, be another manifestation of the recently coined “hepatoadrenal syndrome”.

238 BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS IS A BETTER METHOD FOR NUTRITIONAL ASSESSMENT IN PATIENTS WITH LIVER CIRRHOSIS
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Background and Aim: Traditional methods for nutritional assessment such as conventional bioimpedance analysis (BIA) and anthropometric measurements are unreliable due especially to ascites and peripheral edema. Bioelectrical impedance vector analysis (BIVA) has been used successfully for body composition and nutritional assessment in patients with fluid overload. The aim of this study was to evaluate the usefulness of BIVA for nutritional assessment compared to traditional methods.

Methods: We included 308 patients with liver cirrhosis. Nutritional status was evaluated by Body Mass Index (BMI) and BMI with ascites cutoffs, triceps skinfold thickness (TSF), mid arm muscle circumference (MAMC), serum albumin, conventional BIA and BIVA. We used descriptive statistics and Hoteling’s T2 was used to compare bioelectrical impedance vectors subgroups.

Results: Mean age was 52 years, the main etiologies were HCV, alcohol and cryptogenic. Table 1 shows prevalence of malnutrition according to each method; we found a wide-ranging prevalence of malnutrition among the different methods, the lowest prevalence was found with BMI and the highest with BIVA. Figure 1 shows RXc graph by Child–Pugh where we found malnutrition and subclinical fluid overload in early stages of the disease especially in males, and more marked malnutrition and fluid overload as the disease progressed both in males and females.

Table 1. Prevalence of malnutrition by each method

<table>
<thead>
<tr>
<th>Method</th>
<th>BMI</th>
<th>TSF</th>
<th>Ascites</th>
<th>MAMC</th>
<th>Albumin</th>
<th>BIA</th>
<th>BIVA</th>
</tr>
</thead>
</table>
| Child A        | 1.8%| 2.8%| 3.6%    | 16.9%| 28.6%   | 15.3%| 47.7%
| Child B        | 2.1%| 15.5%| 12.5%   | 37.8%| 85.4%   | 15.3%| 79.9%
| Child C        | 3.8%| 31.4%| 34%     | 48.6%| 93%     | 22.6%| 88.7%
| Females        | 3.6%| 6.7%| 15.4%   | 25.8%| 68.3%   | 19.5%| 62.7%
| Males          | 0.7%| 19.6%| 30.1%   | 37.4%| 67.7%   | 12.9%| 78.4%
| Total          | 2.3%| 13.8%| 13%     | 32.1%| 68%     | 16.5%| 69.8%

Figure 1. RXc graph by Child–Pugh.

Conclusion: BIVA was able to assess malnutrition without being biased by fluid retention and it was even able to evaluate hydration status in these patients, which suggests that this method is better than traditional methods and could be the standard method for nutritional assessment and fluid monitoring in cirrhotic patients.
INFLUENCE OF BMI ON THE RESPONSE TO BETA-BLOCKER THERAPY IN PRIMARY AND SECONDARY PROPHYLAXIS OF VARICEAL BLEEDING IN LIVER CIRRHOSIS


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Background: Patients with cirrhosis and high BMI have a higher rate of hepatic decompensation. So far no study evaluated the influence of the BMI on the response to beta-blocker therapy.

Methods: Retrospective analysis of patients scheduled for HVPG measurement for primary or secondary prophylaxis of variceal bleeding were included in this study. HVPG response was defined as decrease to <12 mmHg or >=20%.

Results: 239 patients (78.3% male) with a median BMI of 25.3 (IQR 22.9–29.0) were included. 4 patients were underweight (1.7%; BMI<18.5), 106 normal weight (45.3%; BMI 18.5–24.9), 77 patients overweight (32.9%; BMI25–29.9) and 47 obese (20.1%; BMI>30). Median baseline HVPG in these groups was 18 mmHg (IQR 14.3–22.5); 19 mmHg (IQR 16–22.3); 19 mmHg (IQR 16–24) and 19 mmHg (IQR 15–23), respectively (p=0.8).

In total 46.4% responded either to propranolol or carvedilol. There were 3 (75%) responder in the overweight group. 56 (52.8%) responder in the normal weight group, 28 (36.4%) in the overweight and 22 (46.8%) in the obese group (p=0.1).

Median overall HVPG drop in the different BMI groups were 3 (75%) responder in the underweight group. 56 (52.8%) responder in the normal weight group, 28 (36.4%) in the overweight and 22 (46.8%) in the obese group (p=0.1).

Median overall HVPG drop was 3 mmHg, median HVPG drop was 106 (55.3); 19mmHg (IQR 16–22.3); 19mmHg (IQR 16–24) and 19mmHg (IQR 15–23), respectively (p=0.8).

Conclusion: High BMI does not influence the response to beta-blocker therapy for primary and secondary prophylaxis of variceal bleeding in cirrhotic patients.

MEASUREMENT OF LIVER STIFFNESS IN CHRONIC LIVER DISEASES VERSUS SHEAR WAVE ELASTOGRAPHY (AIEXPLORER®): A VALID SUBSTITUTION FOR TRANSIENT ELASTOGRAPHY (FIBROSCAN®)?

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Background: Shear Wave Elastography (SWE; Aixplorer®, SuperSonic Imaging Aix-en-Provence) is a new method of liver stiffness measurement (LSM). Aim of this study was to compare SWE to transient elastography (TE; FibroScan®, Echosens, Paris).

Methods: SWE and TE were performed in the same session after an overnight fast. LSM was performed with the right arm in maximal abduction in the intercostal space of the mid-axilla line. For FibroScan 10 measurements were performed and median and IQR were calculated automatically. 3 measurements were performed with Aixplorer and the mean of all measurements was calculated.

Results: A total of 36 patients were included in this study. 80.6% of patients had viral hepatitis, 2.8% alcoholic liver diseases, 5.6% of patients non-alcoholic steatohepatitis (NASH), and and 11.2% cryptic liver disease. TE tracing was not possible due to obesity or ascites in 6. SWE could not be performed in one patient.

Mean TE value was 15.68 kPa SD 18.42 and mean SWE 15.09 kPa SD 14.11 (p=0.8).

There was a significant correlation between TE and SWE values (r=0.847; p<0.001).

Conclusion: Aixplorer and TE show comparable results for the discrimination of fibrosis stages. In obese patients and patients with ascites, the use of Aixplorer could lead to higher success rates.
INTRODUCTION: Hyponatremia (HN) is a common electrolyte abnormality and an independent predictor of increased mortality among patients (pts) with cirrhosis.

BACKGROUND: The HN Registry is a multicenter, prospective, observational study designed to collect data in pts with euvolemic and hypervolemic (US only) HN (serum sodium concentration ([Na⁺]) ≤130 mmol/L) due to SIADH, cirrhosis, congestive heart failure, and nephrotic syndrome.

METHODS: Enrollment will be 5000 pts from community, tertiary and academic medical centers in the USA and Europe between September 2010 and May 2013. A subset of 272 patients of 1860 (∼14%) pts had cirrhosis and sufficient data for analysis. Results from are presented.

RESULTS: HN in the majority (65%) of patients was chronic (>48 hours) compared to acute (<48 hours) and was present at admission in 78.7% of cases. A history of HN was reported in 55% of cases. HN was present at discharge in 51.8% of patients.

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Male</th>
<th>166 (61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥65</td>
<td>56 (20)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>197 (72.4)</td>
</tr>
<tr>
<td>Child Pugh Score, Grade A</td>
<td>19 (7)</td>
</tr>
<tr>
<td>Child Pugh Score, Grade B</td>
<td>65 (24)</td>
</tr>
<tr>
<td>Child Pugh Score, Grade C</td>
<td>166 (61.5)</td>
</tr>
<tr>
<td>MELD</td>
<td>N = 118, score 22.06 ± 7.8</td>
</tr>
<tr>
<td>MELD-Na</td>
<td>N = 118, 28.43 ± 5.9</td>
</tr>
</tbody>
</table>

Table 2. Outcomes

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>N / Median LOS days / Median Na at start of Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN untreated</td>
<td>68 / 7 / 128</td>
</tr>
<tr>
<td>Pharmacologic Rx</td>
<td>8 / 8.5 / 127.5</td>
</tr>
<tr>
<td>Other Rx</td>
<td>4 / 9 / 128.5</td>
</tr>
<tr>
<td>Tolvaptan</td>
<td>4 / 7 / 124</td>
</tr>
<tr>
<td>Fluid Restriction</td>
<td>88 / 7 / 127</td>
</tr>
<tr>
<td>Normal Saline</td>
<td>33 / 8 / 125</td>
</tr>
</tbody>
</table>

CONCLUSIONS: Based on interim data, a large number of patients with cirrhosis are untreated for their hyponatremia. Fluid restriction is the most common treatment. Although data is limited, LOS trends are favorable despite the severity of the hyponatremia. Additional data will be forthcoming as enrollment and analysis continue.

243 ALBUMIN FOR ACUTE EPISODIC HEPATIC ENCEPHALOPATHY (ALFAE STUDY)

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BACKGROUND: Episodic hepatic encephalopathy (HE) is frequently precipitated by factors that may induce circulatory dysfunction, cause oxidative stress-mediated damage or enhance astrocyte swelling. The administration of albumin could modify these factors and improve the outcome of HE.

AIM: Assess the efficacy of albumin on episodic HE in a multicenter, prospective, double-blind, placebo-controlled trial (ClinicalTrials.gov number, NCT00886925).

METHODS: Cirrhotic patients with an acute episode of HE (grade II-IV) were randomized to receive albumin (15 g/Kg) on inclusion – day 0 – and 1.0 g/Kg on day 2) or isotonic saline, in addition to the usual treatment (laxatives, rifaximin 1200 mg per day). The primary end point was the proportion of patients in which HE was resolved on day 3. The secondary end points included survival and the mean length of hospital stay.

RESULTS: Fifty-six patients (HCV=18, alcohol= 24; males= 42; age= 65±10 years) were randomly assigned to albumin (ALB, n=26) or saline (SAL: n=30) stratified by the severity of HE (II-III vs IV). Both groups were comparable with regard to demographic data, liver function (MELD 16.5±4.5), precipitating factors (44.6% infections) and characteristics of the HE episode. The percentage of patients without HE at day 3 did not differ between both groups (ALB: 62.5% vs. SAL: 57.1%; p > 0.05). Differences were not found neither in the mean duration of the HE (ALB: 4.12 days vs. SAL: 3.42 days; p > 0.05) nor in the mean length of stay (ALB: 8.6 days vs. SAL: 10.3 days; p > 0.05). However significant differences in mortality were found in the follow-up at 1.5 months (ALB: 7.7% vs. SAL: 36.7%; p = 0.01) and at 3 months (ALB: 24% vs. SAL: 50%; p = 0.048).

CONCLUSION: Albumin does not improve the evolution of HE during hospitalization. However, differences in survival after hospitalization suggest that the development of HE may identify a subgroup of patients with advanced cirrhosis that may benefit from the administration of albumin.

244 INCREASED GUT PERMEABILITY, ELEVATED ENDOTOXIN RELATED PROTEINS AND NEUTROPHIL DYSFUNCTION IN LIVER CIRRHOSIS

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INTRODUCTION: Infection is the most common precipitant of deterioration of liver function in cirrhosis. Endotoxin, derived from gram-negative organisms in the gut, can enter the circulation due to increased gut permeability, and contributes to endotoxemia and dysfunction of the innate immune system in alcoholic cirrhosis. The aim of this study was to assess gut permeability, endotoxin and related proteins as well as neutrophil function in patients with liver cirrhosis.

METHODS: 40 patients with liver cirrhosis (24 alcoholic, 16 non-alcoholic) and 8 healthy controls were studied. Gut permeability
was assessed by differential sugar absorption and diaminooxidase (DAO) serum levels. Endotoxin was measured by the limulus amoebocyte lysate assay and lipopolysaccharide binding protein (LBP) and sCD14 levels were measured by ELISA. Neutrophil phagocytic capacity was determined by FACS analysis.

**Results:** DAO serum levels, saccharose excretion and lactulose/mannitol ratio in the urine were elevated in patients compared to controls. sCD14 levels were significantly higher in patients compared to controls. LBP did not differ between patients and controls. Age, gender and aetiology did not influence gut permeability, sCD14 and LBP levels. Free endotoxin could not be detected in any samples. There was a trend to reduced neutrophil phagocytic capacity in patients with liver cirrhosis (significant for those with Child–Pugh score >7, \( P < 0.05 \)).

**Discussion:** Gut permeability is increased in liver cirrhosis and might therefore account for translocation of bacterial products into the circulation. Although we could not find free endotoxin in the serum, the increase in sCD14, a mediator in endotoxin clearance, indicates an activation of the immune system in liver cirrhosis due to endotoxin. This is associated with a decrease in neutrophil phagocytic capacity as a possible pathophysiological basis for the increased susceptibility to infections in cirrhosis.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=8)</th>
<th>Patients (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAO U/ml</td>
<td>10.6±3.6</td>
<td>20.1±11.7*</td>
</tr>
<tr>
<td>Saccharose %</td>
<td>0.03±0.06</td>
<td>0.55±1.50*</td>
</tr>
<tr>
<td>Lactulose/mannitol ratio</td>
<td>0.02±0.05</td>
<td>0.11±0.17*</td>
</tr>
<tr>
<td>sCD14ng/ml</td>
<td>1432.8±5370</td>
<td>4787.1±1068.7*</td>
</tr>
<tr>
<td>Phagocytosis %</td>
<td>100.1±53.9</td>
<td>80.2±42.8</td>
</tr>
</tbody>
</table>

\( \text{mean±SD, } * P < 0.01 \)

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**SPLENO-SYSTEMIC SHUNTS AND COVERT HEPATIC ENCEPHALOPATHY**

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**Background and Aims:** Portal-systemic shunts can involve blood from the intestine (patent paraumbilical vein, inverted left gastric vein) or from the spleen (spleno-systemic shunts [SSS]). A peculiar condition is that of total shunt in patients with inverted portal flow. The relationship between portal-systemic shunt and the occurrence of overt hepatic encephalopathy has long been known. However, that between SSS and the subtle neuropsychiatric alterations termed covert hepatic encephalopathy (CHE) is less clear. The aims of this study were to: i) assess the likelihood of CHE screening in relation to the presence of SSS; ii) evaluate the relationship between SSS and quantitative CHE indices.

**Methods:** Three-hundred-and-thirty-one patients with cirrhosis, independently referred for hepatic Doppler-US between Jan-2009 and August-2012 were enrolled. They were qualified as having SSS if convoluted, anechoic spleno-renal and spleno-retroperitoneal channels were detected, and venous flow confirmed by colour-Doppler. Flow direction within the portal vein was also established. Information was obtained on independent referral of the same patients for CHE screening, including electroencephalography, within 6 months of Doppler-US.

**Results:** Eighty-eight/331 (27%) patients were qualified as having SSS; this was spleno-renal in 17 (19%) and spleno-retroperitoneal in 71 (81%). Eight/331 (2%) patients, all with SSS, had inverted portal flow. Forty-three/331 (13%) patients underwent CHE screening, the prevalence of which was higher in those with SSS (34 vs. 5%; \( \chi^2 = 47.2, P < 0.0001 \)). Significant differences in spectral EEG features were observed between patients with/without inversion of the portal flow in the entire population (EEG frequency: 8.3±2.5 vs. 10.6±1.7 Hz, \( P < 0.05 \); slow delta activity: 18±17 vs. 6±5%, \( P < 0.01 \)) and in the SSS group (EEG frequency: 8.3±2.5 vs. 10.3±1.7 Hz, \( P < 0.05 \); slow delta activity: 18±17 vs. 7±5%, \( P < 0.05 \)). In patients without portal flow inversion, no differences in EEG parameters were observed in relation to SSS.

**Conclusion:** A significant association was observed between SSS presence and the likelihood of CHE screening. However, EEG parameters were not different in patients with/without SSS. In contrast, the EEG was slower in patients with inverted portal flow compared to those with SSS only, suggesting that flow inversion is a risk factor for CHE.

**Table 1**

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\( \text{mean±SD, } * P < 0.01 \)

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**CXCL9 AMONG OTHER CHEMOKINES DERIVE FROM PORTAL BLOOD AND CORRELATE WITH THE OUTCOME IN PATIENTS WITH LIVER CIRRHOSIS AND PORTAL HYPERTENSION**

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**Background and Aims:** In cirrhosis portal hypertension is a consequence of increased hepatic resistance and increased portal venous inflow. Besides fibrotic tissue deposition and remodelling processes, hepatic and extrahepatic angiogenenesis is also involved in the pathogenesis of portal hypertension and its complications of cirrhosis and portal hypertension. Chemokines play an important role for induction of experimental fibrosis and angiogenesis, however their role in the human cirrhosis remains to be established.

**Methods:** 42 patients with liver cirrhosis who had received TIPS (transjugular intrahepatic portosystemic shunt) were included in this study. The TIPS-indication was either refractory ascites or recurrent bleeding. During the TIPS procedure portal and hepatic venous blood samples were obtained. ELISA assays for VEGF, MCP1, CXCL9, CXCL10, CXCL11 and angiogenin were measured and correlated to clinical parameters.

**Results:** Portal pressure before TIPS-placement and Child-Score of patients correlated with serum levels of CXCL9, CXCL10 and MCP1 in both compartments. Hepatorenal-syndrome and creatinine levels correlated with circulating levels of CXCL9 and CXCL11. The levels of VEGF, MCP1 and angioenin were significantly (\( p < 0.05 \)) higher in hepatic venous blood than in portal venous blood, suggesting hepatic synthesis and release of these chemokines. Interestingly, the levels of CXCL9 and CXCL10 were higher in portal venous blood than in hepatic venous blood, which may be caused by hepatic clearance, but also points to the liver as site of action for these two chemokines. Of note, survival of patients correlated significantly with circulating levels of CXCL9 and CXCL11.

**Conclusions:** High chemokine levels are associated with complications of patients with cirrhosis and severe portal hypertension. The investigated proangiogenic chemokines are partly liver-derived. But they also target the liver and might thereby induce angiogenesis and fibrogenesis, mechanisms that aggravate portal hypertension.
247 HIGH LEVELS OF SOLUBLE TNF-ALPHA-RECEPTOR-I ARE ASSOCIATED WITH POOR OUTCOME IN CIRRHOSIS WITH PORTAL HYPERTENSION

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Background: TNFα levels are increased in liver cirrhosis even without obvious infection signs probably by continuous endotoxin influx into portal blood, as shown recently (Trebicka et al. Eur J Gastroenterol Hepatol 2011). Soluble TNFα receptors (sTNFR type I and II) are useful tools to investigate TNFα release, which itself has a short half-life. TNFR-II levels correlate with mortality in liver cirrhosis (Grünhage et al Clin Gastro Hep 2008). Here, we investigated circulating levels of soluble TNFR-I and -II in patients receiving TIPS.

Methods: 41 patients with liver cirrhosis and portal hypertension (12 viral, 29 alcoholic) received TIPS. Portal and hepatic venous blood was withdrawn in these patients during the TIPS-procedure and in the invasive control two weeks later. In these samples levels of sTNFR-I and sTNFR-II were measured via ELISA.

Results: We found no significant difference of sTNFR-I and sTNFR-II levels in portal and hepatic venous blood. Both sTNFR-I and sTNFR-II levels correlated directly with the MELD-score (p=0.001) and creatinine (p=0.0001), as well as inversely with albumin (p=0.02) and cholinesterase (p=0.01). The portal pressure measured during TIPS-procedure did not correlate with sTNFR-I levels. In contrast, portal pressure during the invasive control two weeks after TIPS correlated directly and systolic arterial pressure inversely with hepatic venous levels of sTNFR-I. Interestingly, mortality after TIPS correlated with hepatic venous levels of sTNFR-I at the invasive control (p=0.007).

Discussion: This study shows that hepatic venous levels of soluble TNF-receptor type I correlate with severity of hyperdynamic circulation, as well as with increased mortality in patients receiving TIPS. This parameter could represent a prognostic marker for patients with severe portal hypertension receiving TIPS.

248 FASTING AMMONIA (NH₃) AS A PREDICTOR OF HEPATIC ENCEPHALOPATHY (HE) EVENTS

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The utility of plasma NH₃ in managing cirrhotic patients with hepatic encephalopathy (HE) is controversial. Our recent multicenter, randomized, double-blind, placebo-controlled 16-week study of 178 cirrhotics with ≥2 overt HE events in the prior 6 months showed that glycerol phenylbutyrate (GPB; HPN-100), an investigational NH₃-lowering agent, significantly reduced both the proportion of patients with HE events and total HE events (Hepatology 2012; 56:248A).

Aim: To test the hypothesis that NH₃ predicts future HE events in HE-prone cirrhotic patients in remission.

Methods: We analyzed fasting plasma NH₃ levels at baseline and after 7 and 14 days of treatment, and we tested the predictive value of NH₃ in different ranges using a negative binomial model that adjusted for duration of treatment.

Results: HE events correlated strongly with NH₃ both at baseline and after 7 and 14 days of treatment. Covariate analysis detected no evidence of drug effect independent of NH₃ on days 7 or 14. The relationship between NH₃ and HE events was nonlinear; instead, serum NH₃ ≥1.5× the upper limit of normal (ULN) was associated with a statistically significant several-fold increased risk of HE events. Table 1 summarizes the findings on study day 7; findings were similar at baseline and on day 14.

Table 1

<table>
<thead>
<tr>
<th>Event Rate*</th>
<th>Probabilities of an individual patient having 0, 1, or multiple HE events during the study*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma NH₃</td>
<td>No. of HE events/year**</td>
</tr>
<tr>
<td>0–1.5×ULN</td>
<td>1.13</td>
</tr>
<tr>
<td>&gt;1.5×ULN</td>
<td>6.85</td>
</tr>
</tbody>
</table>

*All comparisons significant at p<0.01 **Annualized based on 16 week study.

Conclusions: Fasting plasma NH₃ levels are strongly predictive of future HE events. The effect of GPB on HE events is explained entirely by its effect on NH₃. Furthermore, the risk of multiple future HE events is particularly high for patients with an NH₃ ≥1.5×ULN, suggesting that achieving a fasting level <1.5×ULN may be beneficial in management of patients with cirrhosis and prior HE events.

03c. LIVER TUMOURS: MANAGEMENT

249 HCC DIAGNOSED ON SURVEILLANCE PROGRAMMES: IMPACT ON STAGE AND OUTCOME

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Background and Aims: Surveillance of cirrhotic patients for HCC is recommended by numerous national and international guidelines. However many patients are still diagnosed de novo with this malignancy. Data on the benefits of surveillance remains relatively limited. Our aim was to compare stage at diagnosis and patient outcome for those diagnosed on surveillance and those who were not.

Methods: Using our regional HCC MDT database, we analysed patients diagnosed with HCC between January 2009 and January 2012. All patients were staged using the Barcelona Clinic Liver Cancer (BCLC) system. We compared the stage at diagnosis, the treatment strategy after MDT discussion, and the survival in those diagnosed in surveillance with those diagnosed de novo. Statistical comparisions were made using CHI-squared or Kaplan Meier analysis as appropriate.

Results: 189 patients were diagnosed with HCC at MDT during the study period. We had full follow-up data on 169 patients which were used for analyses, with mean follow up 90 weeks. Mean age was 68 years and 82% were male. Aetiology was alcoholic liver disease in 32% and HCV in 15%. 37 (22%) patients were in surveillance programmes at diagnosis of HCC and 132 (78%) were not. Tumours were BCLC stage A at diagnosis in 29.7% patients in
surveillance, compared with 6.1% not in surveillance (p = 0.0003). 13.5% those diagnosed in surveillance underwent transplantation or resection, compared with 2.3% who were not (p = 0.01). Survival for those diagnosed in surveillance was greater than those diagnosed de novo (p = 0.02).

Conclusions: Most patients diagnosed with HCC in our region were not in surveillance programmes. Patients diagnosed on surveillance were more likely to have potentially curative disease and had higher overall survival.

Reference(s)

250 RELATIONSHIP BETWEEN PLASMA CONCENTRATION AND DAILY DOSE OF SORAFENIB IN CIRRHOTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)
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Pharmacokinetics and dose finding studies on sorafenib were conducted on a heterogeneous group of patients with solid tumors. Portal hypertension, gut motility impairment and altered bile enterohepatic circulation may explain different sorafenib toxicological profile in cirrhotic patients.

Aim: Evaluation of sorafenib plasma concentration in an homogeneous group of cirrhotic patients with HCC.

Methods: Sorafenib plasma concentrations were determined by liquid chromatography every 2 weeks, at 3 and 12 hours since the last administration for a mean of 6 months (range 2–21) in 12 consecutive patients with HCC and Child–Pugh A cirrhosis. The samples collection began after 2 weeks of therapy without dose modifications (800 mg per day or, if not tolerated, 400 mg per day).

Data were estimated using the generalized estimating equation (GEE) and the level of statistical significance was placed at alpha = 0.05.

Results:

i. There were not significant differences between plasma concentrations in patients who tolerate the full dose versus patients in which the dose was reduced from 800 mg daily to 400 mg daily (p = 0.148) due to toxicity.

ii. Sorafenib plasma concentration decreases overtime (p < 0.005).

Conclusions: Our data confirm the variability both in the maximum tolerated dose and in plasma concentration. Low starting dose at the beginning of the therapy, followed by a rapid dose escalation after few weeks of clinical observation may be a strategy to avoid severe early drug toxicity. Long term responder patients who eventually progress may benefit from sorafenib dose escalation in order to restore an adequate drug exposure.
252 IMPROVING PROGNOSIS OF HCC IN THE REAL-WORLD CLINICAL PRACTICE: AN ITALIAN EXPERIENCE

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Background and Aims: Sporadic data suggest HCC outcome is improving over time. We compared two independent cohorts of patients recruited on field at 10 years distance each with the aim of assessing if changes occurred on: epidemiologic, etiologic and clinical characteristics; characteristics of HCC at presentation and outcome.

Methods: The study was performed in two Italian multicentre cohorts of consecutive new HCCs in cirrhosis detected in two time periods: cohort 1 (C1) included 327 patients (Jan-Dec 1998) and cohort 2 (C2) 718 patients (Sept 2008-Dec 2011). HCC was diagnosed according to the current criteria at that period and HCC managed accordingly. Patients of both cohorts were stratified according to Child–Pugh and MELD score. HCC was staged according to TNM classification and BCLC system. Each centre was free in clinical decisions.

Results: C1 patients were significantly older and non-viral-non-alcoholic cirrhosis doubled in C2. At presentation, in C2 liver function was better and comorbidities were more frequent. In both cohorts BCLC very early/early stages were prevalent being significantly higher in C2. BCLC stage B and C were equally distributed, while patients in BCLC stage D were significantly more frequent in C1. In C2 HCCs were more frequently detected under regular US surveillance and were significantly smaller and more frequent in C1. In C2 HCCs were more frequently detected under regular US surveillance and were significantly smaller and unipaucinodular. As a whole treatment, no matter which, was more frequently offered to C2 patients (76.5% vs 52.3%; p < 0.001) and in C2 significantly increased the rate of patients treated by TACE. Ablation was more frequently employed in C2 (p = 0.07). PEI ablation significantly decreased across C1 and C2 while RF became the prevalent ablative method in C2. Median survival was significantly higher in C2 (25.3 vs 18.4 mos; p = 0.05). Cumulative C1 and C2 survival at 1 and 3 years was 65% and 22% and 67% and 37%. HCC size (<3 cm), BCLC stage A and to be treated independently predicted survival.

Conclusions: This study confirmed that HCC outcome improved over time mainly because: better profile of cirrhosis; better profile of HCC at presentation; increasing rate of treated cases. These data should encourage further efforts to implement HCC approach in every day clinical practice.

253 COMPARISON OF SURVIVAL OUTCOMES BETWEEN PATIENTS WITH VIRAL AND NON-VIRAL RELATED HEPATOCELLULAR CARCINOMA: A LARGE MULTI-CENTRE STUDY

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Background: While chronic viral hepatitis B (HBV) and C (HCV) are major risk factors for hepatocellular carcinoma (HCC), it is unclear if the overall survival and recurrence-free survival of patients with viral hepatitis-related HCC differs from those with non-viral disease. We therefore compared survival outcomes between patients with HBV- and HCV-related HCC to those with non-viral related HCC.

Methods: A multi-centre study was conducted on HCC cases seen at three large tertiary academic hospitals from 1994–2012. Patient demographics, severity of liver disease, tumour characteristics, and patient outcomes were retrieved from the HCC database and computer records. Survival outcomes were compared between the three cohorts of HBV-, HCV- and non-viral related HCC and predictors of survival were determined using Kaplan Meier analysis, log-rank test and Cox proportional hazard model.

Results: 734 HCC patients were identified: 81% male, 21.8% HBV, 39.6% HCV, 38.6% non-viral related HCC. Overall survival was better in those with viral related HCC compared to those with non-viral related disease on univariate analysis (p < 0.01). On multivariate analysis age at diagnosis (p < 0.01), extra-hepatic spread (p = 0.04), BCLC stage (p = 0.01), size of largest lesion (p < 0.01), presence of screening for HCC (p < 0.01) and Child Pugh Classification (p < 0.01) were associated with survival outcomes with viral aetiology no longer significant. Of the 260 patients with a complete response to therapy, recurrence-free survival was better in those with HBV-related HCC compared to those with HCV- and non-viral related disease (5 year recurrence free survival was 34.5% vs 6.8% vs 6.1% respectively, p < 0.01). On multivariate analysis only the presence of HBV-related HCC (p = 0.02) and the number of HCC lesions (p < 0.01) predicted recurrence-free survival.

Conclusion: In this large multi-centre study, while overall survival appears similar between patients with HBV- and HCV-related HCC compared to those with non-viral HCC, HBV is an independent predictor of recurrence free survival. This could relate to the ability to medically suppress HBV replication. Further studies are warranted to confirm these findings.

254 SINGLE HEPATOCELLULAR CARCINOMA SMALLER THAN 2 cm: ARE PERCUTANEOUS ETHANOL INJECTION AND RADIOFREQUENCY ABLATION EQUIALLY EFFECTIVE?

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Background and Aims: Aim of this study was to compare percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA) in the treatment of single hepatocellular carcinoma (HCC) ≤2 cm emerging in liver cirrhosis.

Methods: Two hundred forty-four patients with Child A/B cirrhosis (165 men, HCV positive cases 70.5%, median age 68 years) and a single “treatment-naïve” nodule of HCC ≤2 cm who underwent PEI (108 patients) or RFA (136 patients) during the period 1998–2001 were enrolled in this multicentric retrospective analysis. All patients showed complete short-term HCC necrosis.

Overall survival (OS) and HCC recurrence were estimated using the Kaplan–Meier method and Cox regression models were performed to identify factors associated with OS rates.

Results: RFA and PEI groups did not differ significantly in term of demographic parameters, except for male sex (83% in PEI group and 55.1% in RFA group), and clinical features of liver cirrhosis. The mean length of follow-up was 37.1 months both in the PEI
group (range 2–189) and in the RFA group (range 6–111) (p=0.99). No procedure related mortality was noted. Major complications occurred only in 1 patient for both treatment group (p=0.87). OS rates at 1-, 3-, 5-years were 97%, 83.3% and 64.6% in PEI group, and 97.7%, 77.1% and 62.3%, in RFA group (p=0.16). HCC-related death occurred in only 42.3% of PEI and 25.6% of RFA patients (p=0.15). HCC recurrence rates at 1-, 3-, 5-years were 16.1%, 61.4%, 79.4% in the PEI group and 20.4%, 47.7%, and 57.4% in RFA group (p=0.28). Furthermore, cumulative local tumor progression and distant intrahepatic recurrence rates were not different between the two populations. Local tumor progression was diagnosed mostly in the first 2 follow up years (77.7% in PEI group and 71.4% in RFA group).

Multivariate analysis revealed that ascites was the only pre-treatment risk factor negatively associated with OS in both groups (OR=13.6, 95%CI 2.5–73.3, p=0.002 for PEI group and OR=8.7, 95%CI 2.8–27, p<0.001 for RFA group).

**Conclusions:** PEI and RFA are equally effective in the treatment of 
HCC ≤2cm in terms of OS, local tumor progression and overall HCC recurrence.

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**A CASE–CONTROL STUDY EVALUATING THE EFFICACY OF ANTI-VIRAL THERAPY AS SECONDARY PROPHYLAXIS OF HEPATOCELLULAR CARCINOMA (HCC) RECURRENT AFTER CURATIVE TREATMENT IN SUBJECTS WITH HCV-RELATED CIRRHOSIS**


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**Background:** Patients with HCV-related cirrhosis developing HCC have an increased risk of HCC recurrence. The efficacy of Pegylated Interferon (PegIFN) and Ribavirin (RBV) as secondary prophylaxis after curative therapy for HCC has never been evaluated.

**Aim:** To assess whether the treatment with PegIFN+RBV is able to reduce the HCC recurrence rate and to increase survival after curative treatment of HCC in HCV compensated cirrhotic patients.

**Methods:** 60 patients who had undergone treatment for HCC (liver resection [LR] or radio-frequency ablation [RFA]) were enrolled in this prospective, non-randomized, match-controlled study.

**Results:** The cases consisted of 20 subjects receiving standard schedule of PegIFN+RBV for 48 weeks while controls were represented by 40 HCV cirrhotics who had not received antiviral therapy after HCC treatment. Cases and controls were adequately matched (1:2 proportion) for sex, age at LR (or RFA), Child-Turcotte–Pugh class and MELD score. The patients were observed for a median period of 43 months (mo). The efficacy of antiviral treatment on HCC recurrence and survival was assessed at 12, 24 and 36 mo. HCC recurrence was lower in cases compared to controls (10 vs 37.5%, p=0.034 after 12 mo; 20 vs 50% p=0.029 after 24 mo; 40 vs 55%, p<ns, after 36 mo, respectively) while the contrary was observed regarding survival (100 vs 97.5%, p<ns after 12 mo; 100 vs 87.5%: p<ns after 24 mo; 100 vs 77.5%, p=0.023 after 36 mo, respectively). Also the overall survival of the cases was higher than that of the controls (mo, M±SD: 73±40 vs 37.5±32 p=0.002, respectively). Finally, the time to recurrence of HCC was longer in the cases compared to controls (mo, M±SD: 33±18 vs 15±17 p=0.010, respectively). In the treated group 8 (40%) subjects obtained a sustained virological response (SVR). However, the intra-group analysis showed that the achievement of this outcome did not influenced the HCC recurrence rates and survival at 12, 24 and 36 mo.

**Conclusions:** Antiviral treatment with PegIFN+RBV allows to decrease the rate of HCC recurrence and to increase the survival in subjects with HCV cirrhosis treated with LR or RFA. This advantage is independent from the achievement of SVR.

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**LONG TERM CLINICAL AND BIOCHEMICAL COMPARATIVE ANALYSIS AFTER SELECTIVE INTERNAL RADIOTHERAPY WITH YTTRIUM-90 MICROSPHERES IN HEPATOCELLULAR CARCINOMA A. El Fouly,1 J. Ertel1, S. Mueller1, T. Lauenstein1, A. Bockisch1, G. Gerken1, A. Dechêne2, J.F. Schlak1. 1Hepatology and Gastroenterology Department, Essen University, Essen, Germany; 2Radioisotope Department, Egyptian Atomic Energy Authority, Giza, Egypt. 1Institute for Nuclear Medicine, 2Institute for Diagnostic and Interventional Radiology and Neuroradiology, Essen University Hospital, Essen, Germany

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**Background:** More than 90% of the accidentally diagnosed patients have non resectable HCC. Sorafenib is the only standard line of treatment that is recommended for advanced stage HCC according to BCLC staging system. Radioembolization with Yttrium-90 microspheres is a novel transarterial approach for patients with advanced HCC not eligible for Tranarterial Chemoembolization (TACE) or not tolerating adverse events of Sorafenib. The aim of this study was to validate an evidence on the safety, long term efficacy and survival probabilities with subgroup analysis of this treatment among a European cohort of patients with locally advanced HCC.

**Patients and Methods:** Starting from November 2006 till March 2012, almost six years of experience in treating 205 patients with unresectable hepatocellular carcinoma in this prospective study. Yttrium-90 microspheres radiotherapy was performed in a lobar fashion through the right or left hepatic artery. Overall survival rate was considered as a primary endpoint with further subgroup analysis. Radiological response rate was evaluated using modified RECIST criteria. Safety and (AEs) were evaluated in respect to the terminology of (CTCv3).

**Results:** Among 205 patients with advanced HCC treated by 334 sessions of radioembolization using Yttrium-90 glass microspheres, with mean observation period of 11.3 months during follow up. The baseline HCC Staging at time of presentation was 99 (48%) stage B and 106 (52%) stage C according to BCLC staging system respectively. Portal vein thrombosis was presented in 31% of the whole cohort. The median overall survival probability was 18 months (95%CI: 12.5–23.4), within further subgroup analysis; the median survival probability of stage B (BCLC) patients was 19.7 months (95%CI: 10.1–29.8), while in stage C (BCLC) was 12 months (95%CI: 2.5–21.5). The main obvious adverse events were a transient fatigue syndrome and lymphopenia without any clinical impact.

**Conclusions:** Radioembolization with Yttrium-90 glass microspheres is a safe and effective locoregional therapy for patients with advanced HCC even with or without portal vein thrombosis. Since long term survival probabilities seem comparable to standard-lines of therapy, larger studies against or in combination with Sorafenib and TACE are warranted.

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**SORAFENIB VERSUS RADIOEMBOLIZATION WITH Y90: CLINICAL EVALUATION AND SURVIVAL IN PATIENTS WITH ADVANCED HEPATOCELLULAR CARCINOMA AND CIRRHOSIS**

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**Background and Aims:** Sorafenib and Trans-Arterial-Radio-Embolization-Y90 (TARE) are the optional therapies for advanced hepatocellular carcinoma (HCC). Aim of this study was to evaluate survival and tolerability of sorafenib or TARE in patients with advanced HCC.
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Methods: Data from our prospective electronic database were analyzed. Inclusion criteria: Child–Pugh A liver cirrhosis with mono-lobar HCC; stage B or C (according to Barcelona Clinic Liver Cancer classification) with or without portal thrombosis; M0, N0 or N1; treatment with sorafenib for more than 60 days or with TARE (1 or 2 sessions) with a follow-up longer than 180 days. Liver related complications, side effects and survival (Kaplan–Meier curves) were analyzed.

Results: Between January 2008 and November 2012, 137 patients received sorafenib, 30 met the inclusion criteria and were included: 26 male; median age: 69 years (range: 32–82); ECOG 0; median tolerated drug dose: 800 mg (200–800); median treatment time: 4.7 months (range: 2–11). Between May 2011 and November 2012, 29 patients underwent TARE, 19 met the inclusion criteria and were included: 19 male; median age: 69 years (range: 51–79); ECOG 0; treated with 1 (18 patients) or 2 sessions. Among patients treated with sorafenib, 93% (28/30) complained of mild to moderate side effects (mostly asthenia, hand-foot skin syndrome, diarrhea, nausea). Afer TARE 21% (4/19) of patients experienced mild abdominal symptoms (nausea, vomiting). Liver function worsened (Child Pugh score ≥B) in 26% (8/30) of sorafenib and in 16% (3/19) of TARE group after 30–60 days from therapy. Overall 28 deaths occurred (22 sorafenib, 6 TARE). Median overall survival was 10.7 months (95% CI: 8.9–12.5) with sorafenib and 12.2 months (95% CI: 8.3–16) with TARE (p = 0.05). Cumulative probability of survival at 6–12–16 months was 83.3–41.8–23.9% with sorafenib versus 94.6–39.1–39.1% with TARE (p = 0.41). Conclusions: Patients treated with sorafenib for more than 2 months had a higher rate of side effects comparing with TARE. Our preliminary data showed that median overall survival in sorafenib group was comparable to the literature data and similar to those of TARE group. Further investigations are needed to evaluate clinical results and costs.

258 HYPOFRACTIONATED CARBON ION THERAPY DELIVERED WITH SCANNED ION BEAMS FOR PATIENTS WITH HEPATOCELLULAR CARCINOMA – FEASIBILITY AND CLINICAL RESPONSE


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Introduction: Photon-based radiation therapy does currently not play a major role as local ablative treatment for hepatocellular carcinoma. High doses are required for long-term control of HCC-lesions but are difficult to apply because of a relatively low radiation tolerance of the normal liver tissue, even with modern radiation techniques. Therefore carbon ions offer distinct physical and biological advantages. Due to their inverted dose profile and the high local dose deposition within the Bragg peak, precise dose application and sparing of normal tissue is possible. Furthermore, carbon ions have an increased relative biological effectiveness (RBE) compared to photons. A phase-I clinical trial evaluating toxicity and therapy outcome for HCC-patients treated with carbon ions was initiated at our institution (PROMETHEUS-01, NCT 01167374).

Patients and Design: A total of six patients (n = 6) with one or more HCC-lesions (n = 7) were treated with carbon ions delivered by raster-scanning technique at the Heidelberg Ion-Beam Therapy Center (HIT) according to the PROMETHEUS-01 clinical trial protocol. Diagnosis of HCC was confirmed by histology or two different imaging modalities (CT and MRI) according to the AASLD-guidelines. Applied fractionation scheme was 4 x 10 Gy (RBE). Correct dose application was controlled by in-vivo PET measurement of β+-activity in the irradiated tissue shortly after the treatment session.

Results: Patients were observed for a median time period of 11.0 months (range, 3.4–12.7 months). Imaging studies showed a partial response in 4/7 lesions and a stable disease in 3/7 lesions in follow-up CT- and MRI scans. Local control was 100%. One patient with multifocal intrahepatic disease underwent liver transplantation 3 months after carbon ion therapy which was intended as a bridging therapy. During radiotherapy and the current follow-up period no severe adverse events have occurred. Two of the patients reported fatigue symptoms, no further new symptoms developed during the follow-up.

Conclusion: We report the first clinical results of patients undergoing carbon ion therapy using the raster-scanning technique at HIT. All patients are locally controlled and experienced no higher toxicities in a short follow-up period. Further patients will be included in the prospective Phase-I PROMETHEUS clinical trial.

259 DETERMINANTS OF BLEEDING FROM ESOPHAGEAL VARICES IN PATIENTS WITH ADVANCED HEPATOCELLULAR CARCINOMA TREATED WITH SORAFENIB

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Background and Aims: Sorafenib is the standard of care for patients with advanced hepatocellular carcinoma (HCC) and preserved liver function. No data is available on the risk of bleeding from esophageal varices (EV) during treatment. Aim of the study was to assess the prevalence of EV and the rates and risk factors for EV bleeding during sorafenib treatment.

Methods: Starting 2008, all compensated patients with advanced or intermediate HCC not eligible to or failing ablative therapies who were consecutively enrolled in a prospective study of safety and effectiveness of sorafenib. Pretreatment all underwent diagnostic upper gastrointestinal endoscopy.

Results: 108 patients (61% HCV, 72% advanced HCC) received sorafenib for 4.6 (95% CI, 3.3–5.6) months. At baseline, 44 (41%) patients had no EV (group A), 64 (59%) patients had EV [43 (40%) small EV (group B) and 21 (19%) medium/large EV or endoscopically down-sized EV (group C)]. All patients with medium/large EV and those with a previous bleeding were treated with propranolol at the maximal tolerated dose. Eight patients (7%) bled from EV after 64 (18–260) days of treatment and were discontinued. One died for a new episode of bleeding 117 days after sorafenib discontinuation. Bleeding on sorafenib occurred in 3/18 group B patients with neoplastic portal vein thrombosis (nPVT), in 4/10 group C patients with nPVT and 1/11 group C patients without nPVT (p < 0.0001). By multivariate analysis, nPVT (HR = 10.67, 95% CI 1.29–87.94) was the only independent predictor of bleeding.

Conclusion: nPVT is the determinant of bleeding risk in compensated patients with advanced HCC exposed to sorafenib, independently on the size of EV.
260 RANDOMIZED CONTROLLED TRIAL FOR PROTON BEAM RADIOTHERAPY VERSUS TRANSARTERIAL CHEMOEMBOLIZATION FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA; PRELIMINARY RESULTS
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Objectives: This trial aims at comparing the efficacy of Transarterial Chemoembolization (TACE) and Proton Beam Radiotherapy (PBR) in HCC patients who meets Milan and San Francisco criteria (SF). Interim analysis is triggered because of the difference in number of death between two groups.

Methods: This is a single center RCT. Included patients are cirrhotics with tumor burden that meets either Milan or SF criteria who are qualified to receive both TACE and PBR. HCC diagnosed radiologically or by biopsy. Exclusion criteria: surgical candidate for resection, metastatic HCC, prior locoregional treatment, prior liver transplantation, Child–Pugh class C. MELD score >25, active substance abuse, other comorbidity that may impact the survival. Patients were randomized blindly to receive either TACE or 15 sessions of PBR. Primary end point is overall survival. Secondary end points are tumor progression, and histological response on explant.

Results: Forty patients were randomized: 22 in PBR and 18 in TACE group. There is no difference in demographics, MELD score and tumor burden between two groups. Three patients in TACE group are within SF and 15 within Milan. Four patients in PBR are within SF and 18 within Milan. Medium tumor size is 3.1 cm. Medium MELD score 11.2. Tumor regression was determined in 7 patients in TACE and 5 patients in PBR group. One patient in each group had tumor progression. Rest of patients had stable tumor size. Five patients died in PBR group and 8 in TACE group. Median survival in PBR group was not reached but in TACE group it was 23 months. Two-year survival is 77% in PBR and 65% in TACE (p=0.32). Fourteen patients had subsequent liver transplantation at 3–23 months (Median 13.6 month) 7 in PBR and 7 in TACE group. Nine patients had no residual tumor on explant (5 PBR and 7 patients in TACE and 5 patients in PBR group. One patient in each group had tumor progression. Rest of patients had stable tumor size. Five patients died in PBR group and 8 in TACE group. Median survival in PBR group was not reached but in TACE group it was 23 months. Two-year survival is 77% in PBR and 65% in TACE (p=0.32). Fourteen patients had subsequent liver transplantation at 3–23 months (Median 13.6 month) 7 in PBR and 7 in TACE group. Nine patients had no residual tumor on explant (5 PBR and 7 patients in TACE and 5 patients in PBR group. One patient in each group had tumor progression. Rest of patients had stable tumor size. Five patients died in PBR group and 8 in TACE group. Median survival in PBR group was not reached but in TACE group it was 23 months. Two-year survival is 77% in PBR and 65% in TACE (p=0.32). Fourteen patients had subsequent liver transplantation at 3–23 months (Median 13.6 month) 7 in PBR and 7 in TACE group. Nine patients had no residual tumor on explant (5 PBR and 4 TACE).

Conclusion: There is a trend toward improving survival in HCC patients who are within SF and Milan criteria and treated with PBR compared to TACE. There is no difference in histological response between PBR and TACE. Larger sample size is needed to determine the statistical significance of this finding.

261 VASCULAR ENDOTHELIAL GROWTH FACTOR AS A PREDICTOR OF RESPONSE TO THE COMBINED THERAPY WITH SORAFENIB AND TRANSARTERIAL CHEMOEMBOLIZATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA
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Background and Aims: Sorafenib, a dual Raf kinase/vascular endothelial growth factor (VEGF) receptor inhibitor, currently sets the new standard for the first-line treatment of advanced hepatocellular carcinoma (HCC). In this study, we investigated whether baseline and post-treatment changes of serum VEGF level are associated with the response of the combined therapy with sorafenib and transarterial chemoembolization (TACE) in patients with HCC.

Methods: A total of 59 consecutive patients with HCC of intermediate stage (BCLC B) were subjected. All the patients were treated with 400 mg of sorafenib twice daily in combination with TACE. Tumor response was assessed according to the modified RECIST. Serum VEGF level was measured using ELISA in the samples obtained just before and 1 month after the therapy. Response rate (RR) and time to progression (TTP) were compared in relation to baseline and post-treatment serum VEGF level. RR was defined as the percentage of patients with either complete response (CR) or partial response (PR) at 1 year after the therapy.

Results: Out of 59 patients, 23 (39%) achieved CR and 7 (12%) experienced PR at 1 year after the combined therapy. The 1- and 3-year overall survival rates were 92% and 70%, respectively. The median TTP was 14 months. The RR of high-VEGF group, whose baseline serum VEGF level is higher than the median value, was significantly higher than the rate of low-VEGF group (67% vs. 37%; P<0.05). In addition, the RR of patients with decreasing serum VEGF level (>10% decrease at 1 month after the therapy) was significantly higher than the rate of the patients with increasing/stationary serum VEGF level (75% vs. 41%; P<0.05). Also, the TTP of high-VEGF patients and patients with decreasing serum VEGF level were longer than the TTP of low-VEGF group and patients with increasing/stationary serum VEGF level, respectively (P<0.05). In multivariate analysis, VEGF overexpression was a significant predictor of a favorable clinical outcome following the combined therapy with sorafenib and TACE.

Conclusions: Serum VEGF expression may be a useful predictor of the response to the combined therapy with sorafenib and TACE in patients with HCC.

262 SURVIVAL OF PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC) IS AFFECTED BY COMPLETE RESPONSE TO RADIO-FREQUENCY THERMAL ABLATION (RFTA)
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Background and Aims: The effect of completeness of response to RFTA on the length of survival in patients with HCC and Child–Pugh A-B cirrhosis is still unclear. We aimed to assess in a large consecutive cohort the prognostic value of a complete tumor necrosis after RFTA, evaluated with EASL criteria for assessment of treatment response by CT or MR.

Methods: A cohort of 179 consecutive patients (147 Child–Pugh class A 5–6 and 32 Child–Pugh class B 7) admitted to our Unit from 2000 to 2011 was prospectively assessed. A single lesion was observed by CT or MR in 132/179 (73.7%), two lesions in 38/179 (21.2%) and three lesions in 9/179 (5%) of patients. In 150/179 of patients (83.8%) tumor size was ≤3 cm.

Results: One procedure-related death occurred. The proportion of major complications after treatment was 3.1%. Eighty-three patients, including the former (53%), died during follow-up. The median length of follow-up was 15 month (6% of patients lost on follow-up), and the median overall survival was 47.6 months. Overall survival in follow-up was 38% (C-P A 46% at five years, C-P B 23% at three years). In 78% of patients (139/179), we obtained a complete radiological response by CT or MR. In 90.6% of patients complete tumor necrosis was obtained after a single treatment
session. The remaining 40/179 of patients (22.3%) had a stable disease (SD) or a progressive disease (PD) after at most two sessions of RFTA. Overall recurrence rate in patients who achieved a complete response by one or two sessions of RFTA was 69%. By Cox regression analysis, survival was independently predicted by tumor size <3cm, complete radiological response at 1 month after treatment, high albumin levels and no former treatment for HCC.

Conclusions: Patients with the longest survival are those with a baseline HCC ≤3 cm, normal albumin and no former treatment, who have a complete response at imaging 1 month after treatment. Complete necrosis of the primary lesion after one or two sessions of RFTA is related to a long survival and may be considered as a strong surrogate endpoint for efficacy of treatment.

### 263 RADIOEMBOLIZATION WITH YTTRIUM-90 FOR ADVANCED HEPATOCellular CARCINOMA: A CANADIAN EXPERIENCE

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**Background and Aim:** Hepatocellular carcinoma (HCC) ranks among the most common cancers and is a leading cause of cancer-related death. Yttrium-90 (Y90)-labeled microspheres are administered via the transarterial route in a procedure similar to that used for transarterial chemoembolization, allowing specific and targeted therapy. In this study we aimed to establish the efficacy and safety of this procedure.

**Patients and Methods:** Seventy-one patients with unresectable HCC who received radioembolization with Y90 between December 2006-September 2012 were retrospectively analyzed.

**Results:** Sixty-four patients (90%) were males and mean age was 62±2 years. Thirty patients had multiple tumors, mean maximum tumor diameter was 9.8±4.5 cm, and 22 (31%) patients had portal vein thrombosis. Mean survival was 20±3 months. After the first radioembolization and during a mean follow-up of 12±2 months 42 patients (59%) died, 4 (6%) received liver transplant, and 25 (35%) were alive. Two patients (3%) developed gastric ulcers, treated conservatively, 3 had bleeding from femoral puncture, another 2 had transient worsening of the liver biochemistries, 1 developed pleural effusion, and 1 had significant abdominal pain. In the univariate Cox analysis presence of ascites (HR 2.18, P=0.01), hepatic encephalopathy (HR 8.59, P<0.001), INR (HR 1.53, P=0.03), albumin (HR 0.52, P=0.006), bilirubin (HR 1.01, P=0.001), MELD score (HR 1.16, P<0.001), and Child–Pugh (HR 3.26, P<0.001), were significantly associated with mortality. Maximum tumor diameter (HR 0.96; P=0.3), presence of multiple tumors (HR 1.71, P=0.09), alphafetoprotein (HR 1.0; P=0.5), and portal vein thrombosis (HR 1.27, P=0.5) were not associated with mortality. By multivariate Cox analysis MELD (HR 1.01, P=0.02), and Child–Pugh (HR 2.54, P=0.002) were independently associated with mortality. Patients with MELD <12 had a mean survival of 24±3 months, and those with MELD≥12 had mean survival of 8±2 months (Log Rank=0.001). Also, patients with Child–Pugh <8 points (Early B) had a mean survival 23±3 months, compared to Child–Pugh ≥8 who had mean survival 9±3 months.

**Conclusions:** Radioembolization with Y90 is an effective treatment for unresectable HCC. This procedure is also safe and was not associated with major adverse events; however, should be used preferentially in patients with preserved liver function.

### 264 PATTERN OF RECURRENTCE AND PROGNOSIS OF PATIENTS WITH HEPATOCellular CARCINOMA UNDERGOING HEPATIC RESECTION, WITH PARTICULAR REFERENCE TO THE HEPATITIS B AND C VIRAL INFECTION STATUS

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**Background and Aims:** Although the high frequency of posthepatectomy recurrence is a drawback in the treatment of hepatocellular carcinoma (HCC), various modalities, including repeated hepatectomies, have been employed effectively to treat recurrences, according to the tumor recurrence status. Therefore, the overall long-term prognosis of patients with HCC depends largely on the pattern of recurrence/treatment. In this study, we investigated the patterns of recurrence and prognosis in HCC patients, especially in relation to the hepatitis virus infection status.

**Methods:** The study population comprised 244 consecutive patients with HCC treated by hepatectomy. Curative treatments, including repeated hepatectomies, were performed for recurrences, whenever possible. Detailed information on recurrences was collected until the recurrences exceeded Milan’s criteria.

**Results:** The 3- and 5-year disease-free survival rates, survival rates within the Milan criteria, and overall survival rates in the 244 patients were 51.5% and 38.4%, 66.8% and 56.3%, and 84.8% and 74.5%, respectively. In the comparison between patients with hepatitis C virus-related HCC (HC-HCC: n=111) and hepatitis B-related HCC (HB-HCC: n=45), patients with HC-HCC showed lower disease-free (30.2% vs. 40.7% at 5 years, P=0.061) and overall (65.7% vs. 89.7% at 5 years, P=0.011) survival rates; these patients also showed a higher incidence of multinodular (>4) intrahepatic recurrences (19.4% vs. 5.3% at 3 years, P=0.010) as compared to those with HB-HCC. On the other hand, the incidences of recurrences that exceeded the Milan criteria because of other components falling outside the criteria were comparable between the two groups. During the follow-up period, patients with HC-HCC showed a higher incidence of intrahepatic recurrences characterized by multiple lesions and the difference became increasingly more pronounced with time.

**Conclusions:** The Milan criteria served as discriminants to determine whether the recurrent disease was controllable or not controllable. Patients with HC-HCC showed a higher incidence of carcinogenesis in the background liver than those with HB-HCC, and this difference was aggravated with time after hepatic resection.

### 265 COST-EFFECTIVENESS OF HEPATIC RESECTION VERSUS PERCUTANEOUS ABLATION FOR HEPATOCellular CARCINOMA WITHIN THE MILAN CRITERIA

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**Background and Aims:** Both hepatic resection and radiofrequency ablation (RFA) are considered curative treatments for hepatocellular carcinoma (HCC), but their economic impact still remains not clearly determined. The aim of the present study was to analyse the effectiveness and cost-effectiveness (CE) of these two strategies in early stage HCC (Milan criteria).

**Methods:** A Markov model was constructed considering data extracted from a systematic review of the pertinent literature of the last decade and consequent metaanalysis. Patient and disease-free survival were calculated for each treatment by meta-analysis of the
review results using a random-effects model. Costs were assessed from the perspective of the health care providers. A Monte Carlo probabilistic sensitivity analysis was used to estimate outcomes with distribution samples of 1,000 patients for each treatment arm.

Findings: Two randomized controlled trials and fifteen observational studies fulfilled the inclusion criteria; 8,420 patients were included: 3,996 patients underwent resection and 4,424 underwent RFA for early HCC. In a 10 year perspective, for very early HCC (single nodule ≤2cm) in Child–Pugh class A patients, RFA provided similar life-expectancy and quality-adjusted life-expectancy at a lower cost than resection and was the most cost-effective therapeutic strategy. For single HCCs, 1–3 cm and 3–5 cm in diameter, resection provided better life-expectancy and was more cost-effective than RFA, at a willingness-to-pay above €4,200 per quality-adjusted life-year for nodules 3–5 cm. In the presence of two or three nodules ≤3 cm, life-expectancy and quality-adjusted life-expectancy were very similar between the two treatments, but cost-effectiveness was again in favour of RFA, given its lower costs.

Interpretation: For very early HCC and in the presence of two or three nodules ≤3 cm, RFA is more cost-effective than surgical resection; conversely, for single larger early stage HCCs, surgical resection remains the best strategy to adopt as a result of better survival rates at an acceptable increase in cost.

266 COMBINED PERCUTANEOUS MICROWAVE ABLATION (MWA) AND TRANSDURAL CHEMOEMBOLIZATION (TACE) FOR HEPATOCELLULAR CARCINOMA

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Background and Aims: Locoregional therapies are useful for the treatment of unresectable hepatocellular carcinoma (HCC). Radiofrequency thermal ablation (RFA) is considered the standard of care for patients with lesions smaller than 3 cm in diameter not suitable for surgery. However, the likelihood of complete ablation using RFA declines rapidly as tumor diameter is greater than 3 cm. The combination of RFA and transarterial chemoembolization (TACE) has resulted in higher percentage of complete necrosis of the HCCs over 3 cm. Microwaves ablation (MWA) has recently emerged as a new technique promising larger and faster ablation areas without some of the RFA limitations. There is only one report in literature regarding the use of MWA in association with TACE in the treatment of liver lesions; herein we report our preliminary results on feasibility and effectiveness of the combination of thermal ablation with a new 2.45-MHz generator of microwave and TACE in unresectable HCCs larger than 3 cm.

Methods: Thirty-six nodules (size 3–11 cm, mean 4.78 cm, DS=2.09) of HCC were treated with a combination of percutaneous US-guided MWA and TACE (one treatment of ablation and one session of TACE for each lesion). Abdominal contrast enhanced CT scan was carried out 1 month after treatments, and then every three months to assess efficacy. "Technique effectiveness" was defined as complete absence of contrast enhancement with homogeneous hypodensity in treated area.

Results: Technique effectiveness was achieved in 83.3% of the lesions; intermediate-sized HCCs obtained 100% of complete necrosis. Local tumor progressions were found in 3 treated lesions (8%) a median of 9 months after the procedures (range 7–19). Treatments were followed by few adverse effects (AEs), without G4 AEs, according to CTCAE 4.0; particularly, we found hypertransaminasemia G3 in two cases (5%), without any worsening of liver function according to Child–Pugh Score. No deaths, or other major complications occurred.

Conclusion: Our preliminary data showed that the combination of MWA and TACE for the treatment of intermediate and large-sized HCCs is a feasible and safe method, not burdened by an increase of toxicity, with encouraging results in terms of efficacy.

267 DERMATOLOGIC ADVERSE EVENTS WITHIN THE FIRST 60 DAYS OF SORAFENIB TREATMENT ARE ASSOCIATED WITH BETTER OVERALL SURVIVAL (OS) IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)

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Background: Sorafenib [Sor] improves the OS of patients with advanced HCC. Currently, there are no clinical data or markers to predict better survival. The majority of Sor adverse events (AEs) [dermatologic, gastrointestinal or cardiovascular], appear within the first 60 days of treatment and they may influence patients management and outcome.

Aim: To analyze in a cohort of HCC patients treated with Sor the association of dermatologic AEs emerging within the first 60 days (AED60) with the outcome in terms of time to progression (TTP) and OS.

Methods: We prospectively included patients Child–Pugh A/B7 without ascites/encephalopathy, PS 0–1, and without contraindication or risk for Sor. Follow-up included monthly clinical and laboratory monitoring and tumor staging at week 4 and every 8 weeks.

Results: We included 147 patients [Oct. 2007-Jul. 2011] (97% cirrhotic, 46% HCV+, 64yrs, 82% Child–Pugh A, PS 0 84%, BCLC-B 78, BCLC-C 69. With a median follow up of 11.6 months and a treatment duration (mTD) of 6.7 months, TTP and OS were 5.1 months and 12.7 months, respectively. 79 patients presented AED60 and 37 patients needed Sor dose modification due to AED60. Median TTP (6.3 vs. 2.7; p=0.02) and OS (18.2 vs. 9.9; p=0.001) were significantly better in patients with AED60, while mTD was similar [EAD60: 7.6 vs. nonEAD60: 5.5; p=NS]. However, patients with EAD60 presented more dose modifications [3 vs. 2 (p=0.006)]. Results were similar when excluding the 8 patients that died during the first 60 days. Other early AEs categories did not have an impact in outcome. Baseline PS (p<0.001), BCLC (p=0.009) and EAD60 of any grade (p=0.04) were associated with better OS in the multivariate analysis of the whole cohort. Nevertheless, when excluding early deaths, the predictive value was restricted to EAD60 > grade 1 which determines Sor dose modification (p=0.03).

Conclusion: Development of dermatologic adverse events within 60 days of sorafenib initiation is associated with better survival. Therefore, this should not to be taken as a negative event and discourage treatment maintenance. Likewise, 2nd line clinical trials should be designed and/or evaluated considering this information to avoid significant bias.
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SAFETY AND TOXICITY OF THE COMBINATION OF Y90-RADIOEMBOLIZATION AND SORAFENIB IN ADVANCED HCC: AN INTERIM ANALYSIS OF THE EUROPEAN MULTICENTER TRIAL SORAMIC
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Background and Aims: SORAMIC is a randomized phase II-study in HCC composed of three substudies:
- b. RFA plus Sorafenib or placebo for time-to-recurrence;
- a. Gd-EOB-DTPA MRI versus MSCT for stratification to a palliative or a local ablation treatment strategy;
- c. overall survival for Y90-radioembolization plus sorafenib versus sorafenib alone. A planned interim analysis was performed to assess the safety of the combination of SIRT and Sorafenib.

Methods: Eligibility criteria for the palliative study arm included bCLC B/C not suitable for TACE, no lung metastases, Child Pugh ≤7 points, bilirubin within 1.5 times upper limit. In the combination group, radioembolization was performed sequentially at an interval of 4–6 weeks if both liver lobes were involved. Sorafenib was started at 200mg bid day 3 after the last radioembolization and increased to 400mg bid day 10. In the control arm Sorafenib was started at 200mg bid and increased to 400mg bid day 8. Safety data was assessed from the first 40 patients assigned to combination treatment (n=20) or control (n=20). All patients had completed at least the first 8 week follow up after Sorafenib initiation.

Results: Median follow up was 8.3 months. 3 patients in the combination arm displayed partial or complete portal vein thrombosis, 7 in the control arm. The Y90-dose in the combination arm was 1.9Gbq at median (range: 0.5–2.4Gbq). The sorafenib dose administered was 614mg at median (range: 45–793) in the combination group (median duration: 8.5 months) and 557mg (range: 284–792) in control (median duration: 9.7 months). We encountered a total number of AE’s of 196 and 220 in the combination and control arm with grade 3–5 reaching 42 and 49, respectively (p<0.05). One patient died due to brain hemorrhage in the control arm, one due to GI-bleeding in the control arm. No significant differences in the number of total or grade 3–5 toxicities were recorded in the categories bilirubin, albumin, liver enzymes, ascites, Child–Pugh, fatigue, HFSR, blood pressure or diarrhoea (p<0.05).

Conclusion: The interim safety analysis reassures that Y90-radioembolization as a sequential approach followed by an escalation scheme for Sorafenib does not lead to increased toxicity. SORAMIC continues to recruit.

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INTRAHEPATIC CHOLANGIOCARCINOMA (I-CC) OR MIXED HEPATOCELLULAR–CHOLANGIOCARCINOMA (HCC-CC) IN PATIENTS UNDERGOING LIVER TRANSPLANTATION. A SPANISH CASE CONTROL MULTICENTER STUDY
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Background and Aims: Information about the outcome of cirrhotic patients transplanted for suspected HCC and diagnosed of HCC-CC or I-CC at explant pathology is limited. Our aim was to evaluate the outcome of patients with HCC-CC or I-CC at explant pathology after LT for suspected HCC.

Methods: Multicenter, retrospective, case–control study 1:2. The study group (n=42) was formed by patients with HCC-CC or I-CC in the pathology examination. The control group (n=84) was formed by patients with HCC in the pathology examination, matched by number and size of the nodules (±8mm) and etiology of liver disease. The I-CC subgroup (27 patients with I-CC or separate I-CC and HCC vs. 54 controls) and HCC-CC subgroup (15 patients with HCC-CC vs. 30 controls) were also analyzed separately.

Results: The median posttransplant follow-up was 51 (3–142) months. No differences were found in terms of age, sex, etiology, Child–Pugh, MELD, AFP, waiting time, and treatment before liver transplantation between the study and control groups. One-, 3- and 5-year actuarial survival differed between study and control groups (83%, 70% and 60% vs. 99%, 94%, and 89%, respectively, p<0.001). Differences were found in 1-, 3- and 5-year actuarial survival between the I-CC subgroup and their controls (78%, 66% and 51%...
Conclusions: Patients with HCC-CC have similar survival to patients transplanted with HCC. Preoperative diagnosis of HCC-CC should not prompt the exclusion of these patients from transplantation.

270 TREATMENT OF EARLY STAGE HEPATOCELLULAR CARCINOMA IN CHILD–PUGH A CIRRHOTIC PATIENTS NOT CANDIDATES FOR LIVER TRANSPLANTATION: RESECTION VERSUS RADIOFREQUENCY, OUTCOME AND COSTS

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Background and Aims: Surgical resection (SR) and radiofrequency ablation (RFA) are well established curative therapies for the treatment of early stage hepatocellular carcinoma. The aim of our study was to compare the long-term evolution and the cost of treatment with SR or RFA in cirrhotic patients with early hepatocellular carcinoma not candidates to liver transplantation.

Methods: Cohort study. From 2006–2011, 538 patients with hepatocellular carcinoma have been studied in our center. As initial treatment, 49 patients (9.1%) were treated with SR and 78 (14.5%) with RFA. Only cirrhotic patients with a single nodule <4 cm and Child–Pugh A that were not candidates to liver transplantation were included. We studied 20 resected patients and 59 treated by radiofrequency. Actuarial survival, disease-free survival, successive treatments and the overall cost of the treatment at follow-up in regard to anti-tumor treatment were calculated.

Results: The mean follow-up was 32 ± 19 months. Patients treated with SR were younger and have a greater number of platelets and minor bilirubin and albumin but are comparable in nodule size, Child–Pugh stage (5–6), performance status and stage of BCLC. Actuarial survival in the SR group at one, three and five years was 100%, 76.4% and 57.35% and in the RFA group 94.4%, 64%, and 55.4% respectively (p = 0.05). One, three and five year disease-free survival was 94.4%, 66.1% and 43.47% in the CIR group and 91%, 52.4% and 37.5% in the RFA, respectively (p < 0.03). In the SR group recurrences were treated with 1 RF, 2 transarterial chemoembolization and 3 treatments with sorafenib. In the RFA group recurrences were treated with 2 transarterial chemoembolization, 7 RFA and 1 patient with sorafenib. The cost of treatment considering all procedures was 2479 euros/patient/year in the SR group and 1406 euros/patient/year in the RFA group.

Conclusions: In our series, Child–Pugh A cirrhotic patients with a single tumor less than 4 cm when treated with surgical resection or radiofrequency have comparable actuarial survival and disease-free survival. The cost of antitumor treatment per patient and year is higher in the surgery group.

271 EFFICACY AND SAFETY OF PERCUTANEOUS MICROWAVE ABLATION FOR HCC >3 cm. A SINGLE-CENTER EXPERIENCE

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Background: The incidence of Hepatocellular Carcinoma (HCC) is increasing worldwide. Only 30–40% of cirrhotic patients are eligible to resection, liver transplantation and ablation, according to BCLC staging system. Radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) obtain a high rate of complete necrosis (CR) in tumors of 2–3 cm. However, RFA has some limitations. Recently, microwave thermal ablation (MWA) has been proposed in order to achieve the destruction of liver tumors ≥3 cm or close to vascular structures.

Methods: From April 2010 to October 2011, 53 consecutive treatment-naive patients (M/F: 37/16; mean age 70.6 years) were treated. All had cirrhosis (Child–Pugh A/B: 40/13). 47 patients had 1 nodule >3 cm; 6 had 2 nodules, major >3 cm. None were eligible to surgery. Tumor size ranged 3.1–7 cm (mean 4.4 cm). All were treated with percutaneous MWA using 14G needles at an operating frequency of 2450 MHz (AMICA-GEN, HS, Italy). Treatments were performed in a single center with pluriannual experience of RFA and PEI. A written informed consent was obtained from all patients. Treatment efficacy was evaluated using triphasic CT by a single radiologist. CR was defined as absence of contrast enhancement in treated nodules at f-u ≥6 months (mean f-u 13 months, range 7–24). The number and type of complications and side effects were recorded after the procedure and at f-u.

Results: Ablation through microwave achieved a CR in 39 patients (73.6%). 11 patients (8CR) received a second session of treatment after a month. Complete response was achieved only on 2/6 patients with two nodules. Also, 33/40 patients (82.5%) with a single HCC of 3–4 cm showed a CR, while 6/13 with a single tumor >4 cm or of two nodules had a CR (p = 0.02). No deaths and tumoral seeding were reported. A case of biloma was the only major complication occurred (1.8%). Periprocedural side effects as fever, pain and AST/ALT increase were common but did not require extended hospitalization.

Conclusions: Our results suggest that MWA is effective in HCC of 3–4 cm. A CR is achievable also in nodules >4 cm. The procedure was found to be highly safe.

272 MORPHOPHENOTYPIC TACE PREDICTORS IN HEPATOCELLULAR CARCINOMA

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Introduction: Transarterial chemoembolization (TACE) is a locoregional treatment for hepatocellular carcinoma (HCC). It is indicated to control unresectable HCC or as bridging therapy to...
surgery. The individual response to TACE is mostly unpredictable, and to date, only the tumor diameter has been suggested as a helpful predictor, with ≤3 cm HCC less likely to respond. Aim of the present study is to identify tissue TACE predictors of response, to be used in the clinical practice, possibly also on pretreatment liver biopsies.

Materials and Methods: Forty-one patients with HCC underwent liver explantation or resection following TACE. Clinico-pathological data and surgical specimens were available in all the cases. Nineteen HCC liver biopsies obtained before TACE from the same patients were also available. We investigated the expression of markers involved in adaptive mechanisms to hypoxia (CD34 for microvessel density, HIF-1α, VEGF, CAIX) and then we evaluated their association with tumor necrosis, histologically assessed, in the resection specimens. On the basis of the extent of necrosis we defined two groups of nodules, namely the TACE low responders (TLR, ≤50% necrosis) and TACE high responders (THR, >50% necrosis).

Results: In surgical specimens 45/103 (44%) nodules were classified as TLR. Of these 45 nodules, 33 (73%) had a diameter ≤3 cm, 28 (62%) showed a high microvessel density, 30 (66%) CAIX hyperexpression and 40 (89%) VEGF hypoexpression (p < 0.05). While none of these parameters, individually taken, was able to predict with sufficient accuracy a TACE low response, their combination resulted more effective. The association of at least 3 of these parameters, regardless which one, was able to predict a TACE low response with 73% sensitivity, 76% specificity and 74% accuracy. When also applied to pre-TACE liver biopsy the model showed 100% sensitivity, 78% specificity and 89% accuracy.

Conclusions: This study identifies potential tissue markers (VEGF, MVD, and CAIX) able to predict, in combination with the tumor size, the effectiveness of TACE in the treatment of individual HCC. The evaluation of these markers on liver biopsies before chemoembolization may be helpful to optimize patient selection and the recall policy during follow up.

273 COMBINATION OF SORAFENIB AND TRANSARTERIAL CHEMOEMBOLISATION (TACE) IN ADVANCED STAGE HEPATOCELLULAR CARCINOMA (HCC): A RETROSPECTIVE COHORT STUDY AT THREE GERMAN LIVER CENTERS

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Sorafenib is the standard of care (SOC) in patients with advanced Barcelona Clinic Liver Cancer (BCLC) stage C, defined by portal vein infiltration (PVI), extrahepatic tumour manifestation (EHM) and/or a reduced ECOG performance status. Although prospective, randomized results on the use of selective, lipiodol-based TACE either alone or in combination with sorafenib in BCLC stage C are lacking, TACE has been reported to allow a better local tumour control in selected patients.

Methods: Patients with BCLC stage C, who where treated with sorafenib and TACE were retrospectively analysed at three German liver centers. BCLC stage C patients, who were treated either with TACE or sorafenib in the same period of time (January 2007 till October 2012) outside a clinical trial were analysed for comparison. The ethical committee at the University Hospital Frankfurt approved the study.

Results: A total of 134 patients with BCLC stage C were identified, who were treated with sorafenib and TACE (group A; n = 50), TACE (group B; n = 50) or sorafenib (group C; n = 34). The median age of patients was 62, 66 and 63 years, and 88%, 70% and 91% of patients were male, respectively. In groups A, B and C PVI was present in 32%, 72% and 56% and EHM was present in 60%, 6% and 47%. The median CLIP score was 2.1, 2.4 and 2.4. Time-to-progression was 6.3 months (95% CI: 4.3–8.3), 5.4 months (95% CI: 3.5–7.3) and 3.5 months (95% CI: 2.9–4.1) in groups A, B and C. Those with time to progression to AFP as a predictor of survival.

274 TIME TO PROGRESSION TO AFP AS A PREDICTOR OF SURVIVAL IN PATIENTS WITH HEPATOCELLULAR CARCINOMA TREATED WITH SORAFENIB

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Background: Sorafenib (SOR) is the treatment of choice for advanced hepatocellular carcinoma (HCC). Time to radiographic progression (TTP, according to mRECIST) can be a surrogate marker of survival, but its evaluation is complex and costly. It has been suggested that some initial adverse events (hypertension, hand-foot syndrome), as well as changes in AFP during treatment, may have prognostic value.

Aim: To identify clinical/analytical variables with prognostic value in HCC patients treated with SOR.

Methods: Single-center, observational, prospective study of HCC treated with SOR, excluding clinical trials, post-transplant recurrence and combinations with Y90/stereotactic radiotherapy. Clinical data, adverse events (CTCAE v3.0 scale) and SOR changes over time, were prospectively collected from the start of SOR until second-line therapy or death.

Time to progression to AFP was defined from the start of SOR until the time in which AFP rises after nadir; in those with normal AFP: from the start of SOR until the point in which AFP > 20 ng/ml (measurement monthly).

Results: From Jul-07 to Oct-12, 122 patients were included: mean age 63 years, 102 males, 102 cirrhosis. Etiology: alcohol 50% (41%), hepatitis C virus 33% (27%), mixed (HCV + alcohol) 14% (12%), B virus 5% (4%). Staging: BCLC-A 1, BCLC-B 28, BCLC-C 93, Child–Pugh class A 96, asymptomatic 78 (ECOG-PS 0). Median duration of treatment was 7.9 months. Median overall survival (OS) 10.9 months; OS in those starting SOR before 1-Jan-12 (n = 99): 13.7 months (95% CI 10.9–16.4). Low dose and short-term discontinuation of SOR in 82 (68%) and 52 (43%) patients, respectively; full dose maintained in 34 (29%). Severe adverse events (CTCAE grade 3–4): 17 cardiovascular, 12 skin, 11 gastrointestinal bleeding (8 variceal/portal gastropathy), 12 ischemic pancreatitis, 11 decompensation of cirrhosis, 11 infections.

In our cohort, nor hypertension nor skin effects CTCAE >2 during the first three months were predictor of survival. By contrast, 75 patients (62%) increased AFP (median time 129 days). Those with time to progression to AFP > 3 months (n = 28)
when including patients receiving LT, controls had higher survival actually underwent transplantation. risk of death (HR 2.89; 95%CI, 1.12–7.49) compared to those who stage (A/B vs. C/D), MELD score, and largest tumor size, treatment models also inclusive of cirrhosis, race (Asian vs. non-Asian), BCLC in pre-MELD era (Figure). In multivariate cox proportional hazards methods also inclusive of cirrhosis, race (Asian vs. non-Asian), BCLC models also inclusive of cirrhosis, race (Asian vs. non-Asian), BCLC in pre-MELD era (Figure). In multivariate cox proportional hazards models. using Kaplan Meier methods, log-rank test, and multivariate cox proportional hazards models. survival was evaluated using Kaplan Meier methods, log-rank test, and multivariate cox proportional hazards models. results: our study included 171 patients (57 cases and 114 controls). Compared to controls, cases had lower rates of cirrhosis (61.4% vs. 90.4%, p < 0.001), lower MELD (8.9 vs. 11.2, p < 0.001), and greater post-treatment tumor recurrence (43.9% vs. 12.9%, p < 0.001). Using ITT, no survival difference was observed between cases and controls in post-MELD exception era (5-year survival: 69.5% vs. 66.6%, p = 0.87), but a trend towards higher survival was seen in pre-MELD era (5-year survival: 69.5% vs. 46.7%, p = 0.09). When including patients receiving LT, controls had higher survival in post-MELD era (5-year survival: 100% vs. 69.5%, p = 0.04), but not in pre-MELD era (Figure). In multivariate cox proportional hazards models also inclusive of cirrhosis, race (Asian vs. non-Asian), BCLC stage (A/B vs. C/D), MELD score, and largest tumor size, treatment with surgical resection was associated with significantly higher risk of death (HR 2.89; 95%CI, 1.12–7.49) compared to those who actually underwent transplantation.

conclusion: using an ITT model of transplant-eligible patients, no significant difference in survival was observed between patients treated with resection and those listed for LT. however, patients who received LT had significantly better outcomes, and resection was a significant independent predictor for poorer survival. LT is superior to surgical resection in treating HCC in transplant-eligible patients if patients can successfully undergo LT.

A comparison of primary surgical resection to liver transplantation (LT) among transplant-eligible hepatocellular carcinoma (HCC) patients using an intention-to-treat model

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background and aims: the efficacy of LT in the management of HCC is limited by scarce organ availability, resulting in disease progression and mortality on transplantation waitlists. Surgical resection is generally limited to treatment of small, localized tumors in patients with well-preserved hepatic function. Data comparing resection with LT among transplant-eligible patients is limited.

methods: we performed a retrospective case cohort study comparing transplant-eligible patients (by Milan criteria) who underwent resection (cases) with those listed for LT (controls) in an intention-to-treat (ITT) model over a 15-year period. Controls included two patients for every case, matched by age ± 5 years, sex, and HCC etiology (HCV vs. non-HCV). Survival was evaluated using Kaplan Meier methods, log-rank test, and multivariate cox proportional hazards models.

results: our study included 171 patients (57 cases and 114 controls). Compared to controls, cases had lower rates of cirrhosis (61.4% vs. 90.4%, p < 0.001), lower MELD (8.9 vs. 11.2, p < 0.001), and greater post-treatment tumor recurrence (43.9% vs. 12.9%, p < 0.001). Using ITT, no survival difference was observed between cases and controls in post-MELD exception era (5-year survival: 69.5% vs. 66.6%, p = 0.87), but a trend towards higher survival was seen in pre-MELD era (5-year survival: 69.5% vs. 46.7%, p = 0.09). When including patients receiving LT, controls had higher survival in post-MELD era (5-year survival: 100% vs. 69.5%, p = 0.04), but not in pre-MELD era (Figure). In multivariate cox proportional hazards models also inclusive of cirrhosis, race (Asian vs. non-Asian), BCLC stage (A/B vs. C/D), MELD score, and largest tumor size, treatment with surgical resection was associated with significantly higher risk of death (HR 2.89; 95%CI, 1.12–7.49) compared to those who actually underwent transplantation.

Conclusion: Using an ITT model of transplant-eligible patients, no significant difference in survival was observed between patients treated with resection and those listed for LT. However, patients who received LT had significantly better outcomes, and resection was a significant independent predictor for poorer survival. LT is superior to surgical resection in treating HCC in transplant-eligible patients if patients can successfully undergo LT.

OUTCOMES IN PATIENTS WITH SMALL HEPATOCELLULAR CARCINOMA AND CHRONIC HEPATITIS C INFECTION TREATED WITH RFA AND ANTIVIRAL THERAPY

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Introduction: Hepatocellular carcinoma (HCC) is the third most common cause of death worldwide. The risk of developing HCC in patients suffering from cirrhosis is increased in the setting of chronic HCV.

Objective: To determine the tumor recurrence, safety, and survival outcomes of HCC patients with chronic hepatitis C (genotype 1) infection after receiving radiofrequency ablation (RFA) and antiviral therapy using peg-alfa interferon and weight based ribavirin.

Methods: Using our institution’s database, we identified all patients with chronic Hepatitis C (HCV) genotype 1 and small HCC (less than 3.0 cm) between December 2007 – December 2010. The following data was extracted; sustained virological rate (SVR), tumor necrosis rate and tumor recurrent rate, and 1-year survival rate. HCC recurrence and monitoring was done using serum a-fetoprotein (AFP) test and radiological findings.

Results: During the study period, there were 75 patients (42 males, 33 females, age 43 years (32–54) with HCC (≤3cm) and HCV (genotype 1). We divided the patients into two groups: control group (n = 33) received RFA only and treatment group (n = 42) received RAFa and peg-alfa interferon with weight based ribavirin. The tumor complete necrosis rate at three months in the control group was 24.24% versus Rx group was 50% (P < 0.05). The one-year viral suppression in the control group was 30.3% versus Rx group 64.28% (P < 0.05). The HCC recurrence rate in the control group was 38.39% versus Rx group 71% (P < 0.05). The one-year survival rate was 30.3% in control group versus Rx group 61.9% (P < 0.05).

Conclusion: The above results demonstrate potential benefits of adding antiviral therapy and suppressing HCV virus in patients with compensated cirrhosis and small HCC undergoing RFA. Further trials involving larger number of patients are needed to delineate the overall impact of HCV eradication in the patient with compensated cirrhosis and HCC. As the antiviral therapies continue to evolve future trials may offer an opportunity at viral eradication prior to LTx thus improving long term outcomes.

Sorafenib enhances effects of transarterial chemoembolisation for hepatocellular carcinoma: a systematic review and meta-analysis

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Objective: Combination therapy of sorafenib and transarterial chemoembolization (TACE) showed benefits for hepatocellular carcinoma (HCC). This systematic review aims for evaluation of efficacy and safety between sorafenib plus TACE and TACE alone for HCC.

Methods: We systematically searched multi-databases to identify eligible studies. Studies comparing sorafenib combined with TACE and TACE alone for HCC were included.
Background and Aims: Regulated necrosis is arising as a prominent pathological feature of inflammation-driven liver diseases. Morphology is similar to that of oncocytic necrosis. In addition, bile acids are well-established modulators of hepatocyte apoptosis, which may influence regulated necrosis. We aimed to: i) evaluate whether toxic bile acids, namely deoxycholic (DCA) and glycochenodeoxycholic (GCDCA) acids induce regulated necrosis; and ii) assess if ursodeoxycholic acid (UDCA), an hepatoprotective bile acid, can modulate regulated necrosis in primary rat hepatocytes. In HepG2 cells, bile acid-induced caspase-3-independent cell death in primary rat hepatocytes, which was inhibited by necrostatin-1. Similar results were obtained when using concanavalin A or TNFalpha as toxic stimuli. In agreement, cellular release of HMGB1 and RIP3 expression increased in the presence of bile acids plus zVAD-fmk. Pretreatment with UDCA significantly attenuated cell death by GCDCA and DCA in the presence of zVAD-fmk. Finally, DCA and GCDCA triggered conversion of LC3I to LC3II, although bile acid-induced regulated necrosis appeared to proceed normally in the presence of 3-MA.

Conclusions: Our results suggest that GCDCA and DCA induce regulated necrosis in primary rat hepatocytes, which in turn is inhibited by UDCA. In addition, toxic bile acids may trigger autophagy as a pro-survival mechanism during apoptosis, but not during regulated necrosis. A further understanding of the intricate network of cell death mechanisms in the liver may open the door for novel therapeutic approaches.


279 AUTOPHAGY NEGATIVELY REGULATES THE MESENCHYMAL PROGRAM IN HEPATOCYTES BY PROMOTING Snail DEGRADATION

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Background and Aims: In eukaryotic cells, autophagy is a highly conserved self-digestion process that promotes cell survival in response to nutrient starvation and other metabolic stresses. It has been known for many decades that autophagy is highly active during differentiation and development. Deregulation of autophagy is linked to susceptibility to various liver diseases including metabolic syndrome, infectious diseases and cancer. However, the significance of autophagy in modulation hepatic differentiation is still unclear. Our work has investigated whether autophagy plays a role in hepatocyte de-differentiation during the epithelial to mesenchymal transition (EMT).

Methods: The EMT was induced by TGFβ treatment in immortalised but not transformed hepatocytes. The influence of TGFβ on autophagy was monitored at mRNA and protein levels. The effects of autophagy on TGFβ-induced EMT was investigated either inhibiting or stimulating the autophagic flux. The interactions between autophagy and EMT-related proteins were investigated by immunoprecipitation, confocal microscopy, gene silencing and overexpression experiments. The data obtained in vitro were confirmed in animal models of autophagy.

Results: Firstly, we observed that in not transformed hepatocytes, differently from what reported in hepatoma cells, TGFβ negatively regulates autophagy, inducing the accumulation of the two autophagic markers LC3 (microtubule-associated protein 1 light chain 3) and p62. Moreover, we found that autophagy negatively regulates EMT in hepatocytes, mediating the degradation of snail. Interestingly, snail proteins is normally degraded by autophagy in hepatocytes, both in vitro and in vivo.

Conclusions: Our data show that in hepatocytes autophagy has an active role in the maintenance of epithelial identity inhibiting the mesenchymal program, through the protein degradation of Snail. Moreover, they suggest that TGFβ induces EMT in hepatocytes by inhibiting the autophagic process. The role played by the deregulation of the balance between autophagy and mesenchymal program in tumorigenesis and metastasis is currently under investigation.
ROLE OF cMYC IN CELL DORMANCY DISRUPTION IN LATENT TUMORS ESTABLISHED FROM HUMAN HEPATOCELLULAR CARCINOMA (HCC) CELL LINES

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Quiescence is a strategy that cancer stem cells (CSCs) adopt in stressful environments such as those triggered by antineoplastic treatments. CSCs form micrometastases that stay dormant for variable time periods and regain proliferation after unknown stimuli. cMYC induces pluripotency in differentiated cells and may have a role in dormant cells activation.

Aim: To analyze cMYC role in the switch from dormant (generated by HCC line BCLC5) to growing tumors formed by BCLC5 cells transfected with cMYC (BCLC5-cMYC), compared to BCLC9 already expressing cMYC.

Methods: Creation of a stable BCLC5 cell line expressing cMYC by transfection of pCMV6-cMYC, selection of cells expressing cMYC was done by G418 treatment. cMYC levels were confirmed by PCR/Western Blot (WB). Subcutaneous (s.c.) injection of cells into SCID mice: 10^6 BCLC9, BCLC5 and BCLC5-cMYC cells were s.c. injected. Stable cell lines derived from xenograft tumors: eight lines (11 to 18) were derived from a BCLC5-cMYC tumor and 3 cell lines (c1, c2, c4) from tumors produced by injection of BCLC5-cMYC t7 cells. Gene expression of proangiogenic genes (VEGFA, CD13), epithelial–mesenchymal markers (CDH1, VIM), invasion markers (NM23-H1, cMET, ITGB1, MMP2, MMP9) and genes associated with cell pluripotency (OCT4, SOX2, NANOG, CD133, EP Carm) were assessed by real-time PCR, immunohistochemistry and WB.

Results: BCLC9 cells produced tumors in SCID mice while BCLC5 cells developed viable tumors after 6 months of injection. BCLC5-cMYC cells generated tumors with high proliferation and cell lines showed increased expression of VEGFA, CD13 and CDH1 and decreased VIM, cMET and MMP2 compared to BCLC5. Tumor derived cell lines keep expressing pluripotency related genes and acquire EP Carm’s, which was originally expressed only by BCLC9. Second generation of tumors BCLC5-cMYC and their cell lines showed increased expression of proangiogenic markers, genes associated to epithelial phenotype and those related to CSC while markers associated with invasive behavior decreased.

Conclusion: cMYC activation induces angiogenesis and epithelial markers while preserving pluripotent phenotype and reducing invasive potential. These changes consolidate along tumor evolution explaining the aggressive behavior of residual disease after antineoplastic treatment.

INTRAHEPATIC ULTRASOUND-MEDIATED GENE DELIVERY

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Background and Aims: Transient ultrasound-induced increase in permeability of cell membrane and in some cases may enhance gene transfer. The objective of this work is to evaluate transfection efficiency and safety for intrahepatic gene delivery by sonoporation.

Methods: Polyplexes of expression vector DNAs complexed to galactose-bearing polyethylenimine have been used. Expression vectors are two plasmid containing expression cassette for full-size human preproinsulin gene controlled with CMV promoter and flanked by inverted terminal repeats of AAV (pTRhins) and the same cassette for marker gfp gene (pTRegfp). Animals were injected under US guidance with polyplexes in a dose of 40 µg pTRegfp/0.7 ml (rat) and of 15 µg pTRhins/0.15 ml (diabetic mouse) into the liver parenchyma of subdiaphragmals segments using 31 G needles. Afterwards injection locus in depth of 1 cm during 180 sec was ionized by 130 Db ultrasound using multifrequency 3–8 MHz probe.

Results: Sonoporation is able to enhance polypeptide intrahepatic gene delivery to transfecting liver cells in vivo. Flow cytometry analysis of primary hepatocytes isolated from the liver of experimental rats showed that ultrasound-enhanced polypeptide gene transfer was highly localized, and was superior to all controls. At least 42% of the liver cells in vivo can be transfected in this way with ultrasound exposure versus 1.2% without it. Hypoglycemic effect of the insulin gene delivery followed by 3 min US exposure was observed on the third day: glucose level of diabetic mice (hyperglycemic 6-week) decreased on average by 30%. A week after the procedure serial sections from rat liver injected with polyplexes containing 40 µg marker plasmid or saline solution alone exposed to US and without it were analysed for the presence of inflammation. Pathamorphological and histological analysis of experimental livers revealed no inflammatory processes in tissues, and any detectable side effects of US-enhanced gene delivery were seen. Experimental mice and rat liver DNAs were positive in transgene PCR for 1 month after gene delivery.

Conclusions: Our results demonstrate that polypeptide gene transfer by US exposure is effective, robust and feasible and can become the new relevant technology for gene therapy.

CELL CYCLE DEREGLERATION BY HCV PROTEIN EXPRESSION, A POTENTIAL HEPATOCARCINOCGENIC TRIGGER

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Chronic infection by hepatitis C virus (HCV) is a major risk factor for the onset and progression of hepatocellular carcinoma (HCC), which appears to be principally related to chronic local inflammation and fibrosis. Nevertheless, in vitro studies have shown that HCV proteins can directly interact with cell cycle regulators which might trigger carcinogenic processes.

Our goal was to assess in vivo hepatocyte cell cycle perturbation(s) by HCV proteins after an acute liver injury (CCL) using the FL-N/35 transgenic mouse model expressing the full HCV-ORF. Early after CCI, challenge, no differences in the expression of immediate-early genes, growth factors or cytokines were observed between FL-N/35 mice and wild-type littermates (wt), suggesting that cell cycle initiation steps are not perturbed by HCV protein expression.

However, cyclin-A expression and BrdU incorporation at cell S-phase entry were delayed in FL-N/35 mice compared to wt. At cell S-phase entry, Retinoblastoma protein (Rb) phosphorylation was reduced in FL-N/35 mice, suggesting a G1/S transition impairment in the liver of these mice.

We recently published that FL-N/35 mouse livers displayed high levels of DNA-damage. It has been established that the ATM pathway is activated by DNA double-strand breaks and leads to cell cycle arrest. We observed that Chk2 and p53 phosphorylation and p21^waf/cip1 expression, three actors of the ATM pathway, were significantly higher in FL-N/35 mice than in wt mice at G1/S transition. These results suggest that HCV-induced DNA-damage
might impair hepatocyte cell cycle G1/S transition via, at least in part, the activation of the ATM pathway. In addition, histological quantifications showed that mitotic hepatocytes were significantly less abundant in the parenchyma of transgenic mice than in their wt counterparts after CCl4 injection.

Conclusions: The expression of HCV proteins in the liver of HCV mice, in the absence of local inflammation or immune response, induces inhibition of the G1/S transition which could result from HCV-induced DNA damage/ATM pathway activation. This perturbation is a potential hepatocarcinogenic trigger.

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PREDICTION OF miRNA TARGET GENES INVOLVED IN LIVER CANCER PATHWAYS AND ITS VALIDATION
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Background and Aims: MicroRNAs are small 19–25 nucleotides which have been shown to play important roles in regulation of gene expression in many organisms. Phenotypic and expression analysis suggests an important role of microRNAs during development, differentiation, signaling and cancer. Downregulation or accumulation of miRNAs implies a tumor suppressor or oncogenic function. The prediction of miRNA target genes involved in liver cancer pathways and its validation was the aim of this study.

Methods: In this study, two miRNAs deferentially expressed in hepatocellular carcinoma (HCC) (hsa-miR-199a-3p and hsa-miR-195) were identified and analyzed. The prediction was done using a consensus approach of tools (e.g.: DIANA, miRanda, TargetScan, PicTar and RNAhybrid). The validations of the prediction were done at two different levels: (1) In silico was done through the 100 time shuffling for the miRNA sequences and statistical analysis for calculation of Z score and P-value of them followed by Gene Ontology analysis for the selected targets. (2) In vitro was done through different steps starting from targets isolation from Huh-7 cell line using pre-designed specific primers, through purification for these targets and sequencing using API sequencer followed by Gene expression analysis suggests an important role of microRNAs which have been shown to play important roles in regulation of gene expression in many organisms. Phenotypic and expression analysis suggests an important role of microRNAs during development, differentiation, signaling and cancer. Downregulation or accumulation of miRNAs implies a tumor suppressor or oncogenic function. The prediction of miRNA target genes involved in liver cancer pathways and its validation was the aim of this study.

Results: Ten different genes were successfully predicted (p < 0.005). Hsa-miR-195 targets seven highly significant different genes and Hsa-miR-199a-3p targets two different significant genes. Most of these genes are involved in important pathways in cancer like MAPK signaling pathway, Jak-STAT signaling pathways, regulation of actin cytoskeleton, angiogenesis, Wnt signaling pathway and TGF-beta signaling pathway. The predicted targets were FGFR, GHR, PCMT1, CITED2, PEX5, PEX13, NOVA1, AXIN2 and TSPYL2.

Conclusions: The target genes were found to control almost all the hallmarks of liver cancer and therefore could be used as therapeutic targets in cancer treatment.

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A NOVEL ROLE FOR BMP9 AS A PROTUMORIGENIC FACTOR IN LIVER
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Background and Aims: TGF-β family members play a relevant role in tumorigenic processes, including the hepatocellular carcinoma (HCC), but the specific implication of the BMPs subfamily is still unknown. BMPs are multifunctional signals that have been described to play a central role during development and they are also key in maintaining the homeostasis in adult organisms. Interestingly, dysregulation of BMP signalling can have pathophysiological consequences. Although originally isolated from fetal liver, little is known about BMP9, a BMP family member, and its role in liver. Here, we have investigated BMP9 signaling role in HCC cells.

Methods: Human immortalized hepatocytes and HCC cell lines were used to study the BMP9 growth effect. For the analysis of cell proliferation and apoptosis, various techniques were utilized, including DNA synthesis analysis by thymine incorporation and flow cytometry. Anchorage independent growth was assayed by monitoring colony formation in soft agar. BMP9 autocrine production was studied by using siRNA, ligand trap and BMP inhibitor approaches. BMP9 expression level in human HCC samples was analyzed by an immunohistochemistry analysis on a liver cancer tissue microarray.

Results: Our results show that BMP9 is a proliferative factor in HCC cell lines, but not in immortalized adult human hepatocytes. In HepG2 cells, BMP9 triggers the canonical pathway, which regulates expression of the Id1 gene. Importantly, we demonstrated that HepG2 cells have an autocrine BMP9 production that support their proliferation and their anchorage independent growth. Additionally, a more detailed analysis of the BMP9 effects in HepG2 cells revealed a remarkable BMP9-mediated survival effect against serum starvation-triggered apoptosis. Finally, BMP9 expression was increased in 40% of human HCC tissues compared with healthy liver as revealed by immunohistochemistry analysis, suggesting that BMP9 signaling may be relevant during HCC pathogenesis in vivo.

Conclusion: All together, our findings provide new clues for a better understanding of BMP contribution in the pathogenesis of HCC that may result in the development of effective and targeted therapeutic interventions.

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PLASMA MEMBRANE PROTEOMICS IDENTIFIES Notch1 AS A POTENTIAL REGULATOR OF Ras-INDUCED SENESCENCE
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Background: Oncogene-induced senescence (OIS) is an intrinsic tumour suppressor mechanism leading to stable cell-cycle arrest in response to unrestricted oncogene activation. OIS is a heterogeneous phenotype involving multiple effector mechanisms; our understanding of the role of OIS is lacking because of the
difficulty in identifying senescence in living tissues. We wished to
establish a cell-surface phenotype of OIS.

Methods: We utilised the well validated ER:Ras IMR90 HDF in
vitro model which undergo OIS after 6 days treatment with 4-OHT.
To establish a cell surface phenotype we utilised SILAC-based
proteomics and 3 labelling conditions (‘Light’, parental IMR90 +
4-OHT; ‘Medium’ (K+4Da, R+6Da) ER:ras IMR90; ‘Heavy’ (K+8Da,
R+10Da) ER:ras IMR90 + 4-OHT, combined with cell-surface
aminooxy-biotinylation prior to streptavidin pulldown. Tryptic
peptides were fractionated by HplP-HplC and then subjected to
LC-MS/MS. Data was processed by MaxQuant and MASCOT. Hits
were validated through FACS analysis, qPCR and immunoblotting.

Results: 899 proteins were identified. From Gene Ontology
annotation, 73% of these proteins were present at the cell
surface. Notch1 was significantly upregulated in senescent cells
when compared to both control conditions (3.1-3.4 fold). This
upregulation was confirmed by both FACS and immunoblotting.
Downstream Notch pathway signalling was also upregulated in
senescent cells. Treatment with the γ-secretase inhibitor DAPT led
to an increase in cells developing both SAHF and SA β-GAL, features
of OIS. However, Notch1 knockdown reduced both SAHF and SA
β-GAL. The effect of DAPT was not mediated through canonical
Notch1 signaling as RBP-j knockdown had no effect upon DAPT-
mediated upregulation of SAHF and SA β-GAL.

A possible explanation for these findings is that DAPT prevents
the γ-cleavage and promotes Notch1 retention on the cell surface.
During the transition to OIS, the canonical Notch pathway inhibitor
Numb is upregulated, leading to simultaneous inhibition of
canonical Notch and promotion of non-canonical Notch1 signaling.

Conclusion: PMP has identified a cell-surface phenotype of
OIS. Notch1 cell surface expression is upregulated in OIS. The
transition to OIS is correlated with a transition from canonical
to non-canonical Notch1 signalling that may be driven by Numb
expression.

286 EFFECTS OF VITAMIN D3 ON THE EXPRESSION OF
CYTOCHROME P450 (27A1 AND 2R1 ISOFORMS) AND CASPASE-3
ACTIVITY IN THE LIVER OF DIABETIC MICE
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Background and Aims: Diabetes mellitus is accompanied by
various biochemical and functional abnormalities in the liver.
Sustained hyperglycemia and increased oxidative stress play
major roles in the development of secondary complications in
diabetes including hyperglycemia-induced hepatic cells apoptosis.
Cholecalciferol (vitamin D3) is currently recognized to exert
a lot of non-calcemic effects such as immunoregulatory and
antiproliferative, but limited data are available at the moment in
a lot of non-calcemic effects such as immunoregulatory and
electrophoresis and immunoblotting.

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various biochemical and functional abnormalities in the liver.
Sustained hyperglycemia and increased oxidative stress play
major roles in the development of secondary complications in
diabetes including hyperglycemia-induced hepatic cells apoptosis.
Cholecalciferol (vitamin D3) is currently recognized to exert
a lot of non-calcemic effects such as immunoregulatory and
antiproliferative, but limited data are available at the moment in
relation to its effects on apoptosis. We therefore investigated the
relationship between vitamin D3 (F3, D3, D3, D3, D3) status, expression of D3, 25-
hydroxylases (CYP450 27A1 and 2R1, mitochondrial and microsomal
isoforms respectively) and caspase-3 activity.

Materials and Methods: Type 1 diabetes was induced in male
C57BL/J6 mice (weighing 25.0±1.5 g) by i.p. injection of multiple
low dose streptozotocin (40 mg/kg b.w.). Control and STZ-diabetic
mice were maintained with or without treatment with D3, at 15
IU/mouse per os, for 8 weeks (a prevention paradigm). Serum 25-
hydroxyvitamin D3 (25OHD3) was assayed by ELISA. The levels of
cytochrome P-450 27A1 (mitochondrial isoform), CYP450 2R1
(microsomal isoform) and caspase 3 (Casp 3) were assessed by
electrophoresis and immunoblotting.

Results: Serum level of 25OHD3, the main circulating metabolite
of D3, was shown to be reduced to 23.8±1.9 in diabetes vs.
39.7±2.9 nmol/l in control, indicative of diabetes-induced D3 deficiency (p<0.05). These changes were accompanied by
decreased expression of hepatic CYP 450 27A1, which is known to
be a constitutive enzyme with the affinity to D3 in higher
concentrations, whereas diabetes-related elevation of inducible
25-hydroxylase isoform 2R1 was observed. Vitamin D3 treatment
calmed the normalizing reciprocal action on D3, 25-hydroxylases
that led to augmentation of vitamin bioavailability. It was shown
Casp3 activation in the hepatic tissue of diabetic mice as compared
to control. Vitamin D3 administration partially normalized the
activity of Casp3, suggesting the role of cholecalciferol in regulation
of antiapoptotic processes in liver.

Conclusion: The study confirmed that diabetes was associated
with the alterations of mitochondrial and microsomal vitamin
D3, 25-hydroxylases expression and caspase-3 activity in liver that
correlated with vitamin D3 deficits. It was demonstrated potential
role of cholecalciferol in the regulation of antiapoptotic mechanisms
in liver tissue.
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Hsp72 OVEREXPRESSION PROTECTS FROM ACETAMINOPHEN-, BUT EXACERBATES Fas-INDUCED LIVER INJURY
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Background: Heat shock protein (Hsp) 72 is a molecular chaperone which is upregulated in response to a variety of stress situations and possesses broad cytoprotective functions. However its hepatic function remains largely unknown.

Aims and Methods: To study the importance of Hsp72 in the liver, we generated transgenic mice overexpressing Hsp72 under the control of a tissue-specific tetracycline-inducible system and crossed them with animals carrying the tetracycline-responsive transactivator under the control of the liver activator protein promotor (Hsp72-LAP mice). Acute liver injury was induced by a single intraperitoneal injection of acetaminophen (800 mg/kg) or the anti-CD95 antibody Jo2 (0.30 μg/g). The severity of experimental liver injury was determined via serological and histological analyses.

Results: Hsp72-LAP mice displayed doxycycline-regulated, robust Hsp72 overexpression in hepatocytes, but not in the other tissues tested. 18 hours after acetaminophen injection, a significantly lower liver injury was noted in Hsp72-LAP mice compared to single transgens (ALT: 933 vs. 1977, p < 0.05). No differences in acetaminophen metabolism were seen. On the other hand, injection of anti-CD95 antibody resulted in a more profound injury in Hsp72-LAP vs. single transgenic animals (ALT level: 6435 vs. 2574, p = 0.004).

Conclusions: Hsp72-LAP mice represent a unique tool to study the role of Hsp72 in the liver in a time- and cell-type-specific manner. Our results suggest that Hsp72, dependent on context, both ameliorates and promotes liver injury.

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THE OVER-EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE INDUCES MITOCHONDRIAL DISFUNCTION AND CELL DEATH IN HepG2 CELL LINE
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Introduction: Inducing nitric oxide (NO) production has proven to be a potential therapeutic strategy for the treatment of experimental hepatocarcinogenesis. We have recently proved that stable over-expression of endothelial nitric oxide synthase (NOS-3) increases expression of CD95 in the human hepatocarcinoma cell line HepG2, making the cells to be more susceptible to anti-Fas-induced cell death. In addition, NOS-3 has been localized in mitochondria and it has been related to cellular oxygen consumption through inhibition of the oxidative phosphorylation system. The objective of the present study was to determine the effect of NOS-3 over-expression in anti-Fas-induced cell death and at mitochondrial level.

Material and Methods: The study was conducted from HepG2 cell line stably transfected with plasmid pcDNA4/TO (Invitrogen) containing the cDNA of NOS-3 (ImaGenes). Cell death was induced by anti-Fas agonist (0.5 mg/ml; MBL) and was evaluated by caspase-3 and caspase-9 activation, and cytochrome c release into the cytosol. Under these conditions, we studied the cellular localization of NOS-3 by confocal microscopy, and by western-blot in mitochondria isolated by ultracentrifugation. Similarly, we evaluated the activity of the electron transport chain, ATP levels and cellular oxidative stress, by polarography and spectrophotometry. The post-translational modifications were assessed by western-blot analysis of the mitochondrial fraction.

Results: The over-expression of NOS-3 was associated to mitochondrial biogenesis increase, higher activity of the respiratory complexes II+III, reactive oxygen species production, ATP generation, and cytochrome c release. It was also observed an increase of protein nitration related to NOS-3 over-expression. Immunolocalization experiments showed that NOS-3 is mainly found in plasma membrane and perinuclear area, but also in the outer mitochondrial membrane of the transfected cells. In the presence of anti-Fas, it was detected an cellular increase of NOS-3 expression/stability that coincided with a lower localization of the protein in the mitochondrial fraction. Similarly, the higher caspase-3 and-9 activities in the presence of anti-Fas were associated to NOS-3 over-expression.

Conclusion: According to previous studies in endothelial cells, NOS-3 was located in the mitochondrial outer membrane of human hepatocarcinoma cells. Over-expression of NOS-3 was associated to mitochondrial activity increase and susceptibility to cell death in the presence of anti-Fas.

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THE INTERACTION BETWEEN NF-kB ACTIVATION AND PPAR γ ACTIVATION IS REGULATED BY Cks1–Skp2–p27(kip1) PATHWAY AND MEDIATES TUMOR PROGRESSION IN HUMAN HEPATOCELLULAR CARCINOMA
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Background and Aims: NF-kB is known as one of the most important transcriptional factors to promote HCC progression. Recently, we have reported that PPARγ also regulate HCC progression through its activation (DDW 2012). In brief, PPARγ activation inhibits tumor cell proliferation and suppresses tumor growth in clinical cases of HCC. Moreover, low PPARγ expression in HCC was an independent predictor of poor prognosis. In addition, it is reported that PPARγ negatively regulates NF-kB activation in inflammatory injured models. However, no precise mechanisms of how this interaction is induced have been revealed. The aim of this study is to evaluate the negative correlation between PPARγ activation and NF-kB activation, and to reveal the key molecules to regulate this interaction.

Materials and Methods: Fresh surgical specimens were collected from 63 patients with HCC. PPARγ activation and NF-kB activation were assessed by EMSA. Cell proliferation was evaluated by Ki-67 immunostaining. Expression of Cks1, Skp2, and p27(kip1) was determined by western blot to evaluated cell cycle progression. HCC cells were treated with PPARγ agonist, 15d-PGJ2, and activation of PPARγ and NF-kB were evaluated.

Results: PPARγ activation was negatively correlated with NF-kB activation. Ki-67 expression was significantly increased in HCC with high NF-kB activation. However, Ki-67 expression was significantly decreased in HCC with high PPARγ activation. In HCC with high PPARγ activation, expression levels of Cks1 and Skp2 were decreased and thereby p27(kip1) expression was increased. Moreover, HCC cells treated with PPARγ agonist increased PPARγ activation and inhibited HCC cell proliferation in a dose dependent manner in vitro. Interestingly, PPARγ agonist also inhibited NF-kB activation in a dose dependent manner. Significant negative correlation was seen between PPARγ activation and NF-kB activation in HCC treated with PPARγ agonist.
Conclusions: We first demonstrated the interaction between PPARγ activation and NF-kB activation in clinical cases of HCC. Cks1-Skp2-p27(kip1) pathway seems to be the most important cascades for regulating this interaction. Moreover, PPARγ agonist positively regulates PPARγ activation and negatively regulates NF-kB activation. Therefore, Cks1-Skp2-p27(kip1) pathway is a potential therapeutic target for patients with HCC.

291 Bcl-3 MODULATES GalN AND LPS-INDUCED LIVER INJURY IN-VIVO
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Background: Acute liver injury exhibits a high mortality rate and despite recent advances in the underlying pathomechanisms, therapeutic options are still very limited. Increased activation of apoptosis signaling pathways from loss of anti-apoptotic factors or induction of pro-apoptotic factors has been described. Bcl-3 is a nuclear member of the IκB family and proto-oncogen which contributes to cell proliferation and inhibits apoptosis in vitro involving NFκB.

Methods and Results: To explore the role of antiapoptotic protein in acute liver injury, we generated mice exhibiting increased hepatocyte-specific expression of B-cell leukemia-3 (bcl-3). Bcl-3 overexpression was achieved using the cre-lox P system under control of both the albumin regulatory elements and the a-fetoprotein enhancers (alfp-cre:bcl-3). At the age of 8 weeks no phenotypical differences with regards to the liver histology or transaminases were observed between wildtype and alfP-cre:bcl-3 mice. To induce liver injury, wildtype und alfP-cre:bcl-3 mice were treated with galactosamine N (GalN) and LPS, a model of TNF-activated injury. Liver injury was assessed through measurement of serum ALT at 4 and 6 hours. ALT levels in wildtype mice at 4 and 6 hours were significantly higher than those in alfP-cre:bcl-3 mice (ALT: 453 vs. 807 U/l, wt vs. alfP-cre:bcl-3, n = 14). The degree of apoptosis in the liver was assessed histologically and by activation of caspase 3 and was decreased in alfP-cre:bcl-3 mice. At 6 hours the difference in liver injury from GalN/LPS was less pronounced, suggesting that extrahepatic factors or factors independent from bcl-3 are contributing to cellular injury at this point.

Conclusions: In summary, overexpression of the proto-oncogene bcl-3 in hepatocytes can be achieved using the cre-lox P system. Hepatic bcl-3 expression mitigates liver injury and caspase activation from TNF. These findings imply that hepatocellular bcl-3 is a crucial regulator of the dynamics of apoptosis and cellular injury.

292 PAN-CASPASE INHIBITION PROTECTS AGAINST FIBROTIC NASH INDUCED BY CHOLINE DEFICIENT AMINO ACID DEFINED DIET (CDA)
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Hepatocyte cell death is a key feature of nonalcoholic steatohepatitis (NASH). Emerging data suggest that inhibition of caspases may be an attractive therapeutic approach for patients with NASH. Our aim was to test the hypothesis that the pan-caspase inhibitor IDN-6556 reduces hepatocellular apoptosis and fibrosis in a pathophysiological relevant murine model of human NASH.

Methods: C57BL/6 mice, aged 6 to 8 weeks at the beginning of the study, were fed either a choline deficient amino acid-defined (CDA), a choline-sufficient amino acid-defined (CSA) or a low-fat chow diet for 20 weeks. At 16 weeks the mice fed the CDA diet were treated with 3 mg/kg/day of IDN-6556 (n = 12) or with a placebo (n = 12), via gavage, for 5 weeks. After 5 weeks the mice were sacrificed and their livers and blood were collected. Hepatocellular damage, fibrosis and inflammatory activity were assessed by liver histopathology, hepatic triglyceride (TG) quantification, serum ALT levels, and immunoblotting. Markers of hepatic stellate cell (HSC) activation including alpha-smooth muscle actin (alpha-SMA), collagen 1-alpha (COL1A1), and transforming growth factor-beta (TGFB) were determined by real time qPCR.

Results: Treated mice showed improved liver histology with significant reduction in fibrosis, determined by morphometric analysis of collagen using Sirius red staining (Treated 2.5±0.68 vs. Placebo 5.73±0.5 percentage of fibrotic area, P = 1.01 x 10^-4) when compared to placebo mice. In the treatment group also demonstrated improved insulin sensitivity compared to placebo mice (0.112±0.0041 vs. 0.0084±0.0029 ng/ml, P = 4.1 x 10^-4). Insulin function assessment via homeostatic model assessment (HOMA) displayed ameliorated insulin sensitivity in treated mice compared to placebo mice (P = 0.048). However, ALT, AST, TG, and FFA levels were similar in drug treated and placebo treated mice. In addition, we examined the role of the pan-caspase inhibitor in hepatic apoptosis. Liver mitochondrial fractions from drug treated mice were immunoblotted and showed reduced expressions of the pro-apoptotic proteins, Bid and Bax compared to placebo mice.

In conclusion, we have demonstrated that the pan-caspase inhibitor IDN-6556 improved insulin sensitivity and attenuated hepatic fibrosis and apoptosis in mice with CDA diet-induced NASH.

293 SORAFENIB PROVOKES IMPAIRED LIVER REGENERATION AND NON-APOPTOTIC HEPATOCYTE DAMAGE IN MICE BUT SELECTIVE APOPTOSIS IN ISOGENIC MALIGNANT HEPATOMA CELLS
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Background and Aims: The multikinase inhibitor Sorafenib increases the survival of patients with advanced hepatocellular carcinoma (HCC). Current data suggests that Sorafenib inhibits mitogen-activated protein kinase (MAPK) and promotes apoptosis at least in tumor cell lines. Here, we compare the Sorafenib-signaling in murine hepatoma cells and isogenic healthy hepatocytes with the aim to dissect protective and harmful effects of Sorafenib in the regenerating liver.

Methods: Sorafenib tosylate was provided by Bayer Pharmaceuticals. In vitro studies were performed with the murine Hepa1–6 hepatoma cell line or primary isolated hepatocytes both derived from C57/BL6 mice. For in vivo studies C57/BL6 wildtype mice were treated once a day orally with Sorafenib (100 mg/kg) before and after partial hepatectomy (PH) and subsequently analyzed for hepatocyte proliferation, liver injury and regeneration for up to 96 hours after surgery.

Results: In vitro, Sorafenib inhibited cell cycle both in hepatoma cells and primary hepatocytes, in part via down regulation of cyclins D, E and A through transcriptional and post-translational mechanisms. Similarly, anti-apoptotic proteins such as Akt, Bcl-2 and Mcl-1 were downregulated in hepatoma cells and primary hepatocytes. However, Sorafenib induced caspase-3-dependent intrinsic apoptosis was exclusively detected in hepatoma cells but not in hepatocytes. These results indicate that the pro-apoptotic capacity of Sorafenib is not solely due to reduction of anti-apoptotic signals, but depends on cell malignancy. In vivo, repetitive
application of Sorafenib for up to four days transiently inhibited DNA-synthesis and overall cell cycle activity in hepatocytes after PH. In agreement with our in vitro data we did not detect any signs of apoptosis or caspase-3 activation in Sorafenib-treated livers after PH. Interestingly Sorafenib induced elevated, non-apoptotic liver injury after PH as demonstrated by elevated AST and ALT levels. In addition, Sorafenib-treatment resulted in increased occurrence of hepatocytes showing irregular mitoses and free (cytoplasmic) condensed DNA, indicating non-apoptotic cell death.

Conclusions: Sorafenib induces apoptosis selectively in malignant transformed hepatoma cells but not in proliferating hepatocytes in vitro and in vivo. However, our data suggests that Sorafenib can increase non-apoptotic hepatocyte injury in the regenerating liver.

294 EVALUATION OF SERUM M30 AND M65 CELL DEATH MARKERS AS BIOMARKERS FOR DISEASE PROGRESSION IN PATIENTS WITH HBV-RELATED ACUTE-ON-CHRONIC LIVER FAILURE
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Aim: Our present study was aimed at elucidating whether serum levels of M65 and M30 were changed in ACLF patients and its significance in liver failure progression.

Methods: 31 patients with HBV-related ACLF. 20 patients undergoing chronic hepatitis B (CHB), and 10 healthy controls were enrolled. ACLF patients was divided into improved group (n=20) and deteriorated group (n=11) basis on their clinical outcomes.

Total serum cytokeratin-18 (M65) and caspase-cleaved cytokeratin-18 (M30) were dynamic monitored by ELISA.

Results: At admission, the serum levels of M30 (508.65±340.16 U/L) and M65 (768.75±290.02 U/L) in ACLF patients were significantly higher than those in CHB patients (p<0.05) and healthy controls (p<0.05). Serum levels of M65 and M30 were positively related to model for end-stage liver disease (MELD) scores (r=0.74, p<0.05; r=0.7, p<0.05). In ACLF patients, no significant difference of M30 and M65 were observed between improved group and deteriorated group. In improved group of ACLF patients, a significant decrease (p<0.05) of serum M30 (220.33±81.74 U/L) and M65 (410.37±139.35 U/L) was determined in comparison with levels at admission, and paralleled with decreased serum ALT and TBil level. In deteriorated group of ACLF patients, the serum levels of M30 and M65 kept no changes in comparison with those at admission (555.72±436.99 U/L vs. 436.14±299.53 U/L, p=0.46; 883.28±551.11 U/L vs. 703.90±232.17 U/L, p=0.33).

Conclusion: The serum levels of M30 and M65 are sensitive indicators for hepatocytes necrosis and apoptosis. Dynamic monitor of M30 and M65 levels may serve as potential biomarkers for disease progression of ACLF patients.
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**ISOLATION, CHARACTERIZATION AND HEPATOCYTE DIFFERENTIATION OF HUMAN ADULT PROGENITOR CELLS FROM LIVER AND PANCREAS**

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**Introduction:** During embryogenesis, liver and pancreas are generated from a common endodermic precursor. Furthermore, in vivo in humans and in rodents, and in pathological environment, adult hepatocyte clusters have been observed in pancreatic tumors. These hepatic cells could come from mature pancreatic cell transdifferentiation or from pancreatic progenitor cell differentiation towards the hepatocyte lineage. These observations raised the hypothesis that progenitors from endoderm developmental stage could persist during adult life (unidentified in humans).

In this study we established a similar strategy for isolation, proliferation and differentiation of human progenitor cells from adult liver and pancreas.

**Results:** Organ fragments were dissociated with collagenase and extracted cells were seeded in human adult hepatic progenitor cell proliferation medium. After 4–7 days, various cellular populations appeared. The proliferation medium was replaced at confluence by the hepatocyte differentiation medium. We observed an increased expression of the hepatocyte genes albumin and CYP3A4, and no detection of the pancreatic genes insulin and glucagon. At day 21, clusters of positive cells for albumin and cytokeratins 7 and 8/18 were observed.

In a second set of experiments, we isolated a population negative for CD105 and CD90 antigens after 7–14 days of amplification. The populations from both liver and pancreas had an identical morphology. In the hepatocyte differentiation medium, the expression of albumin and CYP3A4 mRNAs increased whereas insulin and glucagon gene expression was not detected. At day 21 positive clusters for albumin and cytokeratins 7 and 8/18 were observed.

**Conclusion:** In conclusion, we showed the feasibility to isolate an epithelial population containing progenitor cells with hepatocyte differentiation capacity from human adult liver and pancreas. These in vitro results strongly suggest the presence of cells with hepatocyte differentiation capacity in the pancreas, as observed in vivo under particular pathological conditions. In the future, these progenitor populations could be a model for basic research and a cell source for liver and/or pancreas biotherapy.

**Reference(s)**


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**ISOLATION AND CULTIVATION OF PURE HUMAN ADULT LIVER STEM CELL POPULATIONS FROM PATIENT LIVER RESECTATES**

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**Background and Aims:** Currently, freshly isolated or thawed human hepatocytes cannot be kept in vitro for reproducible experimentation or therapeutical use: They dedifferentiate rapidly in the absence of significant cell proliferation. Such a rapid loss of function makes it difficult to apply them for cell based therapies in vivo, or pharmacological and toxicological studies in vitro. This explains the need for the isolation and cultivation of liver stem cells from patient samples that can proliferate without signs of senescence and that could be differentiated to a desired state depending on their future application.

**Methods:** Suspensions of liver cells are received from our clinical partners at MHH following a two-step collagenase perfusion treatment of liver biopsies obtained during liver surgery. After having tested a variety of different coating techniques and culture conditions we are now able to successfully enrich and purify populations of adult liver stem cells from patient specific liver cell suspensions using a highly modified ‘plate-and-wait’ method. The cells can be kept continuously in culture or be stored safely frozen in Nitrogen.

**Results:** The populations obtained can be passaged at low ratios (1:3 every 7–10 days) and (so far) show no signs of senescence. They exhibit stunning morphologic plasticity and sensitivity to the slightest changes of culture conditions, strongly indicating an undifferentiated stem cell phenotype. When comparing expression levels of our stem cell populations to primary hepatocytes, the most striking difference is the progenitor cells can engraft to the liver of severe-combined immuno-deficient (SCID) mice after paracetamol damage.

**Conclusion:** These data show that the B-13 cell line is capable of engrafting to the damaged liver and differentiates into a hepatocyte-like morphology. This ability to transdifferentiate in vivo as well as in vitro brings new insights into the cell line and the isolation of a human equivalent would have great clinical potential for the treatment of liver disease.

**Acknowledgements:** Supported by an MRC ITTP Studentship.
arises in the strong expression of EpCAM, which is upregulated 50-fold in the stem cell populations. Expression levels of Albumin and KRT 8/18/7/19 can be rated as intermediate; AFP is barely detectable and basal levels of so-called liver enriched transcription factors (eg HNF1a, 1b, 4a, 6 and more) are largely similar to primary hepatocyte isolates.

**Conclusion:** We have developed a robust method that allows for the enrichment and maintenance culture of human adult liver stem cells from patient samples. Currently – with more populations in the enrichment stages – we are characterising already existing stem cell populations, mainly with respect to their differentiation potentials towards mature hepatocytes or cholangiocytes by the means of RT-qPCR and Immunofluorescence.

**Conclusion:**

**DOSE-DEPENDENT MODULATION OF NF-κB AND miR-21 BY DEOXYCHOLIC ACID DURING APOPTOSIS OF PRIMARY RAT HEPATOCYTES**

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**Background and Aims:** Both deoxycholic acid (DCA), an endogenous bile acid, and miR-21, an oncogenic miRNA, have been implicated in the pathogenesis of liver and gastrointestinal disorders. Interestingly, we have recently shown that DCA inhibits miR-21 expression in hepatocytes. Therefore, we aimed to clarify the mechanisms by which DCA modulates the miR-21 signaling pathway.

**Methods:** Primary rat hepatocytes were incubated with 25–400 μM DCA from 16 to 48h. miR-21 expression was evaluated by qRT-PCR. Programmed cell death 4 (PDCD4), a miR-21 target, and NF-κB were analysed by immunoblotting. PDCD4 silencing was achieved by transfecting cells with a specific PDCD4 siRNA. NF-κB expression and greater cell death (p < 0.05), and activated caspase-3 and apoptosis (p < 0.05) were detected using BAY 11–7085, a specific inhibitor. Cell death, viability and caspase-3 activity were determined by the ApoTox-Glo™ Triplex Assay.

**Results:** Our results show that DCA modulates the miR-21/PDCD4 pathway in a dose-dependent manner. While lower doses (25 and 50 μM) exhibited no toxicity and tend to activate this survival pathway, moderate to high doses (>100 μM) significantly inhibited miR-21 (p < 0.05), and activated caspase-3 and apoptosis (p < 0.01). In agreement, PDCD4 expression was significantly increased in the presence of DCA concentrations greater than 100 μM (p < 0.05). Importantly, upon PDCD4 inhibition, DCA-induced caspase-3 and apoptosis were significantly reduced, particularly for the 100 μM concentration. Finally, DCA inhibited NF-κB expression (p < 0.05), in a similar pattern to miR-21 inhibition. Cells incubated with an NF-κB inhibitor also displayed lower miR-21 levels, increased PDCD4 expression and greater cell death (p < 0.05).

**Conclusions:** DCA modulation of the miR-21/PDCD4 pathway correlates with its effects on apoptosis. While low doses of DCA activate survival, moderate to high doses significantly inhibit miR-21; PDCD4 is an important functional target. Importantly, DCA appears modulate miR-21 via NF-κB. A better understanding of the mechanisms by which DCA impacts on cell death and survival may allow for the development of new therapeutic tools.


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**IDENTIFICATION OF MATRIX METALLOPROTEASE 10 (MMP10) AS A KEY NEW MEDIATOR OF THE REGENERATIVE RESPONSE OF THE LIVER**

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**Background:** The wound-healing response triggered after liver injury involves a transitory substitution of damaged hepatic parenchyma by extracellular matrix (ECM) that needs to be removed when liver tissue is regenerated. Matrix metalloproteinases (MMPs) provide important functions during this process, acting as regulatory molecules by the selective proteolytic activation of growth factors and cell surface receptors in addition to their ability to degrade ECM constituents. The role of some MMPs in liver regeneration has been studied. However, little is known about the function of MMP10, also called stromelysin-2, in this process. Recent studies have demonstrated the overexpression of MMP10 in different tumors, and its behaviour as a profibrogenic factor. We have characterized the expression and relevance of MMP10 in models of liver injury and regeneration.

**Methods:** MMP10 was analysed in different cell lines and in vivo models of liver injury. Acute administration of CCl4 and alfa- naphthyl-isothiocyanate (ANT), partial hepatectomy (PH) and bile duct ligation (BDL) were performed in MMP10+ and MMP10− mice. Gene expression was analysed by qPCR, western-blotting and immunohistochemistry.

**Results:** MMP10 expression is very low in the healthy liver, but it is significantly up-regulated in all injury and regeneration models tested. The protein was detected in parenchymal cells, biliary epithelium, and inflammatory cells. In the BDL model MMP10 was found in the cytosol and nucleus of hepatocytes and cholangiocytes. The stimulation of cells with ligands of the epidermal growth factor receptor (EGFR and TGFβ), as well as with bile acids and GW4064, an agonist of farnesoid X receptor (FXR), triggers MMP10 expression. In all the models examined the absence of MMP10 caused a significant increase of necrosis and a high mortality after PH. A remarkable intrahepatic accumulation of fibrinogen and fibronectin insoluble complexes was observed in MMP10− mice upon liver injury.

**Conclusions:** This is the first description of the expression and function of MMP10 in liver injury and regeneration. We identified MMP10 as a gene regulated by bile acids/FXR and as a target of liver regeneration-related growth factors. MMP10 is essential for correct liver regeneration after an acute injury. MMP10 profibronlytic activity may be crucial in this process.
COMPARATIVE INVESTIGATION OF DIFFERENTIATION POTENTIALS OF HEPATIC STELLATE CELLS AND MONONUCLEAR HEMATOPOIETIC CORD BLOOD STEM CELLS TRANSPLANTED INTO RATS AFTER PARTIAL HEPATECTOMY

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Background and Aim: High incidence of liver diseases and low efficacy of their treatment require searching of new ways for solving this problem. The different populations of stem/progenitor cells are investigated now as a possible source of cells for stem cells therapy of chronic liver diseases. The aim of our study is to establish the differentiation potential of two cell populations:
1. human hematopoietic mononuclear cells (HMC) isolated from cord blood and
2. rat hepatic stellate cells (HSC), which are considered more and more as hepatic progenitor cells) after transplantation into rats with partial hepatectomy (PH).

Materials and Methods: We studied the ways of differentiation of freshly isolated HMC and HSC with immunohistochemical methods. HSCs were isolated from liver of intact rats; HMCs were isolated from cord blood. Both cell populations were transplanted with Green Fluorescent Protein (GFP) and transplanted into portal vein of rats just after PH.

Results: We observed GFP+ hepatocytes and sinusoidal cells in rat livers after transplantation of GFP-transfected HMC and HSC. The number of GFP+ hepatocytes after HSC transplantation was not less than after transplantation of HMC but they appeared in the liver at more earlier stages (2 days against 5 days after transplantation of HMC). This fact suggests that the use of HSC may be necessary for rapid effective recovery of liver parenchyma (e.g. in case of fulminant hepatitis). In both experimental groups, we observed cytokeratin 19+ small round cells in liver parenchyma, but GFP+ cholangiocytes appeared after transplantation of HMC only. There is no transdifferentiation of transplanted HMC or HSC into myofibroblasts.

Conclusions: HMC have more diversified differential potential (hepatocytes, cholangiocytes, sinusoidal cells), but HSC can restore liver parenchymal cells faster in the earlier stages. The transplantation both HMC and HSC is not increase the risk of hepatic fibrosis development.

HEPATOCELLULAR DIFFERENTIATION OF FRESCLY ISOLATED AND IN VIVO ACTIVATED HEPATIC STELLATE CELLS TRANSPALNTED INTO RATS AFTER CARBON TETRACHLORIDE DAMAGE

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Background and Aim: Hepatic stellate cells (HSC) produce important morphogenic cytokines (Hepatocyte Growth Factor, Stem Cell Factor, etc.) and express some stem cell markers (Okt 4, Bcl-2, C-kit, etc.). Moreover, HSC and hepatic stellate cells share some common markers (e.g. desmin and cytokeratins in prenatal human and rat liver) and very close interactions. So, it is supposed, that HSC can be hepatic progenitor cells. We demonstrated earlier the possibility of hepatocyte differentiation of HSC during embryonic human development and in vitro. The aim of this study is to establish the possibility of hepatocyte differentiation of HSC after transplantation into rats with damaged liver.

Materials and Methods: We studied the hepatocyte differentiation of HSC with immunohistochemical methods. HSC were isolated from:
1. intact rats;
2. rats after single injection of Lead Nitrate (the last one stimulated activation of HSC in vivo).

Freshly isolated HSC were transplanted with Green Fluorescent Protein (GFP) and transplanted into portal vein of rats after acute Carbon Tetrachloride (CT) damage.

Results: We observed GFP+ hepatocytes in rat's livers after transplantation of GFP-transfected HSC to them in all cases. There were less numerous GFP+ hepatocytes in livers after CT damage then after PH (as it was shown in our previous studies). In case of CT damaged liver only single freshly isolated HSCs were differentiated into hepatocyte-like cells, but transplantation of in vivo activated HSC increased significantly the number of GFP+ hepatocytes in damaged liver. Activated HSC start the hepatic differentiation from first day and the most of them are differentiated by 5 day after transplantation. There weren't myofibroblasts or fibrosis in all these livers.

Conclusions: HSC can differentiate into hepatocytes after transplantation into rats with damaged liver. In case of pericentral toxic damage of liver by CT transplantation of activated in vivo HSC is more effective for hepatocytes repopulation than transplantation of freshly isolated HSC. It can be useful for treatment of toxic liver damages.
compacted Golgi, suggesting directionality and polarity during cell migration. In parallel, HGF induces changes in the gene expression profile of metalloproteases and integrins, among other molecules involved in cell–cell and cell-matrix interactions, thus suggesting a profound remodeling of the extracellular matrix during cell movement.

**Conclusions:** Our results suggest that HGF is mainly inducing collective rather than individual cell movement in oval cells. These findings offer new challenges to elucidate the relevance of this mode of migration during liver progenitor-mediated regeneration and hepatocarcinogenesis as well as the role played by the HGF/c-Met axis in these contexts.

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**EGR1 PROMOTES THE DIFFERENTIATION OF BM-DERIVED MESENCHYMAL STEM CELLS INTO FUNCTIONAL HEPATOCYTE WITH MESENCHYMAL-TO-EPITHELIAL TRANSITION**


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**Background and Aims:** Epithelial-to-mesenchymal transition (EMT) drives mammary epithelial cells to de-differentiate into mammary stem cells which are mesenchymal-like. MET and the reverse process, epithelial-to-mesenchymal transition (EMT), both occur in normal tissue, including gastrulating and regenerating tissue. MET in hepatic stem cells is important to multiple processes, including hepatic differentiation and liver development, regeneration.

Early growth response (EGR) 1, transcription factor of early growth response gene family, is induced in the responses to a number of growth and differentiation factors. The aim of this study is to investigate that EGR1 acts as a key regulator of EMT/MET in the process of hepatogenic differentiation from bone marrow-derived mesenchymal stem cells (BM-MSCs).

**Methods:** Based on the 2 step protocol, BM-MSCs were cultured for hepatic differentiation. PAS staining and Urea production test were performed to verify the functionality of hepatic differentiation. To search for novel proteins in BM-MSCs before and after differentiation, protein/DNA array was performed. To determine the effect of EGR1, BM-MSCs were infected by lentiviral EGR1 and lenti-shEGR1. EMT markers (N-cadherin, vimentin, a-SMA) and MET markers (E-cadherin, CK18) were identified during the hepatic differentiation of infected BM-MSCs.

**Results:** During differentiation proceeds, from day 1 to day 28, stem cells change in shape from spindle to polygon with larger size and tighter intercellular connections. Intracellular glycogen and urea production was significantly increased from day 1 to day 28. The mRNA expression of liver-specific genes was upregulated. In order to study key regulator in hepatic differentiation, we performed protein/DNA array. EGR1 in differentiated BM-MSCs were significantly increased during the culture compared to undifferentiated BM-MSCs. Therefore, BM-MSCs were differentiated after infected by lentiviral EGR1 and lenti-shEGR1. The forced expression of EGR1 induced MET related genes and liver-specific genes, whereas downregulation of the EGR1 expression decreased MET related genes and hepatic differentiation.

**Conclusions:** In this study, we identified novel factors in the process of hepatic differentiation through inducing the MET process. Our study suggests that EGR1 were expected to the “transcription factor therapy” as novel strategy in future clinical treatment.

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**HEPATITIS B VIRUS INHIBITS LIVER REGENERATION VIA EPIGENETIC REGULATION OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR**

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Liver regeneration after liver damage caused by toxins and pathogens is critical for liver homeostasis. Retardation of liver proliferation was reported in hepatitis B virus (HBV) X protein (HBx)-transgenic mice. However, the underlying mechanism on the HBx-mediated disturbance of liver regeneration has not been elucidated. We investigated the molecular mechanism on the inhibition of liver regeneration using liver cell lines and a mouse model. We established a mouse model of acute HBV infection through hydrodynamic injection of viral DNA. Liver regeneration after partial hepatectomy was significantly inhibited in the mouse model of HBV infection. Mechanism studies have revealed that the expression of urokinase-type plasminogen activator (uPA), which regulates the activation of hepatocyte growth factor (HGF), was significantly decreased in the liver tissues of HBV or HBx-expressing mice. The down-regulation of uPA was further confirmed using liver cell lines transiently or stably transfected with HBx and HBV genome. Furthermore, we demonstrated that HBx suppresses uPA expression through epigenetic regulation of the uPA promoter in mice liver tissues and human liver cell lines. Expression of HBx strongly induced hyper-methylation of the uPA promoter through recruiting DNMT3A2.

**Conclusions:** Taken together, these data suggest that infection of HBV impairs liver regeneration through epigenetic regulation of liver regeneration signals by HBx.

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**Pin1 IS DECREASED IN ISCHEMICALLY DAMAGED LIVER AND SUPPRESSED LIVER REGENERATION AFTER HEPATECTOMY THROUGH THE INHIBITION OF Pin1-MEDIATED NF-κB ACTIVATION IN HEPATOCYTES**


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**Background and Aims:** NF-κB is important in liver regeneration after hepatectomy. We reported that regeneration was suppressed in ischemically damaged liver through decreased activation of NF-κB. However, the precise mechanism how NF-κB activation is decreased in this model is unclear. Recently, we have reported that Pin1 specifically binds to the pThr254-Pro motif in p65, which induces NF-κB activation during hepatic ischemia/reperfusion injury (J Hepatol 2009). Therefore, we sought to determine whether decreased activation of NF-κB by Pin1 degradation inhibits liver regeneration after hepatectomy in severe damaged liver.

**Materials and Methods:** Mice were received up to 60 minutes of hepatic ischemia of the right lateral lobe (30%) followed by hepatectomy of non-ischemic lobe (70%). Liver regeneration was evaluated by liver/body weight ratio and PCNA immunohistochemistry. Hepatocyte NF-κB activation was assessed by EMSA. Expression of Pin1, Pin1–NF-κB–p65 complex, Csk1, Skp2, and p27(kip1) was detected by western blot. Hepatocyte was treated with H2O2 or Pin1 inhibitor, Juglone. Cell proliferation and NF-κB activation was evaluated.

**Results:** In mice with normal liver, MIP-2 induced liver regeneration through NF-κB activation. NF-κB increased cell cycle progression through increased expression of Csk1 and Skp2 which induced
Background and Aims: Hemophilia A is an attractive target for cell and gene therapy applications. The liver is the main source of FVIII in the body, even though our recent studied demonstrated that bone marrow (BM) transplant correct the bleeding phenotype of hemophilia A in mice. Recent studies reported that liver repopulation by BM cells could rescue the bleeding phenotype in hemophilic mice. The aim of our study was to investigate the role of BM cells in liver repopulation after acute liver failure and their role in the treatment of hemophilia A.

Methods: GFP+ mice served as donors and hemophilia A mice as recipients. Acute liver failure was induced by acetaminophen injection. Total BM cells or hematopoietic stem cells (Lin-) were isolated from GFP+ mice and tail vein injected into acetaminophen-treated mice 24 h later. At several time points after transplantation mice were killed and organs analyzed by FACS analysis, immunofluorescence and PCR. aPTT was performed on transplanted mice to evaluate correction of the bleeding phenotype.

Results: GFP+ cells were detected by FACS analysis in blood and BM of treated mice up to 2 weeks after transplantation, whereas for longer time points the number of GFP+ cells drastically decreased. Rare GFP+ cells were found in liver and spleen of transplanted mice up to 6 months. Donor cells were detected in the hepatic non-parenchymal cell fraction and no GFP+ hepatocytes or endothelial cells were observed in the liver of transplanted mice. Immunofluorescence showed the presence of infiltrating macrophages around GFP+ cells and aPTT did not show correction of the bleeding phenotype.

Conclusions: Injected total BM or Lin- cells did not repopulate the liver of acetaminophen-treated mice nor transdifferentiate into hepatocytes or endothelial cells. The presence of infiltrating native mononuclear cells around transplanted cells suggested an immune response against GFP+ cells. Moreover, the transplantation of BM cells did not ameliorate the bleeding phenotype of acetaminophen-treated mice, whereas our recent studies demonstrated a long term engraftment and proliferation of transplanted cells, no transdifferentiation in hepatocytes or endothelial cells and correction of bleeding phenotype in hemophilic mice with no immune response after total body irradiation followed by BM transplantation.

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**TRANSPLANTATION OF KUPFFER CELLS REPLACES RESIDENT HEPATIC MACROPHAGES CORRECTING THE BLEEDING PHENOTYPE OF HEMOPHILIC MICE**

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Background and Aims: Kupffer cells (KC) represent a major component of the reticuloendothelial system with critical roles in microbial clearance, phagocytosis, inflammation, and innate or acquired immune responses. Major uncertainties remain in the biology of KC, including their origin, cycling, life-span, reconstitution, and therapeutic manipulations. This study was aimed at understanding whether transplantation of mouse KC could replace native KC.

Methods: Primary KC were isolated by immunomagnetic sorting and bone marrow cells were differentiated into macrophages in vitro. Cells were characterized by immunophenotyping and phagocytosis assays. Radiolabeling methods were used to track cell biodistributions and genetic reporters to identify cell engraftment. Phagocytosis and cytokine expression were analyzed in transplanted cells. Effects of gadolinium chloride and clodronate to deplete native KC and partial hepatectomy or carbon tetrachloride-induced changes on engraftment or proliferation of transplanted cells were studied. The ability of cells to engraft in the liver and correct the hemorrhagic phenotype of hemophilia A mice in short-term studies was evaluated.

Results: KC displayed macrophage markers along with phagocytic activity. A fraction of transplanted KC engrafted and survived in the liver over up to 3 months. Gadolinium chloride improved cell engraftment. Transplanted KC did not proliferate even after partial hepatectomy or carbon tetrachloride-induced liver regeneration and/or injury. Transplanted Kupffer cells functioned normally with phagocytosis of bacteria and replacement of interleukin-6 deficient in deficient mice. By RT-PCR and immunofluorescence it was shown that KC expressed FVIII as well as FVIII protein. Moreover, we injected healthy KC from C57BL/6 mice into hemophilia A mice i.v. followed by tail-clip assay after 3 d or 7 d. Several recipients of healthy KC survived tail-clip challenge (5 of 7 mice after 3 d; 4 of 7 mice after 7d). Costaining for GFP and F4/80 showed engraftment of transplanted KC in liver.

Conclusions: Replacement of healthy mouse KC by transplantation demonstrated KC have a long life-span. Moreover, injection of KC, which predominantly originate from bone marrow protected hemophilia A mice from bleeding challenge with appearance of FVIII in blood. Cell therapy approaches to reconstitute liver macrophages will help to define their biological and therapeutic potential.
and regenerative responses. Notch receptors differ in structure and function and in their interactions with the Wnt signalling cascade and regulators such as Numb. Therefore Notch and Wnt signalling may coexist, with distinct roles in coordinating an effective regenerative response from HPCs.

**Methods:** Using a combination of HPC cell lines and a ‘single hit’ genetic in vivo model of hepatocellular injury and HPC activation (AhCre MDM2\(^{1/2}\)), Notch and Wnt activity was analysed over the duration of the HPC response. Small molecule inhibitors were used to establish functionality of these signalling pathways in a time-dependent manner.

**Results:** Hepatocellular injury induces ductular activation followed by sequential HPC expansion, migration then differentiation. Notch and Wnt pathway are expressed differentially over this time-course. In HPC lines active Notch1 marks the smallest most naive HPCs at the centre of colonies whereas Notch3 distinguishes a broader population. In vivo Notch1 correlates with the early ‘activation’ phase and marks HPCs near the duct; expression of Notch3 shows a more protracted course marking a population extending from the duct through the proliferative phase into migratory cords. In vitro HPCs express Notch ligand Dll-1. In vivo both self-expression of Dll-1 and niche-derived expression of Jagged ligands are identified.

In vitro loss of Notch signal in general or Notch3 specifically results in impaired proliferation of HPC lines. Wnt target gene expression and LEF-1 activity is enhanced later in the regenerative response and in HPCs further from the parent duct. Notch3 and active Wnt signalling are identified in the same cell late in the regenerative response, confirming a Wnt/Notch permissive state. Notch and Wnt inhibition at different time points through the regenerative response confirms key time-dependent functions for these signals in HPC-mediated regeneration.

**Conclusions:** We have identified a novel role for Notch in hepatocellular regeneration concomitant with a Wnt/Notch permissive state within HPCs. Temporal variation in activity of Wnt and Notch paralogues highlights the complex dynamics regulating an effective regenerative response.

310 FGF INDUCIBLE PROTEIN 14 IS UPREGULATED IN NEOVESSELS DURING CHRONIC INFLAMMATORY LIVER DISEASE AND PROMOTES INTRAHEPATIC ENDOTHELIAL CELL ANGIOGENESIS IN VITRO FOLLOWING STIMULATION VIA TWEAK

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**TWEAK and Fn14 members of the TNF superfamily of ligands and receptors collectively regulate a diverse range of immune, inflammatory and regenerative responses. Recent studies indicate a potential role of TWEAK and Fn14 in tissue repair following liver injury where they may promote angiogenesis and neovessel growth. TWEAK/Fn14 could therefore facilitate inflammatory cell recruitment and promote portal associated lymphoid tissue development during inflammation.**

**Aims:**
1. To investigate TWEAK/Fn14 expression in human liver tissue during chronic liver disease.
2. To study Fn14 expression in isolated intra-hepatic endothelial cells (IHEC).
3. To determine angiogenic responses of IHEC to TWEAK.

**Methods:** Tissue sections from explanted human livers at the time of hepatobiliary surgery, including normal donor; normal tissue adjacent to malignant lesions; HCV; ALD; NASH; Chronic Allograft Rejection; PSC and PBC, were subjected to immunohistochemistry or dual immunofluorescence using antibodies to TWEAK, Fn14 and phenotypic markers CD31 and CD68. Isolated IHEC were cultured with combinations of TNF\(\alpha\), IFNg, FGF, and assessed for TWEAK/Fn14 expression using flow cytometry. In addition IHEC were incubated with recombinant TWEAK in presence ± TNF\(\alpha\) and assessed using a matrigel angiogenesis assay for vessel formation and branching.

**Results:** Fn14 expression was low in normal tissue in portal vessels and sinusoids, whereas in disease portal neovessels (CD31 \(\text{+ve}\)) were highly positive for Fn14. TWEAK expression was low in normal tissue but highly expressed in CD68\(\text{+ve}\) monocyte cells surrounding areas of neovascularisation and inflammatory cell aggregation. Fn14 expression significantly up-regulated on isolated IHEC when stimulated with the TNF\(\alpha\). Confocal imaging showed that the expression of Fn14 was predominantly cytoplasmic unless stimulated with TNF which enhanced cell surface expression. IHEC were consistently negative for TWEAK and TWEAK stimulation increased IHEC angiogenesis, where a change in cell morphology and enhanced branching of capillary like structures was observed. Pre-incubation with Fn14 antagonistic mAb completely abrogated vessel formation and branching.

These new data show that Fn14 activation stimulates neovessel branching in IHEC and that TNF\(\alpha\) promotes mobilisation of cytoplasmic Fn14 to the cell surface suggesting an important regulatory role for TWEAK/Fn14 in neovascularisation and portal lymphoid aggregation during hepatic inflammation.

311 EX VIVO-EXPANDED PERIPHERAL CD34\(^+\) CELLS FOR CHRONICALLY INJURED LIVER TREATMENT: IN VITRO AND IN VIVO STUDIES

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**Objectives:** Stem cells appear to be a promising therapeutic source for the treatment of liver fibrosis/cirrhosis. However, the quality and quantity of the cells to be used still need to be defined. We investigated the effect of ex vivo-expanded granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral CD34\(^+\) cell transplantation into an immunodeficient rat model with liver fibrosis.

**Methods:** Human G-CSF-mobilized peripheral CD34\(^+\) cells were isolated from total mononuclear cells of liver cirrhotic patients by magnetic cell sorting system. Recipient nude rats were injected i.p. with CCl\(\text{4}\) twice weekly for three weeks before administration of expanded CD34\(^+\) cells. CCl\(\text{4}\) was then re-administered twice weekly for three more weeks, and the rats were sacrificed. Saline, \(5 \times 10^4\), \(2 \times 10^5\), or \(1 \times 10^6\) expanded CD34\(^+\) cells/kg body weight were intrasplenically transplanted after CCl\(\text{4}\) treatment for three weeks. Examination items were as follows. 1) FACS and RT-PCR analysis of freshly isolated and expanded CD34\(^+\) cells, 2) morphometry of fibrotic areas of Azan-Mallory stained liver, 3) immunohistochemistry using anti-collagen-type I, alpha-smooth muscle actin (SMA), and PCNA antibodies, and 4) the expression of metalloproteinase and tissue inhibitor of metalloproteinase (TIMP)-1 by real-time PCR.

**Results:** Seven days in culture, G-CSF mobilized peripheral CD34\(^+\) cells were effectively expanded in the culture medium containing VEGF, stem cell factor, interleukin-6, Flt-3 ligand, and thrombopoietin. Expanded CD34\(^+\) cells were also increasingly characterized as positive for cell surface markers of VE-cadherin, KDR and Tie-2, whereas they were down-regulated for CD34,
CD133 and CD117 as determined by FACS analysis. The expression of VEGF, TGF-alpha, FGF-2, endothelial nitric oxide synthase and angiopeptin-2 in expanded CD34+ cells increased compared with freshly isolated CD34+ cells. Expanded CD34+ cell transplantation had dose-dependently reduced liver fibrosis, with the decrease of collagen type-I and alpha-SMA positive cells after six weeks of CCl4 treatment. In high dose of expanded CD34+ cell transplanted livers, the number of PCNA positive hepatocyte increased significantly at six weeks after CCl4 treatment. The expression of TIMP-1 was significantly decreased in high dose of expanded CD34+ cell transplanted livers.

**Conclusion:** Our data suggest that expanded CD34+ cells may represent a promising strategy for a successful cell therapy.

### 312 PRazoSIN AN ALPHa1 Adrenoceptor ANTAGoNist PROTeCTS LiVER FROM ACETAMiNoPHEN iNDUCED ACUTE LiVER iNURY AND EXPANdS THE PROGENITOR CELL POPULATION

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It has been shown that liver repair is regulated in part by the sympathetic nervous system, (SNS) [1] and that the α1-adrenoceptor antagonist prazosin (PRZ) increases hepatic progenitor cells (HPCs) and reduces liver injury in a model of chronic liver disease (non-alcoholic fatty liver disease) [2]. The effect of α1-adrenoceptor antagonism on acute liver injury and effects on HPCs is not known.

**Method:** Acetaminophen (APAP) induced liver failure was used as a model of acute liver injury (AILI). C57BL/6 mice were treated with 500 mg/kg of APAP. 1 hr after APAP, mice were injected with either vehicle, or the α1-adrenoceptor antagonist PRZ. Liver injury was assessed by ALT and histological assessment for necrotic foci. HPCs were enumerated by flow cytometry (HPCs defined as Lin-ve, Hoechst extruding, Thy1.2 +ve cells) or by immunohistochemistry (HPCs defined as pan-cytokeratin +ve). Mechanistically, the canonical Wnt-β-catenin pathway (CWP) known to regulate HPCs and protect the liver from AILI was investigated.

**Results:** PRZ treatment markedly attenuated APAP induced AILI (ALT: 8831±337 IU/ml, p < 0.0003, n=6/group) with reduced mortality consequent to PRZ treatment. In tandem, hepatic necrosis was markedly attenuated by PRZ. Mechanistically, HPC numbers were significantly increased in the APAP+PRZ group compared to APAP alone group: Western blotting revealed increased β-catenin expression after APAP, with PRZ further upregulating β-catenin expression. Among 16 known Wnt ligands, Wnt2, 6, 10α, 11, & 16 mRNA were upregulated in the APAP+PRZ group compared to the APAP alone group. In contrast, PRZ alone did not induce increased β-catenin expression, Wnt upregulation or HPC expansion.

**Conclusions:** PRZ treatment significantly attenuates AILI in mice treated with APAP with a mechanisms involving expansion of HPC and upregulation of β-catenin. Further studies are required to determine the clinical significance of these findings.

**Reference(s)**


regeneration is insufficient. We here further investigated the expression pattern of the novel oval cell marker Neighbor of Punc E11 (Nope) in liver regeneration after chronic (Mdr2−/) mice and/or acute liver injury (partial hepatectomy) in adult mice.

**Methods:** For analysis of chronic liver injury, liver tissue was extracted from Mdr2−/− of different age and stage of fibrosis. Additionally, Mdr2−/− and Balb/c mice were analyzed for the impact of acute liver injury with or without pretreatment with DNA-alkylating reagents to block hepatocytic proliferation. 24 hours to 6 months postoperatively, mice were sacrificed and the liver tissue was tested for expression levels of Nope via quantitative RT-PCR. Co-stainings were performed for Nope in combination with epithelial-specific marker E-cadherin, the biliary marker CK19 and markers of hepatic stem/progenitor cells (A6, EpCAM).

**Results:** While normal adult liver tissue shows only negligible expression of Nope, chronic liver injury in Mdr2−/− mice leads to a considerably increased expression level of Nope. Co-stainings with CK19 demonstrated a bile-duct-specific expression of Nope in these mice. While partial hepatectomy in the normal adult liver has no effect on Nope, it leads to the development of Nope-positive hepatocytic cell clusters in the chronically injured Mdr2−/− mouse model especially if hepatocyte proliferation is blocked. These Nope-positive clusters are negative for CK19 but stain positive for E-cadherin. Oval cell markers A6 and EpCAM both show coexpression with Nope in proliferating ductular cells, but only a minor hepatocellular cell fraction within Nope-positive clusters also stains positive for A6.

**Conclusion:** We here report the expression of the novel oval cell marker Nope in liver regeneration after chronic and/or acute liver injury. The increase of Nope in chronic liver injury is mainly limited to bile ducts, but especially after additional acute liver injury and blocked hepatocytic regeneration a small population of Nope-positive hepatocytic progenitor cells arises. We conclude that Nope detects a novel progenitor cell population within the regenerating adult liver.

### 315 FoxO3 REGULATES HEPATIC GLUCOSE PRODUCTION

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**Background and Aims:** The FoxO family of Forkhead transcription factors are the key downstream targets of insulin and growth factors regulating cell survival, energy metabolism, and longevity. Under caloric restriction, FoxOs translocate to the nucleus to activate gene expression, while at postprandial stage, they are inactivated.

**Methods:** For analysis of chronic liver injury, liver tissue was extracted from Mdr2−/− of different age and stage of fibrosis. Additionally, Mdr2−/− and Balb/c mice were analyzed for the impact of acute liver injury with or without pretreatment with DNA-alkylating reagents to block hepatocytic proliferation. 24 hours to 6 months postoperatively, mice were sacrificed and the liver tissue was tested for expression levels of Nope via quantitative RT-PCR. Co-stainings were performed for Nope in combination with epithelial-specific marker E-cadherin, the biliary marker CK19 and markers of hepatic stem/progenitor cells (A6, EpCAM).

**Results:** While normal adult liver tissue shows only negligible expression of Nope, chronic liver injury in Mdr2−/− mice leads to a considerably increased expression level of Nope. Co-stainings with CK19 demonstrated a bile-duct-specific expression of Nope in these mice. While partial hepatectomy in the normal adult liver has no effect on Nope, it leads to the development of Nope-positive hepatocytic cell clusters in the chronically injured Mdr2−/− mouse model especially if hepatocyte proliferation is blocked. These Nope-positive clusters are negative for CK19 but stain positive for E-cadherin. Oval cell markers A6 and EpCAM both show coexpression with Nope in proliferating ductular cells, but only a minor hepatocellular cell fraction within Nope-positive clusters also stains positive for A6.

**Conclusion:** We here report the expression of the novel oval cell marker Nope in liver regeneration after chronic and/or acute liver injury. The increase of Nope in chronic liver injury is mainly limited to bile ducts, but especially after additional acute liver injury and blocked hepatocytic regeneration a small population of Nope-positive hepatocytic progenitor cells arises. We conclude that Nope detects a novel progenitor cell population within the regenerating adult liver.

### 316 REVERSAL OF HEPATIC FIBROSIS IN AN INDUCED MODEL OF CHRONIC LIVER INJURY BY G-CSF-, PLERIXAFOR- OR G-CSF+PLERIXAFOR-MOBILIZATION OF HEMATOPOIETIC STEM CELLS

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**Background and Aims:** Bone marrow (bm) could serve as a source of liver repopulating cells in case of liver damage. We investigated the mechanisms involved in the regeneration process of the chronically injured liver in a mouse model after G-CSF- or Plerixafor- or G-CSF+Plerixafor-mobilization.

**Methods:** GFP mice donated bm to lethally irradiated C57Bl6 recipients. Donor chimerism (>90% GFP+) in the recipients’ blood was determined by flow cytometry (FCM), before the initiation of CCl4 in order to induce chronic liver damage. G-CSF was given for 6 or 12 days and Plerixafor during the last 3 days of G-CSF administration. At sacrifice, Lin- sca1+ckit+ cells were measured by FCM to confirm mobilization of Hematopoietic Stem Cells (HSCs). Hepatic fibrosis was measured by the total fibrootic index in Gomori/Mason histostains and by immunohistochemistry (a-sma+). Immunohistochemistry for ck19 was used to detect hepatic stem cells. Bm-derived mature hepatocytes (GFP+/ck8,18+) and liver-committed bm stem cells (GFP+/ck19+) were detected by double immunofluorescence. Angiogenesis was evaluated by ELISA (VEGF). The hepatic mRNA levels of PPARgamma, IL6 and TNFa were determined by quantitative real-time PCR.

**Results:** Fibrosis and a-sma+ cells were significantly decreased in treated mice as compared to CCl4–only mice (p≤0.003). However, this effect was more prominent in the G-CSF-treated groups (p≤0.00000005). In G-CSF groups, we detected higher numbers of liver stem cells, liver-committed bm stem cells and bm-derived mature hepatocytes as compared to CCl4 group. In contrast, Plerixafor or G-CSF+Plerixafor didn’t significantly affect these cell populations. The mean fold change of mRNA levels of IL6 and TNFa in G-CSF-groups was significantly lower as compared to CCl4-group (p≤0.03) and similar to normal animals. Plerixafor or G-CSF+Plerixafor treatment had no impact on the hepatic concentration of these proinflammatory cytokines. On the other hand, the beneficial effect of Plerixafor or G-CSF+Plerixafor in ameliorating hepatic fibrosis was mostly mediated by enhanced angiogenesis (p≤0.002) and PPARg expression (p≤0.05).

**Conclusion:** All mobilizing agents tested may have a therapeutic effect on the injured liver facilitating hepatic reconstitution. However, they function differentially during the healing process. Overall, G-CSF seems to provide a higher therapeutic potential over Plerixafor or G-CSF+Plerixafor.
HEPATIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS IN 3D BIOREACTOR CULTURES
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Background and Aims: Primary hepatocytes are difficult to culture ex vivo and their utility is limited by the lack of available human hepatocytes. These problems provide incentive into finding sources of hepatocyte-like cells from stem cell populations. The hepatic differentiation of human embryonic stem cells (hESC) has been extensively described for 2D cultures, however differentiated cells exhibit poor hepatic function and no typical hepatic architecture is observed.

Methods and Results: Human ESC (WA01, WiCell, James A. Thomson, University of Wisconsin, Madison, USA) were initially subjected comparatively to five established protocols [1–5] for promoting hepatic differentiation. In two of these approaches [1,2] we observed successful differentiation of hESC into hepatocyte-like cells, which were obtained for further analysis and culture. The culture and differentiation of hESC was carried out in a miniaturised bioreactor system with a cell compartment volume of 0.4 ml and 2D cultures were performed in parallel as controls. The differentiation in the bioreactors was performed by adaptation of the protocols published by Hay et al [1,2]. Medium samples were taken daily and analysed for hepatic differentiation markers. At the end of culture immunohistochemistry for hepatic, biliary and pluripotency markers was performed for both bioreactor and 2D cultures. RT-PCR analysis for hepatic-specific genes including albumin and various cytochrome P450 isotypes showed a significant increase in expression in 3D cultures. Furthermore observations of the cellular architecture from bioreactor cultures demonstrated 3D structures that were closer to primary liver cell structures than those observed from 2D cultures.

Conclusions: These data show that hESC can be successfully differentiated in 3D bioreactors where they express a number of hepatocyte-specific genes and form 3D structures. Thus, the bioreactor system provides a new instrument for generating functional hepatocyte-like cells from hESC, which has great potential for clinical and pharmacological studies.

Reference(s)
Concentrations of TNF-α and IL-6 in supernatants and patient sera were assayed by ELISA. We designed a 5-plex Phosflow FACS protocol to determine activation of key transcription factors in innate immune signalling pathways (NF-κb, ERK1/2, STAT1 and STAT3) in CD14+ monocytes following stimulation with LPS and flagellin.

Results: We included 28 patients and 6 normal controls. Patients with compensated cirrhosis had a selective deficit in flagellin-induced IL-6 production (330±146 pg/ml) compared to patients with non-cirrhotic ALD or NAFLD (896±117 pg/ml) compared to patients. We found no differences in flagellin-induced TNFα, IL-6 and RANTES, nor in cytokine production when monocytes from healthy volunteers were incubated with patients’ sera before stimulation with LPS or flagellin. Flagellin-induced ERK1/2 phosphorylation in CD14+ monocytes is greater in non-cirrhotics than in cirrhotics (32.8% vs 10.5%, p = 0.056), but there were no differences in LPS-induced ERK1/2 phosphorylation and LPS- and flagellin-induced NF-κb phosphorylation.

Conclusions: We find selective impairment of TLR5-mediated IL-6 production in patients with compensated cirrhosis compared to non-cirrhotic patients that may be related to defects in ERK1/2 phosphorylation. These data identify potential signalling pathways that may be involved in liver disease progression or in susceptibility of cirrhotic patients to bacterial infections.

320 THE HEPATITIS C VIRUS NON-STRUCTURAL 3/4A PROTEASE MEDIATES AN INTRAHEPATIC T Helper(Th)-1 TO Th2 SHIFT BY CLEAVING THE T CELL PROTEIN TYROSINE PHOSPHATASE E.D. Brenndörfer1, A. Brass1, J. Karthe2, G. Ahlen1, J.G. Bode2, M. Sällberg1. 1Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska Institutet, Stockholm, Sweden; 2Department of Gastroenterology, Hepatology and Infectious Diseases, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany E-mail: erwin.brenndorfer@ki.se

Background: The hepatitis C virus (HCV) non-structural (NS)3/4A protease is not only essential for the viral life cycle but also known to modulate hepatic signaling pathways by cleaving proteins such as MAVS (mitochondrial antiviral signaling protein), TCPTP (T cell protein tyrosine phosphatase) and TRIF (Toll/IL-1 receptor domain-containing adaptor inducing IFN[ ]). However, the global effects exerted by NS3/4A in vivo still remain unclear. This is of high interest because NS3/4A protease inhibitors are currently introduced as part of HCV therapy.

Objectives: We aimed to characterize the mechanisms responsible for the NS3/4A-mediated interference with intercellular signaling and intrahepatic immunity in vivo.

Methods: The intrahepatic immune response of naïve and lipopolysaccharide (LPS)/D-galactosamine (D-galN) treated NS3/4A-transgenic (Tg) mice was determined by western blot, ELISA, Real Time PCR, Flow cytometry and survival analysis. Various MAVS or TCPTP constructs were injected hydrodynamically to study the relevance these NS3/4A protease substrates.

Results: Intrahepatic NS3/4A expression made mice resistant towards TNF[ ]-induced liver damage and caused an alteration of the intrahepatic cytokine (IFN[ ], IL10) and chemokine (CCL3, CCL17, CCL22, CXCL9, CXCL11) profiles towards an anti-inflammatory state. Consistent with this, the number of intrahepatic Th1 cells and IFN[ ]+ T cells in NS3/4A-Tg mice decreased, while the amount of Th2 cells increased. These effects could be reversed by injection of uncleavable TCPTP but not uncleavable MAVS and were absent in a mouse expressing a non-functional NS3/4A protease.

Conclusions: NS3/4A induces a TCPTP-dependent shift of the intrahepatic immune response towards a non-antiviral Th2-dominated immunity. Thus, HCV-mediated TCPTP cleavage could play a crucial role in the establishment of chronic hepatitis C.

321 INDUCTION OF IL-10 PRODUCING T-HELPER CELLS TYPE 1 BY HEPATOCYTES DEPENDS ON NOTCH SIGNALING S. Burghardt1, A. Erhardt1, S. Huber2, G. Tieg1. 1Institute of Experimental Immunology and Hepatology, 2. Medical Clinic, University Medical Center Hamburg Eppendorf, Hamburg, Germany E-mail: s.burghardt@uke.de

Background and Aims: A single injection of concanavalin A (ConA) to mice induces acute Th1-mediated liver injury. Subsequently, liver regeneration occurs and an immunosuppressive state is established. This study was intended to identify the role of hepatocytes (HCS) in ConA-induced immunoregulation.

Methods: HCs or splenic DCs were isolated from saline- or ConA-pretreated wildtype (wt), IFNγ-, or interferon regulatory factor (IRF)-1-deficient mice. Subsequently, HCs or DCs were co-cultured with splenic CD4+ T cells from wt, DEREG or Foxp3-ires-mRFP (FIR) x interleukin-ten ires gfp-enhanced reporter (tiger) mice and stimulated with anti-CD3 for 48 hours. Cytokine release was measured by ELISA. Frequencies of CD4+IL10+, CD4+Foxp3+ Tregs and Notch1+ T cells were quantified by FACS analysis.

Results: CD4+ wt T cells co-cultured with wt HCs from ConA-primed mice showed significantly increased IL-10 levels accompanied by elevated IFNγ levels. In contrast, splenic DCs from ConA-primed mice were not able to induce IL-10 release in naïve wt T cells. CD4+IL10+ cells were largely Foxp3 – indicating an IL-10 producing Th1 phenotype. HCs from ConA-primed IFNγ− and IRF1− mice, which cannot respond to IFNγ, failed to aggravate IL-10 production by CD4+ T cells. HCs from ConA-primed mice showed elevated Jagged1 mRNA expression while CD4+ T cells showed increased receptor density of Notch1. Blocking Notch signaling by inhibition of either ADAM17-mediated receptor cleavage or γ-secretase prevented IL-10 secretion. Furthermore, HCs from ConA-primed mice promoted the TGFβ-driven conversion of naïve wt T cells into Foxp3+ Tregs which was again abrogated by blocking Notch signaling.

Conclusions: We showed that HCs from regenerating mouse livers induce an IL-10-secreting regulatory Th1 phenotype in naïve T cells and convert these cells into Foxp3+ Tregs in the presence of TGFβ. This process depends on an intact IFNγ-dependent Th1 response in vivo and on ligation of Notch1 by Jagged1. The failure of splenic DCs to induce IL-10 expression indicates that the generation of an IL-10 producing T cell subset is restricted to liver-resident non-professional antigen presenting cells favouring the ‘liver tolerance effect’ as well as regeneration in response to inflammation.

322 KIR AND HLA LOCI AS PREDICTORS OF HCC OUTCOME IN PATIENTS WITH HCV INFECTION E. Cariani1, M. Pili2, A. Zerbinì1, C. Rota1, A. Olivani2, P. Zanelli4, A. Zanetti4, T. Trenti1, C. Ferrari2, G. Missale2. 1Clinical Pathology, Ospedale Civile S. Agostino-Estense, Modena, 2U.O. Infectious Diseases and Hepatology, Azienda Ospedaliero-Universitaria di Parma, Parma, 3Clinical Immunology, Azienda Ospedaliero-Universitaria ASMN, IRCCS, Reggio Emilia, 4U.O. Medical Genetics, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy E-mail: ecariani@hotmail.com

Background and Aims: Natural killer (NK) cells are involved in the anti-tumor immune response through the interaction of inhibitory and activating receptors with their ligands. We evaluated the impact of the Killer Immunoglobulin-like Receptors (KIRs) of NK cells and of their Human Leukocyte Antigen (HLA) ligands over the outcome
of HCV-related hepatocellular carcinoma (HCC) after treatment by either surgical resection (SR) or radiofrequency thermal ablation (RTA).

**Methods:** Sixty-one patients with HCV-related HCC were included in this study. Typing of 10 KIR genes was performed by real-time PCR coupled with melting analysis. HLA typing was carried out by PCR-Sequence Specific Priming followed by high resolution typing by PCR-Sequence Specific Oligonucleotide Probes when definition of the HLA-B or C supertypes was ambiguous. The expression of interferon-γ and of CD107a, a marker of cytotoxic function, was evaluated in basal condition and after stimulation. Survival curves were estimated by the Kaplan–Meier method and compared by log-rank test. Cox proportional hazards regression model was used for multivariate survival analysis.

**Results:** Activating KIR2DS5 was associated with significantly longer time to recurrence (TTR) and overall survival (OS) (p < 0.05). Homozygous HLA-C1 (p = 0.01) and HLA-Bw4180 (p = 0.05) were expressed by patients with longer OS. Multivariate analysis identified the type of treatment (SR vs RTA) and HLA-C1 as independently related to longer TTR (both p < 0.05), whereas only KIR2DS5 was an independent predictor of longer OS (p < 0.05). Compound KIR2DL2-C1 and KIR3DS1-Bw4180 genotypes were associated with better TTR and worse OS, respectively (p < 0.05 each). A prevalent cytotoxic NK phenotype was detected in patients with both longer TTR and OS, and cytotoxic capacity was significantly higher in subjects with HLA-C1 alone or combined with KIR2DL2/KIR2DL3 (p < 0.05 and <0.01, respectively).

**Conclusions:** These results support a central role of NK cells in the immune response against HCC, providing a strong rationale for personalized post-treatment monitoring schemes and therapeutic strategies enhancing NK cell response.

**323 DURING PRIMARY EBV INFECTION, THE INTENSITY OF LIVER ABNORMALITIES CORRELATES WITH AGE AND IMMUNE RESPONSE, AND THE USE OF CORTICOSTEROIDS IS NOT DELETERIOUS**

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**Background and Aims:** Severe forms of primary EBV infections (PEI) are increasingly reported in developed countries. We aimed to characterize acute liver disease associated with PEI.

**Methods:** Cohort study conducted in a French Regional Hospital. Using electronic medical records and lab alert system, we identified all cases of in- or outpatients (whatever the age and department) referred for PEI over a 5-year period. We included cases with clinical and biological (anti-VCA IgM+ and anti-EBNA+) evidence for PEI with documented blood and liver (ALT, AST, GGT, bilirubin and alkaline phosphatasches) biology. We collected demographic, clinical, biological and therapeutics data. We compared patients with/without serious liver abnormalities (grade ≥3, i.e. >5-fold normal value, according to the international grading scale) and evaluated the impact of systemic corticosteroids (SCS) on the normalization of liver abnormalities.

**Results:** 179 cases were included. Median age was 17.9 years (interquartile range: 7.7–23.2), Sex-ratio (M/F) was 1.2. Overall, 163 (91%) showed at least one grade ≥1 hepatic abnormality and 75 (42%) had a serious hepatitis. Cytolysis (ALT or AST) was positively correlated with age (r = 0.35, p < 0.0001), immune response assessed by blood activated lymphocytes (r = 0.4, p < 0.0001) and plasma EBV viral load (r = 0.44, p = 0.007). Cholestasis (GGT) also correlated with age (r = 0.41, p < 0.0001), immune response (r = 0.28, p = 0.001) and viral load (r = 0.3, p = 0.08). In a multivariate analysis, age >17 years and blood activated lymphocytes >10% were independently associated with serious hepatitis (OR = 3.9, 95%CI=1.9–8.1, p = 0.0002 and OR = 5.2, 95%CI=2.5–10.6, p < 0.0001, respectively). Overall, 134 patients (75%) were hospitalized, during a median 3 days [2–5]. Hospitalization was a direct result of hepatitis (abdominal pain, jaundice or lab abnormalities) in 22% of cases. No patient died or was transferred in the intensive care unit. Overall, 38% received SCS: typically 1 mg/kg/day, during a median 7 days [3–10]. In a Cox-model, SCS were found to have a beneficial impact on AST normalization (HR = 1.8, 95%CI=1.1–2.9, p = 0.01), but not on ALT or GGT.

**Conclusions:** Serious liver abnormalities are frequent during PEI and correlate positively with age of onset, immune response against EBV and plasma viral load. In this setting, SCS are frequently prescribed and are not deleterious.

**324 EFFECT OF VITAMIN D SUPPLEMENT ON T HELPER1/2 CYTOKINE LEVELS IN CHRONIC HEPATITIS C PATIENTS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PILOT STUDY**

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**Background and Aims:** Vitamin D deficiency has been shown to be associated with poor treatment response of chronic hepatitis C (CHC) to interferon based therapy. Vitamin D has crucial role in immunoregulatory functions and its deficiency results in imbalance in Th1/2 cytokine responses. In chronic hepatitis C infection, most studies demonstrated dysregulated immune responses by increasing in Th1 and decreasing in Th2 cytokine responses. Considering that vitamin D replacement therapy could restore treatment response of CHC. We, therefore, hypothesized and investigated the immune mechanism by which correction of vitamin D deficiency in patients with CHC might restore immune functions through balancing Th1/Th2 cascades and, eventually, promoted clearance of virus during interferon based therapy.

**Methods:** In this double-blind, placebo-controlled, interventional pilot study; we assigned CHC patients with vitamin D deficiency to receive vitamin D supplement or placebo for 6 weeks. The patients were measured 25-hydroxyvitamin D (25(OH)D) levels and Th-1/2 related cytokines including IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IFNg and TNFa at baseline and at 6 weeks. Baseline characteristics of the two groups were similar. The levels of IFNg and TNFa were significantly higher in patients than in controls. At pre and post supplement, the mean 25(OH)D levels in placebo group were 20.59 and 21.22ng/ml respectively, and in vitamin D group were 21.07 and 48.44ng/ml respectively. Significant treatment effects were observed on a change of 25(OH)D levels in vitamin D group (p < 0.001). However, there were no significant differences in serum levels of all Th1/Th2 cytokines studied both in the same and in between groups during pre and 6-week post supplement period.

**Conclusions:** The immunopathogenesis by which vitamin D supplement promoted sustained virological response in interferon based therapy of CHC could not be demonstrated by the changes in systemic Th1/Th2 immune cytokines, studied in this protocol. Other immune mechanism may play an important role in this clinical dilemma and remained to be explored.
325 PROBING HEPATIC ANTIGEN PRESENTATION IN PATIENTS WITH CHRONIC HBV INFECTION
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Background and Aims: Hepatitis B virus (HBV) replicates exclusively in hepatocytes, but a number of antigen presenting cells (APC) in the liver may be capable of taking up soluble HBV antigens or infected cells and cross-presenting them to the immune system. The development of T cell receptor-like monoclonal antibodies (TCR-L mAb) able to bind complexes formed between HLA-A2 and immunodominant HBV epitopes (Satsky J Virol 2011) has allowed us, for the first time, to examine the topography of HBV antigen presentation in human liver tissue ex vivo.

Methods: Sections from frozen liver biopsy material (surplus to diagnostic requirements) from 18 patients with chronic HBV infection (CHB) were analysed by immunofluorescent staining using TCR-L mAbs able to visualise HLA-A2 bound to HBV env183-91 or core18-27. Sections were co-stained with cytokeratin-18 and DAPI and, in selected cases, with anti-CD68 or anti-DC-SIGN/L-SIGN.

Results: The TCR-L mAb were able to stain liver sections from HLA-A2+ patients infected with HBV genotype D in a highly specific manner, regardless of their viral load, sAg titre or degree of liver inflammation (ALT). Staining of HBV epitopes was not homogeneously distributed across the tissue, suggesting patchy foci of preferential antigen presentation. The most striking finding was that HLA-A2/HBV epitope complexes were mainly found lining the liver sinusoids rather than localising to hepatocytes. A minority of CD68+ Kupffer cells co-stained with the TCR-L mAb but the predominant staining pattern was more suggestive of liver sinusoidal endothelial cells (LSEC) in its distribution; in keeping with this, co-staining with DC-SIGN/L-SIGN could be demonstrated.

Conclusions: TCR-L mAbs are powerful tools allowing accurate visualisation of antigen presentation within diseased HBV-infected human liver. Mapping their distribution revealed the first ex vivo evidence for HBV epitopes localising to sinusoidal populations and suggests that Kupffer cells and LSEC are capable of cross-presenting HBV antigen. This has important implications for understanding the tolerisation of T cells perpetuating persistent HBV infection and for the application of novel immunotherapeutic approaches.

326 CHEMOKINE RECEPTOR CCR5 AND ITS CORRESPONDING CHEMOKINE CONTRIBUTES TO VIRUS-INDUCED MURINE FULMINANT LIVER FAILURE VIA NK CELLS RECRUITMENT
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Aims: Previous study shown that NK cells migrate to the liver from the blood, spleen, and BM post MHV-3 infection. Here we aimed to investigate the recruitment mechanisms of NK cells to liver. Thus the role of the CC chemokine receptor 5 (CCR5) on NK cells and its corresponding chemokines MIP-1α, MIP-1β and RANTES were measured in MHV-3 induced fulminant hepatic failure.

Methods: Balb/cj mice (6–8 weeks, female) were intraperitoneally injected with 100 PFU MHV-3. The expression of CCR5 on NK cells in the liver, spleen, BM and blood were analyzed by flowcytometry. The genetic and protein level of CCR5-corresponding chemokines (MIP-1α, MIP-1β and RANTES) were detected by real-time PCR and Immunohistochemistry staining. Transwell migration assay was used to assess the chemotactic effect of MHV-3-infected hepatocytes on the splenic NK cells. Adoptive transfer experiments were used to observe the survival rate of the Balb/cj mouse after anti-ASGM-1 injection and MHV-3 infection.

Results: With MHV-3 infection, the frequencies of NK cells from spleen and BM expressing CCR5 increased at 12h post-MHV-3 infection; meanwhile, they all increased significantly in the liver and peripheral blood at 24h post-MHV-3 infection. Moreover, the hepatic mRNAs level of MIP-1α, MIP-1β and RANTES were significantly elevated post infection, as well as the immunohistochemistry staining. The transwell migration assay demonstrated that MHV-3-infected hepatocytes have the capacity to attract and recruit the splenic NK cells; furthermore, CCR5 and its corresponding chemokine MIP-1β and RANTES may play a key role in the NK cells recruitment. Adoptive transfer the splenic NK cells with CCR5 blockage can prolong the survival time of mouse after anti-ASGM-1 injection and MHV-3 infection.

Conclusion: These results suggest that the CCR5 plays a prominent role in the NK cells recruitment to liver, and this subsequently contribute to hepatocyte injury in MHV-3 induced FHF.

327 INTRAHEPATIC TREGS ARE PLASTIC BUT FUNCTIONAL AND BILARY EPITHELIAL CELLS SUPPORT THEIR FATE
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Background: Regulatory T cells (Tregs) are crucial in maintaining peripheral tolerance by controlling T effector responses. They are implicated in both human and murine models of hepatic inflammation including autoimmune hepatitis, viral hepatitis, liver cancer and post-transplant tolerance. However little is known about their lineage stability, function and fate in the inflamed hepatic microenvironment.

Methods: Human liver infiltrating (LI) lymphocytes were isolated from explanted liver tissues and the chemokine and cytokine receptor expression and intracellular-cytokine secretion of LI-Tregs cells assessed by flow cytometry. Recruitment into the inflamed liver was modelled in vitro by migrating Tregs across human hepatic endothelial cells activated by proinflammatory cytokines and interactions with bile ducts by coculture with primary human cholangiocytes. Function was assessed by suppression assays. Distribution and localisation of LI-Tregs in tissue was determined using dual immunohistochemistry and confocal microscopy. Cytokine levels in liver tissue culture supernatants and biliary epithelial cells conditioned media were measured by Luminex.

Results: LI-Tregs expressed CD39 (57±11%), CD95 (83±4%), CD27 (73±3%), CD44 (90±3%), IL15r (31±15%) and IL6r (17±15%). Liver tissue supernatant contained IL-1β (363±88 pg/ml), IL-6 (8960±59 pg/ml), IL-12 (44±35 pg/ml), IFN-γ (21±8.33 pg/ml) but minimal level of IL-2. Stimulated biliary epithelial cells secrete IL-6 and IL-15. Transmigrated Tregs and Tregs in inflamed liver supernatant has significant reduction in FOXP3 transcription factors (p=0.01 & p=0.001) compared to Tregs in the peripheral blood. Importantly, both post-endothelial transmigrated Tregs and Tregs in the inflamed microenvironment are functional but suppression capacity was significantly reduced (p<0.0001) in Tregs residing in the inflamed liver. LITregs reside closely to bile ducts around portal tract. Co-culture experiment of PET Tregs with biliary epithelial cells suggested that Tregs proliferation or death depends on contact Fas-Fas ligand pathway and soluble cytokines.

Conclusions: LITregs maintain functional suppression despite downregulating FOXP3 in the liver microenvironment. Their survival is increased by inhibiting Fas mediated apoptosis and inhibiting IL-15.
Background and Aims: Persistent HCV infection leads to liver fibrosis and carcinogenesis. Expression of PD-1, an inhibitory molecule, causes T cell exhaustion and failure of HCV eradication. Furthermore, degree of PD-1 expression defines the level of T cell dysfunction (Nakamoto N et al. Gastroenterology 2008; 134: p1927–37). However, little is known about how persistent T cell exhaustion influences clinically during HCV infection.

Methods: Sixty patients of chronic hepatitis C were included. Peripheral blood mononuclear cells were taken after informed consent, and were analyzed by flow cytometry before intervention. Twenty-two patients (37%) were HCC-bearing cases. Clinical laboratory parameters including serum albumin, branch-chain amino acids (BCAA) to tyrosine ratio (BTR), cross-section area of visceral fat (by electrode impedance method), METAVIR staging of recent liver biopsy (available in 29 cases), and clinical staging of HCC were also analyzed.

Results: PD-1 expression of peripheral CD4+ T lymphocytes was significantly elevated in HCC-bearing cases (12.1% vs 7.4%, p < 0.0001), but that of peripheral CD8+ T lymphocytes was not statistically significant. To further evaluate risk factors affecting hepatitis carcinogenesis, we found that PD-1 expression of CD4+ T lymphocytes had no statistical correlation to fibrosis stages of liver biopsy specimen (p=0.98). Elevated PD-1 expression of CD4+ T lymphocytes significantly correlated to lower serum albumin (p < 0.0001, correlation coefficient c.c.= −0.49), lower BTR (excluding BCAA supplemented cases, p = 0.016, c.c. = −0.48), and higher cross-section area of visceral fat to body surface area (BSA) ratio (p = 0.0016, c.c. = 0.55), which suggested that T cell dysfunction significantly correlated to nutritional/metabolic derangements in chronic hepatitis C. PD-1 expression of CD4+ T lymphocytes also increased accordingly to the T factor of HCC (p = 0.035), TNM staging (p = 0.015), and JIS scores (p = 0.013). Furthermore, PD-L2 levels expressed on plasmacytoid dendritic cells (pDCs) were significantly increased as PD-1 expression of CD4+ T lymphocytes elevated (p = 0.002, c.c. = 0.61).

Conclusions: These findings suggest that T cell exhaustion may play a pathological role in carcinogenesis and HCC progression in chronic HCV infection. Correlation of PD-1 expression of CD4+ T lymphocytes and nutritional/metabolic factors also suggests an immunological rationale for nutritional intervention in these patients.

Background and Aims: Patient with chronic hepatitis B virus (HBV) infection are characterized by a profound immunotolerance against viral antigens. To promote sustained viral clearance it is necessary to boost a viral specific immune response of the host. HBV transgenic mice are immunologically tolerant to HBV antigens, representing a good model to test strategies aiming at breaking T cell tolerance. Gene delivery of cytokines to the liver may represent an interesting strategy to maximize its antiviral efficacy and reduce side effects. We provide a novel strategy to induce functional T cell in HBV transgenic mouse model based on the genetic transfer of immunostimulatory cytokines.

Methods: Recombinant adeno-associated viruses (rAAV) expressing interleukin-15 (rAAVIL-15) and interferon-alpha (rAAVFIn-α) were produced. HBV transgenic mice were injected i.v. with rAAVIL-15 or rAAVFIn-α or the combination of both. Ten days after treatment HBV-specific cytotoxic T lymphocyte response in spleen and liver lymphocytes by pentamer staining and FACS analysis and by in vivo killing assay, to evaluate the ability of CD8+ T cells to kill antigen loaded cells. Finally, the antiviral efficacy of the treatment was analyzed.

Results: Sustained liver specific IL-15 expression expands the population of HBV-specific CD8+ T cells in spleen and the liver. However, IL-15 expanded T cells failed to kill peptide loaded cells, indicating that they remained functionally silent. When IL-15 expression is combined with IFN-α, we observed an increase in the number of HBV specific T cell but more importantly those cells are functional, since they were able kill antigen loaded cells and induce the death of all the animals due to acute hepatitis. Furthermore a strong antiviral effect was observed.

Conclusions: Thus, our study defines a potent new approach to brake the tolerance in a model of chronic viral infection by (1) expanding virus-specific CD8+ T cell with IL-15 and (2) blocking negative signals with IFN-α. Such an approach may have broad applications in developing treatment strategies for patients with chronic hepatitis B.
Figure 1. A reduction of circulating CD3+ lymphocytes in patients with HBV-ACLF. A. The absolute CD3+T lymphocyte count, including CD3+CD4+ and CD3+CD8+ T cells (B) in peripheral blood from 3 groups was measured by flow cytometry. The line and error bars represent the median with interquartile range (A, for at least one group data were in skewed distribution) or mean with SEM (B, for all data displayed normal distribution), and the same method with following figures. Statistical comparisons were performed by ANOVA or the non-parametric Nemenyi test. *p < 0.05; **p < 0.01; and not significant (NS), p > 0.05.

In comparison with controls, the CD4+Tregs count remained unchanged while the Tconv count correspondingly declined, promoting elevation of the Tregs-to-Tconv ratio amongst CD4+ T cells in HBV-ACLF patients. Moreover, the number of circulating IL-17-producing CD4+Th17 cells decreased slightly and the CD4+ Treg-to-Th17 ratio increased. Further, we showed that the frequency of activated Treg-II (CD4+CD25+++CD45RA−) subpopulation was elevated and the ratio of Treg-II/I was dramatically higher in HBV-ACLF patients than controls.

Conclusions: The development of CD4+Tregs, in particular the activated Treg II subpopulation with potent suppressive properties, prevails over Tconv, while an exhaustion of circulating CD3+ T lymphocytes exists in HBV-ACLF patients, which probably contribute to immune dysfunction status of HBV-ACLF.

Figure 2. Prevalence of circulating regulatory CD4+CD25++ T lymphocytes (CD4+ Tregs) over Tconv counterparts (CD4+CD25i−/+) in HBV-ACLF patients. (A) Representative flow cytometry analysis of the percentage of CD4+CD25++ Tregs (equivalent to CD4+FoxP3+ Tregs) among CD4+ T lymphocytes. Below is a comparison of the absolute number of circulating CD4+ Tregs and Tconv among different groups. The ratio of CD4+ Tregs to Tconv was detected as well. (B) Representative figure of intracellular staining of FoxP3 and IL-17A in CD4+ Tregs; comparison of the absolute CD4+ Th17 cell count and the ratio of CD4+ Th17/Tregs in indicated numbers of patients with ACLF, patients with chronic hepatitis B and healthy controls were displayed. The absolute number was calculated by multiplying the frequency of CD4+ Tregs or Tconv or Th17 in CD4+ T lymphocytes by the corresponding absolute CD4+ T cell counts.

Figure 3. Change in frequencies of CD4+ Tregs subpopulations among peripheral CD4+ T cells in three groups. (A) Representative flow cytometry analysis (including gating links) and mode pattern of three subsets of CD4+ Tregs defined by the expression of cell surface markers (CD45RA and CD25), as follows: CD25++CD45RA− T cells (Treg-I); CD25+++CD45RA− T cells (Treg-II); and CD25++CD45RA+ T cells (Treg-III). (B) Frequencies of Treg-I, -II and -III subsets among CD4+ T cells and comparisons between groups were made.

331 CO-STIMULATION VIA CD27 MIGHT IMPORTANTLY CONTRIBUTE TO ANTI-HCV ACTIVITY OF NK CELLS
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Background: Genetic and functional data indicate Natural Killer (NK) cells to modulate the natural course of HCV infection by yet incompletely understood mechanisms. Co-stimulation via CD27, a member of the TNF receptor family, has been suggested to be involved in NK cell-mediated innate immunity. Here, we provide first evidence that CD27-mediated activation effectively triggers anti-HCV activity of human NK cells and show that CD27 may be involved in spontaneous clearance of acute hepatitis C.

Methods: A total of 56 persons was enrolled into this study (acute HCV/HIV(+), n=29; chronic HCV/HIV(+) n=10; HCV(−)/HIV(+) n=6; healthy controls n=11). Peripheral NK cells were phenotypically characterized by flowcytometry and functionally analyzed (IFN-γ secretion and inhibition of HCV replication) using the HUH7_replicon (Luc A2 JFH) system in the absence or presence of stimulating (anti-CD27) or inhibiting (anti-CD70, anti-IFN-γ) antibodies.

Results: Analyzing isolated NK cell populations, we found CD27(+) NK cells to be significantly more effective in inhibiting HCV replication as compared to the CD27(−) subset. This effect was mediated via IFN-γ as CD27(+) NK cells displayed stronger IFN-γ production following co-incubation with HCV_replicon-containing HUH7 cells than CD27(−) NK cells (p<0.01) and co-incubation with an IFN-γ specific antibody significantly reduced anti-viral NK cell function. A role for CD27/CD70 interactions in anti-viral NK cell activity was confirmed by the fact that I) NK cell-mediated inhibition of HCV replication could partly be blocked by anti-CD70, and II) pre-stimulation of NK cells with anti-CD27 significantly
increased the in vitro anti-HCV activity of NK cells. A role for CD27 in anti-HCV NK cell activity was furthermore supported by the observation that in HIV(+) patients with acute hepatitis C significantly higher frequencies of CD27+ NK cells were found in patients who were subsequently able to clear HCV infection than in those becoming chronically infected.

Discussion: Our data indicate that CD27(+) NK cells display anti-HCV activity via robust secretion of IFN-γ after binding to CD70. In this way, co-stimulation of NK cells via CD27 may be involved in spontaneous clearance of acute hepatitis C in HIV(+) patients.

332 KUPFFER CELL DERIVED IRAK-M INHIBITS IMMUNE-MEDIATED HEPATITIS BY REGULATING TH1 AND TH17 CYTOKINES PRODUCTION

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Background and Aims: Excessive immune and inflammatory responses are associated with the pathogenesis of acute liver failure. Interleukin-1 receptor associated kinase (IRAK-M) is only expressed in the myeloid lineage cells, and displays a negative regulator of Toll-like receptor signaling to inhibit immune and inflammation. Here we investigated the roles and underlying mechanisms of IRAK-M on Concanaevalin A (Con A)-induced hepatitis.

Methods: Immune-mediated hepatitis was induced in mice by injection of concanavalin A (Con A). Mice were injected with GdCl3 to deplete Kupffer cells (KCs), and then received adoptive transfer of the isolated KCs that were subjected to Ad-α-gal or Ad-IRAK-M or Ad-IRAK-M siRNA, respectively. Hepatitis was evaluated by liver enzymes assay, histological damages, mononuclear cell (MNC) infiltration, cytokines production, and intracellular staining of immune cells.

Results: Depletion of KCs completely abolished Con A-induced hepatitis. Transfer of Ad-α-gal-treated KCs restored the hepatic susceptibility to Con A. However, transfer of Ad-IRAK-M-treated KCs attenuated Con A-induced hepatitis, inhibited MNC infiltration, reduced the number of interferon (IFN)-γ and interleukin (IL)-17 producing CD4+ T cells, and decreased IFN-γ and IL-17 production. Furthermore, transfer of Ad-IRAK-M siRNA-treated KCs developed significantly more severe hepatitis, enhanced MNC infiltration, increased the number of IFN-γ and IL-17 producing CD4+ T cells, and elevated IFN-γ and IL-17 production when compared to transfer of Ad-α-gal-treated KCs.

Conclusions: These findings indicate that KCs-derived IRAK-M protects from immune-mediated hepatitis, which may be via suppression of Th1 cytokine IFN-γ and Th17 cytokine IL-17 production.

333 HCV-INFECTION IS NOT A MAJOR DETERMINANT OF IMMUNESENCE IN AGING DRUG USERS

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Background and Aims: Immunesenescence has been associated with increasing age, impaired immune function and morbidity, and can be accelerated by chronic viral infections. Injecting drug users (DU) are at increased risk for blood-borne infections, including HCV and HIV. We studied the effect of long-term drug use and the presence of HCV monoinfection and HCV/HIV coinfection on immune senescence by studying telomere lengths in different groups of (DU) from the Amsterdam Cohort Studies.

Methods: Four groups were included:
1. healthy individuals (n = 22) recruited outside the ACS;
2. DU from the ACS who were Multiple Exposed Uninfected (MEU) (n = 8);
3. chronic HCV (cHCV) monoinfected DU (n = 21);
4. cHCV/HIV coinfected DU (n = 23).

For each participant we selected an age-matched recent PBMC sample (t=2) and for MEU, cHCV and cHCV/HIV DU a historic sample (t=1). To assess immunesenescence we determined the median telomere length of T cell subsets relative to telomere length of calf thymocytes (RTL) by using flowcytometry and fluorescent in situ hybridization (Flow-FISH).

Results: Median age for all age-matched groups at t=2 was 52.1 years (IQR 47.9-55.9) and 34.4 years (IQR 31.3-38.4) for t=1. In all groups, telomere length within CD8+ T-cells decreased significantly from a RTL of (0.36 (0.24-0.33) to 0.28 (0.24-0.33) in MEU, 0.36 (IQR 0.32-0.41) to 0.30 (IQR 0.26-0.34) in cHCV monoinfected and 0.30 (0.27-0.30) to 0.23 (0.21-0.28) in cHCV/HIV coinfected DU, p < 0.01 within each group. A smaller, but significant decrease in RTL was observed for CD4+ T-cells between t1 and t2, p < 0.01 within each group. Telomere length of CD8+ T-cells in HCV/cHCV infected individuals at t=2 was significantly shorter as compared to those with cHCV infection, MEU or healthy controls, p < 0.01. No statistically significant difference was observed between the latter 3 groups. Telomere length of CD4+ T-cells in HCV/HIV coinfection was significantly (RTL 0.26, IQR 0.23-0.31) reduced as compared to healthy donors (RTL 0.31, IQR 0.27-0.34), p < 0.05.

Conclusions: These data suggest that long-term drug use and chronic HCV do not increase telomere shortening. In contrast, HIV-infection seems to be the major determinant of accelerated ageing of the immune system in HIV/HCV coinfeected individuals.

334 INTERACTION OF PROTECTIVE POLYMORPHISMS IN HCV: A COMPARISON OF SPONTANEOUS AND TREATMENT INDUCED RESOLUTION

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Background and Aims: Only 20% of individuals exposed to HCV achieve spontaneous resolution and many have a suboptimal treatment response. SNPs within both the adaptive and innate immune systems determine these outcomes including IL-28B, KIR2DL3 and HLA-C. Our recent data also implicates Tapasin (a component of the antigen processing pathway) and specific HLA-B alleles. The aim of this study was to determine how these SNPs interact to resolve HCV.

Methods: Overall 358 patients were genotyped for the IL28B rs12979860 polymorphism, KIR, HLA class I and the G/C non-synonymous coding polymorphism in tapasin. Statistical analysis was performed using Chi-squared and Chi-squared test for trend.

Results: Fifty-one individuals spontaneously cleared HCV; 177 had been treated with Pegylated Interferon/Ribavirin of which 88 (49.7%) had genotype 1 infection and 80 (45%) achieved an SVR.

For spontaneous resolution KIR2DL3/HLA-C1 homozygosity (p = 0.03), IL28B-CC (p = 0.006) and tapasin G heterozygosity in individuals with an HLA-B allele with aspartate at residue 114 (p = 0.029) or in the presence of HLA-B heterozygosity (p = 0.012) protected against CHC. Spontaneous resolution was strongly
associated with the number of protective factors possessed; 3 factors >2>1<0 (p < 0.0001, chi squared test for trend). In the treatment cohort overall only IL-28B (p = 0.03) was associated with SVR, but not KIR, HLA-C or Tapasin. However, sub-analysis by HCV genotype indicated that in individuals with HCV G2/3, IL-28CC was not protective, being found in 10/26 (38.5%) of those without SVR vs 23/60 (38.3%) with SVR. Conversely the compound KIR genotype was protective in these individuals (p = 0.04). For HCV G1 infection, IL-28B (p = 0.03), but not KIR was associated with SVR. Tapasin was not associated with SVR in either cohort. **Conclusions:** Spontaneous resolution of HCV is associated with multiple factors which may interact to enhance protection against chronic infection. Conversely, in treatment induced resolution, host genetic protective factors are discrete, dependent on the HCV genotype and do not act synergistically. Spontaneous and treatment-induced resolution of HCV infection have discrete immunogenetic pathways.

335 HBV INHIBITS THE AIM-2 INFLAMMASOME IN HUMAN KUPFFER CELLS
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The human Hepatitis B virus (HBV) is a major health problem with hundreds of millions of people chronically infected worldwide who are at high risk for liver cirrhosis and hepatocellular carcinoma. The role of innate immunity for the clearance of HBV infection remains controversial. The innate immune response requires a panel of immune pathogen recognition receptors that sense infection by recognizing microbial molecular motifs resulting in type-I interferon and pro-inflammatory cytokine secretion. One potent and recent discovery in innate immunology is the so-called “inflammasome response”. The inflammasome is a multiprotein complex whose assembly leads to the caspase-1 dependent maturation of the pro-inflammatory cytokines IL-1β, IL-1α and IL-18. To date the most characterized inflammasome responses are mediated by the innate sensors NLRP3 and AIM-2 described in differentiated human monocytes (THP-1). However very little is known on the inflammasome function in the liver and its role in response to HBV infection. In this study we address the inflammasome response in the liver using ex vivo cultured primary human cells (PHC) derived from hepatectomy. PHC are constituted mainly of hepatocytes (PHH), but contain also abundant immune cells including Kupffer cells (KC), the liver resident macrophages. Our results show that PHC have active AIM-2 and NLRP3 inflammasomes but that such activity is due to the contaminating KC population and not PHH. The inflammasome response in freshly purified human KC resulted to be comparable to the one assessed in THP-1. We next assessed the inflammasome response during HBV infection. The incubation of HBV with THP-1 or KC resulted to be comparable to the one assessed in THP-1.

**336 INTERFERON LAMBDA-PRODUCING CD141+ DENDRITIC CELLS ARE ENRICHED IN HEALTHY HUMAN LIVER AND PRIME INTERFERON-GAMMA AND IL-17-SECRETING T CELLS**

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**Background and Aims:** Extensive populations of liver immune cells detect and respond to homeostatic perturbation caused by damage, infection or malignancy. Dendritic cells (DCs) are central to these activities, having roles in mediating innate and adaptive immunity as well as helping create the tolerogenic environment characteristic of healthy liver. However, DCs in healthy human liver are poorly studied. Circulating DC populations are diverse, including plasmacytoid DCs (pDCs) which are potent interferon alpha (IFN-α) producers, and myeloid DCs (mDCs), powerful antigen-presenting cells which also produce IFN lambda (IFN-λ).

**Methods:** Here we characterised mDC and pDC populations in perfusates from 22 healthy donor and 12 diseased explant livers collected during transplantation.

**Results:** Significant populations of viable mDCs and pDCs were present in all perfusates and their relative distributions differed significantly from peripheral blood DC populations. Almost one third of CD11c+ mDCs from healthy liver expressed CD141 (~27.4%) as compared to ~5% of circulating mDCs. Hepatic CD141+ mDCs demonstrated potent pro-inflammatory function in allogeneic mixed lymphocyte reactions (MLRs), inducing CD4 T cell proliferation and production of IFN gamma (IFN-γ) and interleukin (IL-17) but no IL-10. CD8 T cell proliferation and IFN-γ secretion was also observed. The majority of healthy liver mDCs expressed the tolerogenic markers immunoglobulin-like transcript 3 (IILT3) and IILT4 although ~50% of CD141+ DCs were negative for these markers, indicating differentially activated CD141+ subpopulations. CD123+ pDCs and CD123+ mDCs were significantly expanded in perfusates from diseased livers (p = 0.0188; p = 0.0003) whereas CD141+ DCs were significantly depleted. Despite their depletion in the diseased state, CD141+ DCs isolated from explant livers produced markedly increased poly(I:C)-induced IFN-λ when compared with donor liver DCs.

**Conclusion:** Our results indicate that healthy liver has a significant population of CD141+ DCs which have a potent pro-inflammatory phenotype likely to play an important role in hepatic anti-viral immunity. Our data highlight the possibility of several different functional roles for liver DC populations which could provide important targets for the development of successful therapeutic and preventative vaccines.

337 EXPRESSION OF PD-1 AND CTLA-4 IS INCREASED DURING SYMPTOMATIC PHASE OF ACUTE HEPATITIS A

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**Background:** Inhibitory molecules such as programmed death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)
are associated with antiviral effector T-cell dysfunction, which influences on T-cell exhaustion and persistent viral infection. These PD-1 and CTLA-4 are up-regulated in chronic viral infection such as chronic hepatitis C, chronic hepatitis B and human immunodeficiency virus infection but there is no report about the role of PD-1 and CTLA-4 in patients with symptomatic acute hepatitis A. We investigated the expression of PD-1 and CTLA-4 during symptomatic and convalescent phases of acute hepatitis A.

**Methods:** Seven patients with symptomatic acute hepatitis A, 5 patients with non-viral acute toxic hepatitis were enrolled for detection of PD-1 and CTLA-4 on T-cell subset of peripheral blood mononuclear cells (PBMC) isolated from these subjects during symptomatic and convalescent phases by flow cytometry. Five as healthy control were also examined for comparison with these patients.

**Results:** Symptomatic acute hepatitis A showed significant increase of PD-1 and CTLA-4 expression compared to non-viral acute toxic hepatitis or healthy control (PD-1: 18.3±15.7% vs. 3.7±3.0% vs. 1.6±1.8%, p<0.05, CTLA-4: 23.5±12.0% vs. 6.1±1.2% vs. 5.9±1.2%, p<0.05) (median ± SD). In addition, highly expressed PD-1 and CTLA-4 were dramatically decreased in convalescent phase of acute hepatitis A.

**Conclusions:** In acute hepatitis A, PD-1 and CTLA-4 are up-regulated during symptomatic phase then down-regulated after recovery. This changing pattern of PD-1 and CTLA-4 expression was not seen in non-viral acute toxic hepatitis. Our findings suggest that PD-1 and CTLA-4 have protective effect as inhibitory molecules to suppress cytotoxic T-cells which induce destruction of viral infected hepatocytes.
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T CELLS REDIRECTED BY A CHIMERIC ANTIGEN RECEPTOR RECOGNIZING HBsAg EFFICIENTLY CONTROL HBV IN VIVO IN TRANSGENIC MICE
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Background and Aims: Current antivirals suppress HBV but do not clear the infection. For virus clearance strong effector T cell responses are needed, which are sparse in chronically infected individuals. Cell therapy using T cells redirected by HBV-specific receptors may clear HBV and help to prevent and treat HBV-associated liver cancer. We designed a chimeric antigen receptor (CAR) that is composed of a single chain antibody fragment binding to HBsAg and CD28/CD3ζ signaling domains. This study aimed to proof feasibility of this approach in vivo addressing the following challenges: (i) T cell-depletion to generate space for cell engraftment in chronic virus carriers is too perilous, (ii) viral antigens circulating in high amounts may inactivate transferred T cells or (iii) trigger uncontrolled immune damage.

Methods: Primary murine CD8+ T cells were isolated, stimulated using an optimized protocol and grafted with CARs by retroviral transduction. A CAR that binds HBV envelope proteins and transmits activation signals to the T cell was compared to a control CAR without a proper signaling domain and a CAR not binding HBV proteins.

Results: CD8+ T cells engineered to express an HBV-specific CAR, which recognizes HBV envelope proteins of various subtypes on infected hepatocytes, were able to engraffe and expand in immune competent HBV transgenic mice. Following adoptive transfer CAR-grafted T cells targeted the liver, remained functional in vivo, rapidly and efficiently controlled HBV replication while causing only transient liver damage. The large amount of circulating viral antigens neither impaired nor over-activated the transferred T cells.

Conclusion: HBV-specific cell therapy with CAR-engineered T cells bears the potential to treat chronic hepatitis B and HBV-associated hepatocellular carcinoma irrespective of the patient’s individual HLA-type.

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CORTICOSTEROIDS AFFECT HEPATITIS C INFECTION BY MODULATING PLASMACYTOID DENDRITIC CELL FUNCTION
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Introduction: Chronic hepatitis C virus (HCV) infection is one of the leading indications for liver transplantation (LTx), but outcomes are often compromised by re-infection of the graft. Several studies have indicated that the use of corticosteroid-based immunosuppression is a risk factor for severe HCV recurrence, but the mechanism for the steroid-mediated effect on HCV is unknown.

Aim: To investigate the effect of steroids on HCV-replication, on the antiviral activity of IFN-α, and on the anti-viral effect of plasmacytoid dendritic cells (pDCs), which are the principal IFN-α-producing cells.

Methods: As a model for HCV replication we used Huh7 hepatoma cells stably transfected with the non-structural coding sequence of HCV directly coupled to a luciferase reporter gene (Huh7-ET). A Huh7 cell line stably transfected with a luciferase gene under the control of an interferon response element (Huh7-ISRE-Luc) was used to investigate effects on IFN-α signal transduction. To study the effect of steroids on inhibition of HCV-replication by pDCs, human pDCs were stimulated with a Toll-Like Receptor (TLR)-7 ligand in the presence or absence of steroids, and conditioned media (pDC-CM) from these cells were added to Huh7-ET cells. In addition, Huh7-ET cells were co-cultured with human pDCs in the presence or absence of steroids.

Results: Dexamethasone and prednisolone did not affect HCV replication directly. IFN-α (10 IU/ml) completely inhibited HCV replication, but steroids did not interfere with the inhibition of HCV-replication by IFN-α. Moreover, steroids had no effect on IFN-α signal transduction as measured in Huh7-ISRE-Luc cells. pDC-CM from TLR-7 stimulated pDCs potently suppressed HCV replication. Interestingly, addition of steroids to PDC during TLR-7 stimulation inhibited IFN-α production and abrogated the antiviral capacity of pDC-CM. Addition of pDCs to Huh7-ET cells significantly reduced HCV replication, and this reduction was almost completely reversed by addition of steroids. Pre-incubation of the Huh7-ET cells with an IFN-α receptor blocking antibody inhibited the antiviral action of pDC-CM.

Conclusion: Corticosteroids do not directly affect HCV-replication and neither interfere with the antiviral action of IFN-α, but inhibit the antiviral capacity of pDCs. Therefore, steroids may promote HCV-recurrence after LTx by suppressing IFN-α production by PDCs.

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INTRAHEPATIC IL-8 PRODUCING CD4+ REGULATORY T CELLS AND FIBROGENESIS IN CHRONIC HEPATITIS C
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Background and Aims: Regulatory CD4+ T cells (Tregs) can suppress T cell functions and thus are considered to potentially alter outcomes of HCV infection. Recently, we detected that Tregs clones from HCV-infected patients produce significant amounts of interleukin 8 (IL-8), which did not interfere with their immunosuppressive function. Here, we analyzed localization and frequency of IL-8+ Tregs in the blood and livers of patients with chronic hepatitis C and studied their effects on fibrogenesis.

Methods: Biopsy and explant livers from 17 patients with chronic hepatitis C were studied by multiple color immunofluorescence histology on cryostat sections. Numbers of IL-8-Foxp3+CD4+ Tregs were determined quantitatively using flow cytometry both in freshly isolated peripheral blood mononuclear cells and transgenic liver specimens. Finally, we analyzed effects of Tregs from hepatitis C on activation of primary human hepatic stellate cells (HSC).

Results: In liver tissue from chronic hepatitis C we detected IL-8-Foxp3+CD4+ T cells in the lymphocytic infiltrates of fibrotic areas at low numbers (median 5 double-positive cells, range 3–15, per 200 μm² visual field) and their position co-localized with that of Foxp3+CD4+ T cells. Overall, percentage of Foxp3+CD25+CD127dimCD4+ Tregs in blood was increased in chronic hepatitis C (4.6±1.4% of CD4+ T cells) as compared to healthy controls (2.8±1.5%; p=0.0087), but we did not find a significant difference in IL-8-Foxp3+CD4+ Tregs between chronic hepatitis C and controls. Of note, simultaneous comparison between blood and liver specimens of patients with chronic hepatitis C demonstrated that IL-8-Foxp3+CD4+ Tregs were markedly enriched in the livers.
Our data demonstrate that in chronic hepatitis C, beta1 and alpha-SMA in resting HSC (p = 0.0005) and their numbers correlated with advanced stages of fibrosis (p = 0.022), but not to inflammation. Finally, in vitro supernatants of Tregs induced mRNA up-regulation of profibrogenic markers TIMP1, MMP2, TGF-beta1 and alpha-SMA in resting HSC (p < 0.05 each).

**Conclusion:** Our data demonstrate that in chronic hepatitis C, Foxp3+CD4+ Tregs are enriched in areas of hepatic fibrosis, exhibit up-regulated IL-8 expression and can activate HSC. Thus, in addition to suppression of inflammation adaptive Tregs in hepatitis C play a dual role as regulators of fibrogenesis.

### 343 HUMAN HEPATOCYTE INDOLEAMINE-2,3-DIOXYGENASE CONTRIBUTES TO ANTIVIRAL DEFENSE AND IMMUNE REGULATION IN HEPATITIS C VIRUS INFECTION

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**Background and Aims:** Chronic hepatitis C virus (HCV) infection is a major cause of liver disease and hepatocellular carcinoma. The mechanisms of viral pathogenesis are only partially understood. Indoleamine-2,3-dioxygenase (IDO) – an enzyme that catabolizes tryptophan – is a central regulator in negative feedback regulation of the immune system in clinically significant conditions, such as tumour-induced immunosuppression. An increased serum kynurenine to tryptophan ratio, which is an index of IDO activity, has been previously demonstrated in patients with chronic HCV infection when compared to patients with resolved HCV infection and healthy individuals. However, the molecular mechanism of IDO induction in HCV infection and its role in viral pathogenesis are unknown.

**Methods:** Using primary human hepatocytes and cell-culture derived HCV JFH1 virus we investigated the effect of HCV infection on IDO expression. Real-time RT-PCR, gene transfection and silencing assays were performed to further identify the underlying molecular mechanisms of hepatic IDO induction. We evaluated the effect of IDO expression on HCV infectivity and the CD4+ T cell response using a hepatocyte co-culture system.

**Results:** We show that HCV infection stimulates IDO expression in hepatocytes. IDO gene induction was transient and dependent on HCV replication and interferon-regulatory factor 1 (IRF1). Overexpression of hepatic IDO impaired HCV replication modestly. Moreover, IDO expression in hepatocytes significantly blocked CD4+ T cell effector function.

**Conclusions:** Hepatic IDO plays a dual and opposing role during HCV infection by retarding viral replication and also regulating host immune responses. The dichotomous nature of IDO in HCV infection may favour HCV persistence within the host over viral eradication. Since IDO-inhibitor drugs have entered phase II clinical trials in cancer patients, the outcome of the ongoing clinical trials may provide valuable information regarding the potential efficacy of these drugs in chronic HCV infection and HCV-induced HCC.

### 344 PERIPHERAL HCV-SPECIFIC CYTOTOXIC RESPONSE DETECTION AT WEEK 12 OF PEGYLATED-INTERFERON alfa-2b PLUS RIBAVIRIN TREATMENT FOR CHRONIC HCV INFECTION CORRELATES WITH SUSTAINED VIROLOGIC RESPONSE DEVELOPMENT

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**Background and Aims:** The second phase of viral load (VL) decay, during Peg-interferon (IFN) plus ribavirin (RBV) treatment for hepatitis C virus (HCV) infection, is probably due to specific immune response. Low HCV VL decrease at week 12 (w12) of treatment has 100% negative predictive value of sustained virologic response (SVR), and this could be related with the absence of HCV-specific cytotoxic T lymphocytes (CTL). The development of SVR after Peg-IFNα2b plus RBV treatment according to the detection of HCV-specific CTL response at w12 was analysed.

**Methods:** A longitudinal cohort study was carried out. 25 HLA-A2+ chronic HCV patients were recruited. Peripheral blood lymphocytes (PBL) were taken at weeks 0 and 12 of treatment. HCV-specific CTL response was tested directly ex-vivo and after specific in-vitro stimulation. HCV-specific CTLs were detected by PBL staining with anti-CD8-Cy5 mAb plus HLA-A2/peptide-PE multimers against two NS3 epitopes and subsequent flow-cytometric analysis. Samples were split into two groups according to the presence of detectable HCV-specific CTLs at w12 (Group 1: detection, Group 2: no detection). SVR rate was compared between both groups and ROC analysis of the ability of w12 HCV-specific CTL detection to predict SVR was carried out.

**Results:** Both groups were similar according to sex, age, basal viral load, HCV-genotype and liver fibrosis. Group 1 and 2 consisted of 14 and 36 samples respectively. SVR was higher in group 1 (93%) than in group 2 (47%) (p = 0.003). In genotype-1 patients, an increase on HCV-specific CTL frequency between base line and w12 of treatment was observed (p = 0.011), but not in group 2. Also HCV-specific CTL proliferation was more frequent in group 1 than in group 2 during treatment (p = 0.025). Detection of HCV-specific CTLs at w12 correlated with the level of HCV viral load decrease between base line and w12 (p = 0.016, r = 0.389). The detection of HCV-specific CTLs at w12 among HCV genotype-1 patients with early virologic response (EVR) had a 100% PPV of SVR. Furthermore, early detection of HCV-specific CTLs at w12 correlated with the development of SVR.

**Conclusion:** In persistent hepatitis C virus (HCV) infection, HCV-specific cytotoxic T cell lymphocytes (CTL) reactivity is impaired and this affects HCV control. Interleukin-7 receptor (CD127) expression on these cells could regulate CTL reactivity through Mcl-1/Bim balance modulation. Bim is a pro-apoptotic molecule blocked by the action of Mcl-1.

**Methods:** Mcl-1/Bim expression and T cell reactivity on HCV-specific CTLs were compared according to CD127 phenotype. Peripheral blood lymphocytes (PBL) from HLA-A2+ HCV+ patients
were obtained. HCV-specific CTLs were visualized by staining PBL with anti-CD8 and HLA-A2/peptide pentameric complexes (pentamer). Mcl-1/Bim/CD127 phenotype of HCV-specific CTLs was tested by staining detectable CD8+/pentamer+ cells with anti-Mcl-1/Bim/CD127 antibodies. HCV-specific CTL proliferation ability after specific in vitro challenge was tested in the presence and absence of pancaspase inhibitor z-VAD-fmk. All stained cells were analysed by flow cytometry.

Results: CD127low-expressing HCV-specific CTLs associated with high HCV viraemia, while CD127high correlated with undetectable viral loads ($p < 0.001$). Directly ex vivo, pentamer+ cell frequency was similar according to CD127 expression level. Nevertheless, CD127low pentamer+ cell proliferation after specific in vitro challenge was impaired ($p < 0.05$), although this was corrected by z-VAD-fmk treatment ($p < 0.05$). Mcl-1 expression was low directly ex vivo ($p < 0.01$), and Bim was up-regulated after antigen encounter ($p < 0.05$) of CD127low pentamer+ cells. The ex-vivo difference between Mcl-1 and Bim expression on pentamer+ cells correlated positively with CD127 expression level ($p < 0.001$) and with pentamer+ cell reactivity ($p < 0.05$).

Conclusions: A low ex vivo Mcl-1 expression and Bim up-regulation after antigen encounter are involved in CD127low HCV-specific CTL hyporeactivity during chronic infection, but it can be overcome by apoptosis blockade.

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COMPRISED FUNCTION OF NATURAL KILLER CELLS IN ACUTE AND CHRONIC VIRAL HEPATITIS

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Background: Natural Killer (NK) cells play an important role in the protection against hepatitis virus infections and are thought to be involved in the pathogenesis of chronic liver disease. Some studies suggested distinct effects of HBV and HCV on NK cells. Here, we aimed to elucidate the phenotype and function of NK cells in acute and chronic viral hepatitis.

Methods: Blood samples from 134 individuals including patients with acute hepatitis B or C, chronic hepatitis B, C or D and healthy blood donors were studied ($n = 13, 25, 12, 18, 34, 32$). Expression levels of activation and inhibitory receptors and multi-functional responses were assessed using 15-color flow cytometry.

Results: The frequency of NK cells was increased in acute and chronic hepatitis C as well as in hepatitis delta compared to healthy controls. Similarly, higher frequencies of CD56dim NK cells were detected in hepatitis patients, with the exception of chronic hepatitis B. Patients with acute hepatitis B and C showed increased frequencies of CD56dim NK cells, with distinct phenotypic changes compared to the responsive chronic infection. CD56dim maturation was not altered in viral hepatitis. This was paralleled by phenotypic changes in the acute compared to the respective chronic infections. CD56dim NK cell maturation was not affected. Multi-functional responses to interferon alpha-stimulation after co-culture with K562 target cells were markedly reduced in all hepatitis patients irrespective of the underlying virus and the phase of infection. Principal component analysis of the generated data, using both clinical and flow cytometry data, revealed a distinct set of parameters separating acute from chronic hepatitis.

Conclusion: Our data suggests a reshaping of the NK cell pool during hepatitis infections, impaired cytolytic activity and reduced cytokine production. In contrast to some previous studies, NK cell alterations were equally observed in HBV, HCV, and HDV infections.

We did nevertheless observe distinct patterns of NK cells phenotype between acute and chronic infections. Thus our data suggests a common mechanism in the alteration of NK cell phenotype and function depending more on disease activity than virus-specific factors.
Conclusions: In contrast to data obtained from cell lines, TLR-induced expression of IFNs and ISGs is higher in patients infected with HCV. This situation could be mimicked in vitro during long-term poly I:C treatment. These findings shed new light on the relevance of TLR3 in the pathogenesis of HCV and may provide a possible explanation for the elevated ISG expression during chronic HCV infection, the so called "IFN-paradox".

348 TOLL-LIKE RECEPTOR 3 STIMULATED PRIMARY HUMAN NON-PARENCHYMAL LIVER CELLS ARE POTENT SUPPRESSORS OF HEPATITIS C VIRUS REPLICATION

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Background and Aims: The role of non-parenchymal liver cells (NPC) as part of the innate immune system in the defense against hepatotropic viruses such as hepatitis C (HCV) is not well understood. Previously, this question has been mostly addressed in murine cells. Therefore, the aim of the study was to characterize the Toll-like receptor (TLR) signaling in primary human NPC.

Methods: NPC were isolated after perfusion and digestion of liver tissue obtained after tumor resections or liver transplantations. Cells were isolated via density centrifugation and MACSbead separation. Cells were stimulated with TLR1–9 agonists for 6h, RNA was extracted and quantitative RT-PCR was performed. HCV-harboring Con1 cells were cocultured with supernatants of NPC stimulated with TLR1–9 agonists for 24h. In addition release of interferons (IFN) and inflammatory cytokines was determined by ELISA.

Results: Kupffer cells (KC; non-HCV n=15; HCV n=7), hepatic stellate cells (HSC; non-HCV n=15; HCV n=10) and liver sinusoidal endothelial cells (LSEC; non-HCV n=15; HCV n=10) showed type-specific elevation of IL-6, TNF-α and IL-10 in response to TLR ligands. KC, LSEC but not HSC from HCV-infected patients showed an elevated IL-6 induction compared to uninfected controls. Supernatants of TLR3-activated KC, LSEC and HSC only contained an antiviral activity against HCV when assessed in the Con1 replicon system. Type I, -II and -III IFN expression was induced in a cell-type dependent manner, with maximum induction of IFN-β and IL-28A in LSEC, whereas IFN-γ was predominantly expressed in poly I:C-activated KC. LSEC isolated from HCV-positive donors showed higher responsiveness to poly I:C, represented by higher expression levels of IFN-β, IL-28A, IL-28B and IL-29 compared to those of uninfected controls. In contrast IFN response in KC and HSC did not significantly differ between these groups. The source of cells, i.e. non-cirrhotic or cirrhotic tissue, had no measurable impact upon any of the results obtained in this study.

Conclusions: Human primary NPC respond to TLR stimulation primarily with induction of pro-inflammatory cytokines in a cell type-specific manner. TLR3 activation of NPC leads to secretion of IFN-β, IFN-γ and λ-interferons, which mediates an antiviral state against hepatotropic viruses such as HCV.

349 DEPLETION OF GUT BACTERIA WITH ANTIBIOTICS PREVENTS CONCANAVALIN (Con) A INDUCED HEPATITIS IN MICE

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Background: Con A administration leads to T cell-mediated hepatitis in mice, which is mediated by the cytokines interferon (IFN)-γ and tumour necrosis factor (TNF)-α. The lipopolysaccharide (LPS)/Toll-like receptor (TLR) 4 signalling pathway is involved in several forms of liver injury. Con A-induced hepatitis is attenuated in mice deficient in TLR4. In the current study, the effect of depleting gut bacteria on Con A-induced hepatitis was investigated. The effect of Con A on gut permeability was also examined.

Methods: Groups of mice (C57BL/6) were treated for 5 days with Polymyxin B (150 mg/kg/day) and Neomycin (450 mg/kg/day) via drinking water. Control mice were given normal drinking water. Following antibiotic treatment, Con A (20 mg/kg i.v.) was administered. Blood and tissue were harvested 8 or 24 hours later. Liver injury was assessed by measuring plasma ALT and histology.

Results: Concentrations of IL-6 were elevated when compared to uninfected controls. In contrast IFN response in KC and LSEC but not HSC from HCV-infected patients showed an elevated IL-6 induction compared to uninfected controls. In contrast IFN response in KC and HSC did not significantly differ between these groups. The source of cells, i.e. non-cirrhotic or cirrhotic tissue, had no measurable impact upon any of the results obtained in this study.

Conclusions: The lipopolysaccharide (LPS)/Toll-like receptor (TLR) 4 signalling pathway is involved in several forms of liver injury. Con A-induced hepatitis is attenuated in mice deficient in TLR4. In the current study, the effect of depleting gut bacteria on Con A-induced hepatitis was investigated. The effect of Con A on gut permeability was also examined.

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increased. In contrast, these changes were less pronounced in IL-6ko-mice. Cytokines—(IL-6, IL-1β) and TNF-α) treatment of rat-hepatocytes showed a downregulation of Fpn-1, Fpn-1a and Fpn-1b and upregulation of hepcidin gene-expression. Moreover, Western-blot analysis of cell lysate of IL-6 treated hepatocytes detected as expected an increase of α2-macroglobulin (positive acute-phase-protein) while albumin (negative acute-phase-protein) and Fpn-1 were downregulated.

Our results demonstrate that liver behaves as a “sponge” for iron under acute phase conditions and Fpn-1 behaves as a negative acute-phase-protein in rat hepatocytes mainly but not exclusively because of the effect of IL-6. These changes could explain iron retention in the cytoplasm and in the nucleus of hepatocytes during APR.

351 COMBINED ADENOVIRUS-MEDIATED microRNAs TARGETING mfgl2, mFas, AND mTNFR1 PROTECT AGAINST FULMINANT HEPATIC FAILURE IN MICE

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Aims: Hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF) has a poor prognosis with a high in-hospital mortality. Hepatic and circulating inflammatory cytokines, such as fibrinogen like protein 2 (fgl2), Fasl/Fas, and tumor necrosis factor α (TNFα)/TNFR1, play a significant role in the pathophysiology of ACLF. This study aimed to evaluate the therapeutic effect of recombiant adenoviral vectors carrying microRNAs (miRNAs) targeting mouse fgl2 (mfgl2) or both mFas and mTNFR1 on murine hepatitis virus-3-induced fulminant hepatitis in BALB/cj mice.

Methods: miRNA expression plasmids against mfgl2, mFas, and mTNFR1 were constructed, and their inhibitory effect on the target genes was confirmed in vitro at both the mRNA level by qRT-PCR and protein level by Western blot analysis. pcDNA6.2-mFas-mTNFR1-mRNA was constructed, expressing miRNAs against both mFas and mTNFR1. To construct a miRNA adenovirus expression vector against mfgl2, pcDNA6.2-mfgl2-mRNA was cloned using Gateway technology. Ad-mFas-mTNFR1-mRNA was also constructed.

Results: After the adenovirus vectors were delivered by tail-vein injection, 8 of 18 mice (44.4%) recovered from fulminant viral hepatitis in the combined interference group compared with 7 of 18 (38.9%) receiving Ad-mfgl2-miRNA treatment and 3 of 18 (16.7%) mice receiving Ad-mFas-mTNFR1-miRNA. These adenovirus vectors significantly ameliorated inflammatory infiltration, fibrin deposition, hepatocyte necrosis and apoptosis, and prolonged survival time.

Conclusions: Our data illustrated that combined interference using miRNAs targeting mfgl2, mFas, and mTNFR1 might have significant therapeutic potential for the treatment of fulminant hepatitis.

Acknowledgments: This work was supported by the Innovation Team Development Plan of the Ministry of Education of China (IRT1131) and the National Science Youth Fund of China (NSFC30700702).

352 ADENOSINE SWITCHES (LIVER) MACROPHAGES TOWARDS A FIBROLYTIC PHENOTYPE

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Background and Aims: Macrophages play an important role in in tissue remodeling, including liver fibrosis, via expression of different matrix metalloproteinases (MMPs). The spectrum of MMPs appears to depend on macrophage polarization. M1 macrophages, induced e.g. by toll like receptor ligands and IFNgamma, express proinflammatory cytokines like IL-6 and markers like iNOS and CD11c. M2 macrophages, which have been associated with fibrogenesis, are induced by IL-4 and IL-13, and are characterized by the expression of Arg1 and Mr1. Adenosine has been implicated as an inducer of M2 polarization. We therefore studied the role of adenosine as a modulator of the M1-M2 transition and as a modulator of potentially fibrolytic MMPs.

Methods: Mouse RAW and TPA-activated human U937 macrophages were stimulated with LPS and IFNgamma to induce M1, and with IL-4 and IL-13 to induce M2 polarization. 4 hrs after stimulation, adenosine was added to culture medium for 24 hrs. When expression of macrophage markers and MMPs was quantified by qPCR and ELISA.

Results: Induced M1 and M2 polarization were confirmed by the expression of IL-6 and Arg1, respectively. In M1-polarized cells, adenosine decreased expression of CCL3, but increased CCL3 expression in M2-polarized cells. Putatively fibrolytic MMP-9 was upregulated along with CCL3, while profibrogenic TIMP-1 was increased 6hr, but down-regulated at 24hrs. Notably, adenosine increased TIMP-1 both in M1- or M2-polarized cells. Adenosine strongly induced MMP-1, MMP-8, MMP-9 and MMP-18 in M1-polarized cells, but markedly suppressed MMP-1 in M2-polarized cells.

Conclusions: Expression of potentially fibrolytic MMPs was largely downregulated, while TIMP-1 mostly upregulated along with M2-polarization of (liver) macrophages upon treatment with adenosine. The involved adenosine receptors are currently under investigation. Adenosine receptor antagonists may be attractive agents to induce macrophage switching towards a fibrolytic phenotype.

353 RNA SEQUENCING ANALYSIS OF HUMAN LIVER SINUSOIDAL ENDOTHELIAL CELLS REVEALS EVIDENCE FOR AN ANTI-INFLAMMATORY ROLE DURING HCV INFECTION

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Background: Liver sinusoidal endothelial cells (LSECs) due to their extraordinary scavenger activity are likely to play critical roles in the uptake of hepatic viral pathogens. They can act as antigen-presenting cells, inducing either inflammation or tolerance under different conditions. While these processes are particularly relevant to HCV infection, the role of LSECs in chronic hepatitis C is not defined.

Aim: To apply systems biology approaches to evaluate the role of LSECs in HCV infection and pathogenesis.

Methods: Poly(A)+ RNAs from HCV, MOCK or LPS treated human LSEC and Huh7.5 cells were analyzed by RNA-sequencing (Illumina). Cell transcriptomes were compared to mild and severe hepatitis C liver biospecimens. Results: We identified distinct gene expression profiles in HCV infected LSECs and hepatocytes, suggesting different roles during HCV infection. Unlike prior RNA-seq analysis of macrophages that demonstrated a broad increase in IL1-b and NFκB-responsive proinflammatory cytokines and chemokines, HCV did not induce a marked increase in such inflammatory signals in both LSECs and Huh7.5 cells. In contrast, HCV in general induced a downregulation of inflammatory signals in LSECs. LSECs overall displayed 754, 245 and 2543 DEGs at 8, 24 and 48 hours after HCV exposure. This study aimed to evaluate the therapeutic effect of recombinant adenoviral vectors carrying microRNAs (miRNAs) targeting mouse fgl2 (mfgl2) or both mFas and mTNFR1 on murine hepatitis virus-3-induced fulminant hepatitis in BALB/cj mice.

Methods: miRNA expression plasmids against mfgl2, mFas, and mTNFR1 were constructed, and their inhibitory effect on the target genes was confirmed in vitro at both the mRNA level by qRT-PCR and protein level by Western blot analysis. pcDNA6.2-mFas-mTNFR1-mRNA was constructed, expressing miRNAs against both mFas and mTNFR1. To construct a miRNA adenovirus expression vector against mfgl2, pcDNA6.2-mfgl2-mRNA was cloned using Gateway technology. Ad-mFas-mTNFR1-mRNA was also constructed.

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biological functions (ACP5 9x, A2M 6x, SERPING1 7x) including TGFβ. Gene pathway analysis highlighted changes associated with angiogenesis (40), adhesion (142), ECM-organization (107), cell proliferation (153), signaling (153), apoptosis (109) and regulation of defense/immune responses (104). Furthermore, analyses of genes commonly expressed in LSECs exposed to HCV with HCV infected liver showed significant overlap of these pathways.

**Conclusions:** This is the first comprehensive gene expression analysis of LSECs that provided insight into the broad portrait of genomic changes associated with the LSEC response to HCV and evidence that LSECs downregulate inflammation during HCV infection. Defining the mechanisms of HCV-LSEC interactions will facilitate the understanding of HCV induced liver disease and the role of LSECs in other inflammatory liver diseases.

354 **PREVALENCE OF AND RISK FACTORS FOR COMBINED cART ASSOCIATED DRUG-INDUCED LIVER INJURY IN HIV/HBV COINFECTED, HIV/HVC COINFECTED, AND HIV MONONINFECTED PATIENTS**

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**Objectives:** Evaluation of the incidence/prevalence of and risk factors for combined antiretroviral therapy (cART) associated drug-induced liver injury in HIV/HBV (HIV/HBV) coinfected, HIV/HCV coinfect (HIV/HCV), and HIV monoinfected patients (HIV).

**Methods:** A total of 267 randomly selected, cART-naïve, HIV/HBV (n=90), HIV/HCV (n=90) and HIV (n=87), who received their first cART at the Medical University of Vienna, were included into this retrospective study. Patients' medical history was searched for the availability of laboratory data from defined time points: Baseline (BL), first laboratory data after the initiation of cART, month3, month6, month12, month24, and month36. DILI was defined according to the DILI Expert Working Group 2011 criteria, while AIDS Clinical Trials Group 1996 criteria was used for severity grading. Significant liver fibrosis was defined as AST to platelet ratio (APRI) >1.5 (AST/[upper normal limit (UNL) × platelet count ×10^{-7}]).

**Results:** 37 out of 267 patients displayed at least one episode of DILI. DILI was observed in 62 (6.1%) visits, while grade3/4 DILI were observed in 14 (1.3%) out of 1015 visits. The incidence/prevalence of DILI varied significantly throughout the infection groups (HIV/HCV: 8.4% vs. HIV/HCV: 6% vs. HIV: 3.4%; p=0.024). Female patients (11.5% vs. 4.2%; p<0.001), and patients with significant liver fibrosis (12% vs. 5.8%; p<0.018) displayed a higher Incidence/prevalence of DILI. The incidence/prevalence of DILI was comparable between different time episodes of cART initiation (before 2000: 5.7% vs. 2000–2005: 6.7% vs. after 2005: 6.1%; p=0.918). A significant correlation between the number of DILI episodes and infection group (r =−0.123; p=0.017), gender (r =0.143; p=0.006), BL CD4+ cell count (r =−0.143; p=0.02), BL AP UNL (r =0.355; p<0.001), as well as BL GGT UNL (r =0.213; p=0.002) was observed. Grade3/4 DILI was more frequently observed in patients with advanced liver fibrosis (5.6% vs. 0.9%; p=0.001).

**Conclusions:** Our findings suggest, that DILI is still a concern in current cART regimens as the incidence/prevalence did not decrease over time. The negative correlation between incidence/prevalence of DILI and BL CD4+ cell count might be attributed to the immune reconstitution syndrome. We were able to identify risk factors for DILI and thereby isolate groups of patients who may profit from closer monitoring. Although grade 3/4 DILI was rare, the long term significance of DILI in regards to it’s possible effect on cART efficacy and development of liver fibrosis/cirrhosis should be investigated.

355 **ENHANCED INTERFERON ALPHA-DEPENDENT TRAIL EXPRESSION BUT COMPRISED CYTOKINE PRODUCTION OF NK CELLS IN CHRONIC HEPATITIS C IN RELATION TO THE IL28B GENOTYPE**

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**Background:** NK cells contribute both to clearance of acute hepatitis C virus (HCV) infection as well as to regulation of liver injury in chronic hepatitis. Interferon alpha-induced expression of TRAIL on NK cells correlates with HCV-RNA decline during therapy (Gastroenterology 2010; 138:1865–1897). However, the role of the IL28B genotype in type-I interferon-dependent regulation of NK cell function is unknown.

**Methods:** NK cells from healthy controls (n=23) and patients with chronic hepatitis C (n=19) were studied in vitro after stimulation with a wide range of concentrations of interferon alpha or IL-2. Upregulation of TRAIL and CD107a as well intracellular cytokine production were investigated by flow-cytometry after coculturing with K562 or Huh7.5 cells. The IL28B SNP rs12979860 was determined by melting curve analysis.

**Results:** NK cells of patients with chronic hepatitis C showed a marked polarization towards cytotoxicity with significantly higher upregulation of TRAIL on both CD56^{high} and CD56^{dim} NK cells after stimulation with rather low concentrations of IFNα (0.5 ng/ml). However, cytokine production was more pronounced in healthy controls as only high doses of IFNα (100 ng/ml) were able to induce cytokines in NK cells from patients. In line with these observations, NK cells from hepatitis C patients had significantly lower expression of Nkp30 and NKG2A but were more frequently NKG2D-positive. Of note, cytokine production but not TRAIL expression in healthy controls was higher in individuals carrying the IL28B-CC genotype. In contrast, IL28B-non-CC hepatitis C patients showed higher interferon-gamma but not TNFa or CD107a expression than IL28B-CC patients. The percentage of interferon-gamma-positive NK cells after IFNα-stimulation correlated with ALT levels only in IL28B-CC genotype (r =0.83) but not in IL28B-CC patients.

**Discussion:** NK cell function in response to type-I interferon stimulation is reshaped in chronic hepatitis C towards a cytotoxic phenotype. Improved cytokine production of NK cells in IL28B-CC healthy controls could be a correlate for a better early defence supporting HCV clearance in acute hepatitis C. However once chronic infection has been established, the increased activity of NK cells from IL28B-CC individuals may contribute to the activity of liver disease.

356 **IgG4 IMMUNOSTAINING IN LIVER ALLOGRAFTS OF HEPATITIS C INFECTED PATIENTS WITH PLASMA CELL HEPATITIS – A POTENTIAL SURROGATE MARKER OF ALTERED AUTOIMMUNE PHENOMENA**

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**Aims:** Posttransplant plasma cell hepatitis (PTPCH) has been described in both pediatric and adult liver transplantation (LT) recipients for various liver diseases. More recently, PTPCH was
described in the setting of recurrent hepatitis C virus (HCV) infection, and associated with worse fibrosis progression and a negative impact on patient outcome. While some suggested that PTPCH was a subtype of recurrent HCV mimicking autoimmune hepatitis (AIH), others concluded that PTPCH was a histologic variant of rejection. Nevertheless, the immunological mechanisms leading to its development remain unknown.

Methods: A search was conducted in our pathology database (2001–2012). Liver biopsies of 3 groups of patients were identified: 21 cases of PTPCH in patients with HCV (M=15; F=6; mean age 58±8.5 yrs), 18 cases of PTPCH for liver diseases other than HCV (M=4; F=14; mean age 24.6±16 yrs), and 20 cases of AIH (M=5; F=15, mean age 44.9±18.4 yrs). Histological assessment was performed with H&E and IgG4 immunohistochemical staining. Disease activity grading was based on portal inflammation, interface hepatitis, perivenular inflammation/necrosis and lobular necroinflammation. The severity of plasma cell infiltrate was semiquantitated and the presence of bile duct damage was noted. The highest number of IgG4+ plasma cells per x40 field in portal areas was recorded. The presence of AIH-related auto-antibodies and elevated IgG or γ-globulin in the serum was documented.

Results: The average number of IgG4+ plasma cells was 27 (0–150) in the PTPCH-HCV+ group compared to 9.69 (0–78) and 6.25 (1–20.5) in the PTPCH-HCV− and AIH patients, respectively (p=0.0503). When a cutoff of 25 IgG4+ plasma cells was chosen, 70% of 21 cases were positive (33.3%) in PTPCH-HCV+ group vs. only 1 of 18 cases (5.5%) in PTPCH-HCV− group and none (0/20) in AIH patients. There was no significant difference in the other histological or serological parameters among the groups.

Conclusions: IgG4-positive plasma cells seem to be increased primarily in HCV-related plasma cell hepatitis. They may serve as a potential surrogate marker of altered autoimmune phenomena. Further research is needed to elucidate the pathophysiologic relevance of this findings.

357 INFECTER GAMMA PROMOTES LYMPHOCYTE TRANSMIGRATION VIA THE TRANSCELLULAR ROUTE ACROSS HUMAN HEPATIC SINUSOIDAL ENDOTHELIAL CELLS

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Introduction: Lymphocyte infiltration of liver tissue is a hallmark of all inflammatory liver diseases and drives the progression of liver fibrosis. Leucocyte trafficking occurs within the low shear environment of the hepatic sinusoids which are lined by hepatic sinusoidal endothelial cells (HSEC). We have recently reported that leucocytes can migrate across this vascular bed via a novel transcellular route but the mechanisms underlying this process are poorly understood.

Aims: To elucidate the mediators of transcellular migration across HSEC.

Methods: Human liver tissue was obtained from explanted livers or margins of hepatic resections from patients undergoing transplantation or surgery at the Queen Elizabeth Hospital Birmingham. Primary HSEC were isolated using density gradient centrifugation and immunomagnetic selection. Lymphocytes were isolated from whole blood and perfused over cytokine stimulated monolayers of HSEC in a flow based adhesion assay. Lymphocytes were visualized adhering and transmigrating across HSEC by phase contrast microscopy. Endothelial monolayers were then fixed and immunofluorescently labeled before visualisation with confocal microscopy.

Results: Using confocal microscopy lymphocytes were visualized transmigrating directly through endothelial cells (transcellular) and between endothelial junctions (paracellular). With tumour necrosis factor alpha stimulation the majority of lymphocytes migrated via the paracellular route (59% of transmigrating cells via the paracellular route and 41% via the transcellular route). With the addition of interferon gamma stimulation this led to a change in the route taken by lymphocytes with the majority migrating via the transcellular route (38% of transmigrating cells via the paracellular route and 62% via the transcellular route). Chemokine receptor blockade with pertussis toxin led to a 50% reduction of transcellular migration. Transcellular migration did not involve junctional adhesion molecules such as JAM-A orZO-1 but did alter actin distribution.

Conclusion: The proportion of lymphocytes transmigrating via the transcellular route across HSEC is much higher than that reported in other endothelial cells. This process appears to be promoted by interferon gamma and is partially mediated by chemokines as well as HSEC cytoskeletal changes. These findings highlight the unique nature of the hepatic sinusoids and their potential as a target for therapy in chronic inflammatory liver disease.

358 EFFECT OF N ACETYLGLYCINE (NAC) IN HYPOXIA INDUCED LIVER INJURY (HILI) – A RANDOMIZED PLACEBO CONTROL CLINICAL TRIAL

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Objectives: HILI is common with a prevalence of 10% in US. Transient shift of intra hepatic hemodynamic compromise leads to tissue hypoxia and induces hypoxia induced protein (HIP), heat shock protein 70 (HSP24,70), Endothelial reticular stress (ER) leading to reperfusion injury (RI). Dramatic rise of transaminases, drastic reversal with restoration of perfusion in weeks follows. In cirrhosis HILI requires liver transplantation. This study evaluated spontaneous recovery and salvage in HILI utilizing NAC.

Methods: Sixty patients (n=60) with mean arterial pressure (MAP) <35% and normal LFTs at base line. Group A (n=28) chronic liver disease (CLD) [alcohol-11/28 (39%), NASH-9/28 (32%), Hepatitis C-4/28 (14%), hepatitis B-2/28 (7%), PBC-1/28 (3%), AIH-1/28 (3%). Group B (n=32) [respiratory failure-12/32 (37%), CHF-8/32 (25%), CVA-2/32 (6%), sepsis-6/32 (19%), post op-4/32 (12%)]. Randomized into Placebo group – A1 (14) & B1 (16) and IV NAC for 48 hours – A2 (14) & B2 (16). Serum Transaminases, Bilirubin, INR, Creatinine and MELD score at 0, 3rd, 6th, 9th and 12th days with MAP and modified Sequential Organ Failure Assessment (SOFA) Score. All patients were allowed standard of care (SOC) and resuscitations if needed.

Exclusions: Organ transplant, Septic shock, Hemodialysis, cancer, acute myocardial Infarct, Tylenol injury, acute viral hepatitis and organ trauma.

Results: Placebo groups A1, B1: Normalized A1-LFTs – on 3rd day-(7%), 6th day-(21%), 9th day-(36%) and 12th day-(21%). 1/14 (7%) died. B1[CLD] LFTs 3rd day-(19%) 6th day-(44%) 9 th (25%), 2/16 (6%) died of sepsis] NAC Groups A2[normalized LFTs 3rd (57%) 6th day-(43%) 9 th day (25%), (7%) one died] B2 [CLD] [Normalized LFTs – 3rd day-(63%), 6th day-(25%) 9 th/16 (6%), one died].

Conclusion: This Study postulates that IV NAC (A2, B2) has efficient spontaneous recovery and salvage in non-CLD sub group B2 (63%) >A2 (57%) in day 3, in CLD NAC (A2) >placebo (A1) clinical recovery over placebo at 3rd day, (44%) over (36%) – 6th day. Larger trial need to establish the routine usage of IV NAC in HILI.
HLA FOOTPRINTS IN HIV/HCV CO-INFECTED PATIENTS

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Background and Aims: HLA-diversity is a major host genetic risk factor in infectious diseases. In particular, several studies have described associations between distinct HLA alleles and outcomes of HCV and HIV infection. The objective of this study was to analyse the distribution of HLA Class I alleles in HIV/HCV co-infected individuals in order to identify HLA-disease associations that reflect the impact of this dual infection.

Methods: Clinical data from 1110 Caucasian patients (HCV: n = 477, HIV: n = 181, HIV and HCV: n = 346, healthy controls: n = 106) were retrieved by retrospective chart review. HLA-A and B allele distribution were determined by molecular methods (SSO and SSP) with HLA-resolution reduced to the 2-digit level to enable uniform comparisons. Fisher’s exact test and Chi-square tests with Yates correction for multiple testing were applied for statistical analysis.

Results: Patients with HIV mono-infection were significantly older than patients in each other group (p < 0.001). MSM was the predominant risk factor for HIV infection (52%), while most HIV/HCV and HCV-infected patients had been infected via blood exposure (83%). The frequency of HLA alleles HLA-A*30 (p = 0.023), HLA-B*08 (p = 0.003), HLA-B*39 (p = 0.003), and HLA-B*49 (p = 0.032) did not differ between the patient groups but was significantly reduced in infected patients as compared to healthy controls irrespective from the underlying type of infection. In contrast, HLA-B*57, which is assumed to increase chances of spontaneous HCV resolution and delay HCV progression, was selectively increased in patients with HIV mono-infection (13%) as compared to each other group (healthy controls: 9%, HIV/HCV and HCV mono-infection: 6% each; p = 0.022).

Conclusion: Reduced detection rates in HIV- or HCV-mono- and HIV/HCV co-infected patients indicate that alleles HLA-A*30, HLA-B*08, HLA-B*39, and HLA-B*49 carry a poor prognosis but do not reflect interactions between HIV- and HCV-infections. Patients with blood-borne infections usually acquire HCV first and HIV later on. Thus, finding a high HLA-B57 carrier rate in HIV+ patients but in none of the other groups indicates a protective role of this allele for both HIV and HCV infection. Taken together long-term exposure of Caucasian patients to HIV and HCV infection seems to induce subtle footprints in their HLA class I repertoire.

CXCL10 GENE POLYMORPHISM PREDICTS RAPID VIROLOGIC RESPONSE TO PEGYLATED INTERFERON/RIBAVIRIN COMBINATION THERAPY IN ASIAN PATIENTS WITH DIFFICULT-TO-TREAT CHRONIC HEPATITIS C GENOTYPE

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Background and Aims: Interferon-gamma inducible protein 10 kDa (IP-10 or CXCL10) is a CXC chemokine that plays an important role of host immune response against hepatitis C virus (HCV) infection. Pretreatment serum IP-10 level correlated with HCV treatment outcome. Recent data suggested that G-201A polymorphism in the CXCL10 promoter region altered function, regulated CXCL10 expression, and were associated with susceptibility to disease progression in chronic hepatitis B carrier. This study aimed to determine the association of CXCL10-201 polymorphisms and treatment response with pegylated-interferon based regimen in Asian patients with chronic hepatitis C (CHC).

Methods: 227 Asian patients with CHC treated with pegylated interferon-alpha and ribavirin were genotyped for the G-201A polymorphism of CXCL10 gene. The definitions of rapid viral response (RVR), early viral response (EVR), end of treatment viral response (EOT) and sustained viral response (SVR) were made according to the European Association For The Study of The Liver (EASL) clinical practice guideline.

Results: Of these, 185 patients achieved SVR. Eighty patients (35.2%) were genotype 1 or 4. The SVR rates of GG, GA and AA genotypes were 80.3%, 86.1% and 100% (p = 0.51) for all HCV genotype, and 55.6%, 63.3% and 73.3%, p = 0.43, respectively for HCV genotype 1/4. There were no differences in age, sex, body mass index, alcohol consumption, baseline HCV RNA viral load and advanced fibrosis between HCV genotype 1/4 infected patients with RVR and non-RVR. RVR rate was not different between non-GG and GG genotype (84.0% vs 69.8%, p = 0.22). However, in subgroup of HCV genotype 1/4, patients with CXCL10 non-GG genotype achieved higher RVR rate than GG genotype (88.9% vs 44.9%, Odd ratio=9.8, p = 0.02, 95% CI 1.13–84.60) (Figure 1). In addition, these polymorphisms were not associated with EVR and EOT.

Conclusions: In patients with chronic hepatitis C genotype 1 or 4, CXCL10 –201, GG genotype was a strong predictor for RVR.

REGULATORY T-CELLS DIFFERENTIALLY REGULATE PROLIFERATION AND CYTOKINE PRODUCTION OF HCC-SPECIFIC CD8+ T-CELLS

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Background and Aims: Despite the presence of IFN-γ-producing tumor antigen (TA)-specific CD8+ T-cells in hepatocellular carcinoma (HCC) lesions and their association with patient survival, we and others have failed to specifically expand these cells in vitro. This is probably due to functional alterations of the CD8+ T-cells, e.g. an impaired proliferative capacity. Several mechanisms have been suggested to contribute to this TA-specific CD8+ T-cell failure, including the expression of inhibitory receptors or the action of regulatory T-cells (Treg). In this study, we used TA-specific tetramers to analyze the presence of TA-specific CD8+ T-cells independent of their functionality and to determine their inhibition by Treg.

Figure 1: Association of CXCL10 –201 promoter polymorphisms.
Methods: TA-specific CD8+ T-cell lines were generated by peptide-specific stimulation of lymphocytes from HCC patients in the presence or absence of CD25+ cells. Epitopes derived from the TAs NY-ESO-1 and MAGE-A1 were used for the analysis of T-cell frequency (tetramer) and functionality (production of IFN-γ) by flow cytometry. Additionally, the presence of Treg (CD4+CD25+Foxp3+ cells) was analyzed in blood, liver and tumor tissue of HCC patients as well as in blood and liver of HCC-negative controls.

Results: As expected, we were unable to detect TA-specific production of IFN-γ after specific expansion of patient-derived CD8+ T-cells. Strikingly, however, despite the lack of IFN-γ producing TA-specific CD8+ T-cells, TA-tetramer binding cells were readily detectable in 20 of 37 patients (54%). In contrast, virus-specific tetramer-positive CD8+ T-cell lines were capable of producing IFN-γ. Depletion of Treg prior to culture increased the proliferation of TA-specific CD8+ T-cells. Importantly, however, it did not restore functionality of TA-specific CD8+ T-cells suggesting that Treg inhibit proliferation but not cytokine production of TA-specific CD8+ T-cells. The biological role of Treg in this setting is supported by their intratumoral enrichment in patients with HCC (p<0.0001 by 1-way-ANOVA).

Conclusions: TA-specific CD8+ T-cells obtained from patients with HCC are severely impaired in functionality although still capable of proliferation. The depletion of Treg enhanced the proliferation of these cells, but did not allow TA-specific CD8+ T-cells to regain effector functions. This suggests that additional mechanisms beside proliferation but not cytokine production of TA-specific CD8+ T-cells. Strikingly, however, despite the lack of IFN-γ production by TA-specific CD8+ T-cells, IFN-γ production was observed in 20 of 37 patients (54%).

Therefore, CD8+ T cells seem to be essential for establishing an efficient virus specific CD8+ T cell response and for clearance of acute LCMV-infection in the liver.

363 THE PRO-INFLAMMATORY ROLE OF GALECTIN-3 IN ACUTE LIVER INJURY

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Background and Aims: We used Concanavalin A (Con A) and α-galactosylceramide (αGalCer) induced liver injury, well established murine models of T/NKT cell mediated hepatitis, to study the role of Galectin 3 (Gal-3) in acute liver pathology.

Methods: We tested susceptibility to Con A and αGalCer-induced hepatitis in galectin-3-deficient (Gal-3−/−) mice and wild-type (WT) C57BL/6 mice, as evaluated by liver enzyme test, histology, cytokine production, intracellular staining of immune cells and percentage of apoptotic mononuclear cell (MNC) in the liver.

Results: Gal-3−/− mice were less sensitive to both Con A and αGalCer-induced hepatitis. The level of tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and interleukin (IL)-17 and -4 in the sera and the number of TNFα-, IFNγ-, IL-17- and -4-producing CD8+ T cells as well as IL-12-producing CD11c+ DCs were lower, whereas the number of IL-10-producing CD4+ T cells and F4/80+ macrophages were significantly higher in livers of Con A treated Gal-3−/− mice compared to WT mice. Significantly higher percentages of late apoptotic Annexin V+ propidium-iodide+ liver-infiltrating MNCs and splenocytes were observed in Con A treated Gal-3−/− mice, compared to WT mice.

The injection of αGalCer induced significantly higher expression of Gal-3 on CD3+ NK11+NKT cells, DCs and NK11+CD11c+ NKDCs in the liver of WT mice. Significantly lower number of CXCR3+ NKT and DCs was noticed in livers of αGalCer-treated Gal-3−/− mice, compared to WT mice. The level of IL-10 in the sera and percentage of IL-10-producing CD3+ NK11+ and CD4+ NK11−CD11c+ cells were significantly higher in αGalCer-treated Gal-3−/− mice. Percentage of liver infiltrating DCs, CD11c+ DCs and TNFα-, IFNγ-, and IL-12-producing DCs was significantly lower in αGalCer-treated Gal-3−/− mice. In vitro, αGalCer-loaded DCs, isolated from livers of untreated Gal-3−/− mice, produced significantly higher amounts of IL-10 and IL-4 and significantly lower amounts of IFNγ compared to DCs from WT mice.

Conclusions: Gal-3 plays an important pro-inflammatory role in acute hepatitis by promoting the activation and migration of T lymphocytes, NKT cells and DCs, secretion of proinflammatory cytokines and apoptosis of MNCs in the liver.

Supported by grants 175069 and 175103 from the Serbian Ministry of Education and Science.

364 MODULATION OF LIVER FIBROSIS BY REGULATING ALTERNATIVELY ACTIVATED MACROPHAGE SIGNALING THROUGH IL-4RAlpha

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Background and Aims: Liver fibrosis progression and regression are modulated by cells of the innate immune system, especially macrophages. Macrophages can be divided roughly into classically activated and alternatively activated macrophages (CAM and AAM, resp.). While AAM have been implicated in fibrogenesis, the role of CAM and AAM as modulators of liver fibrosis is largely unexplored.

The role of CD4+ T cells in acute viral hepatitis is not clear. To evaluate their function, the kinetic of Lymphocytic Th1 and Th2 cells were determined. For this purpose, C57BL/6 wt mice were treated with CD4+ T cell depleting antibody before the infection.

The injection of αGalCer induced significantly higher expression of Gal-3 on CD3+ NK11+NKT cells, DCs and NK11+CD11c+ NKDCs in the liver of WT mice. Significantly lower number of CXCR3+ NKT and DCs was noticed in livers of αGalCer-treated Gal-3−/− mice, compared to WT mice. The level of IL-10 in the sera and percentage of IL-10-producing CD3+ NK11+ and CD4+ NK11−CD11c+ cells were significantly higher in αGalCer-treated Gal-3−/− mice. Percentage of liver infiltrating DCs, CD11c+ DCs and TNFα-, IFNγ-, and IL-12-producing DCs was significantly lower in αGalCer-treated Gal-3−/− mice. In vitro, αGalCer-loaded DCs, isolated from livers of untreated Gal-3−/− mice, produced significantly higher amounts of IL-10 and IL-4 and significantly lower amounts of IFNγ compared to DCs from WT mice.

Conclusions: Gal-3 plays an important pro-inflammatory role in acute hepatitis by promoting the activation and migration of T lymphocytes, NKT cells and DCs, secretion of proinflammatory cytokines and apoptosis of MNCs in the liver.

Supported by grants 175069 and 175103 from the Serbian Ministry of Education and Science.
Methods: Expression patterns of markers related to CAM and AAM were investigated in mice with CCL4-induced liver fibrosis and spontaneous biliary (Mdr2 KO). For functional studies, AAM were inhibited with a blocking IL-4Ralpha antisense oligonucleotide (ASO) in vitro (murine RAW macrophages) and in vivo (6 weeks CCL4-treated and Mdr2 KO mice). Fibrosis and fibrogenesis were measured by collagen quantification, qPCR and immunohistochemistry for inflammation and fibrosis related transcripts.

Results: In the CCL4 model, IL-4Ralpha expression was strongly reduced 2 weeks after the last dose. In Mdr2 KO mice, expression of the AAM inducing receptors IL-4Ralpha gradually increased until age 6-wk, and decreased thereafter. In RAW macrophages the ASO significantly suppressed IL-4Ralpha. When given to CCL4-treated mice twice weekly from week-2 to week-4 after gavage, the ASO suppressed IL-4Ralpha and increased the CAM markers CCL3, MMP-8 and MMP-9, and decreased expression of profibrogenic procollagen alpha1(I) and the AAM markers Arg1 and Mrc1. Mdr2 KO mice that received ASO from week 6 to week 10 of age showed a similar shift from AAM to CAM. These mice displayed a mild increase in ALT due to AAM suppression.

Conclusions: Expression of profibrogenic molecules and fibrogenesis are paralleled by increased hepatic AAM and concomitant IL-4Ralpha expression, suggesting a significant contribution of AAM to hepatic fibrogenesis. Modulation of AAM macrophages by specific pharmacological intervention is a promising antifibrotic approach to inhibit fibrosis progression and induce its reversal.

365 IDENTIFICATION OF PEKIN DUCK INTERFERON LAMBDA-3 AND INITIAL ASSESSMENT OF ITS ANTIVIRAL EFFECTS IN THE DUCK HEPATITIS B MODEL

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Introduction: Interferon lambda-3 (IFN-lambda;3), also known as interleukin-28B (IL-28B), belongs to the recently discovered type III interferons and IL-10 family of cytokines. Type III interferons induce antiviral responses similar to type I interferons but signal through a different heterodimeric receptor complex (IFN-lambda;3/IL10R2). IFN-lambda;3 inhibits replication of many viruses, especially those affecting epithelial cells of the respiratory and gastrointestinal tracts and the liver. The aim of this study was to identify and characterize duck IFN-lambda;3 (duIFN-lambda;3) to study its role in the immunopathogenesis of duck hepatitis B virus (DHBV) infection.

Methods: The duIFN-lambda;3 cDNA was obtained by RT-PCR and RACE. A homology model of duIFN-lambda;3 was constructed based on the human IFN-lambda;3 structure. A eukaryotic expression vector was generated for expression of a C-terminal-His-tagged IFN-lambda;3 protein and culture supernatants of transfected 293T cells were assessed by immunoblot using an anti-His antibody. Primary duck hepatocyte cultures generated from two-week-old DHBV-negative ducklings were infected with DHBV at a MOI of 75 for 24 hours and treated with recombinant duIFN-lambda;3 or control-treated. Intracellular virus (ICV) and 2′,5′-oligoadenylate synthetase-like (OASL) mRNA expression were assessed by real-time PCR/RT-PCR.

Results: The predicted 185 amino acid protein had an amino acid identity of 63% and 37% with chicken and human IFN-lambda;3 proteins, respectively. The duIFN-lambda;3 structure by homology modeling was similar to that of human IFN-lambda;3. Mapping the duIFN-lambda;3 cDNA with duck genomic sequences revealed a five exon-four intron gene structure similar to that of chicken and human IFN-lambda;3 genes. Recombinant duIFN-lambda;3 up-regulated OASL mRNA expression 100-fold by 24 hours but had only a modest effect on ICV after 96 hours of treatment.

Conclusion: Our observations suggest evolutionary conservation of genomic organization and structural features implicated in receptor binding of IFN-lambda;3 and demonstrated bioactivity of the expressed duIFN-lambda;3 protein. The identification and expression of duIFN-lambda;3 will allow the study of the role of type III interferon in the immunopathogenesis of DHBV infection and may facilitate the exploration of novel immunotherapeutic strategies in the duck hepatitis B infection model.
The subset of gd T cells, especially Vd2 gd T cells, plays a critical role in protective immune responses against cancer and viral infections; however, their role in chronic hepatitis B virus infection remains unclear. Here we characterized peripheral and intrahepatic gd T cells, and analyzed their association with liver damage in a cohort of HBV-infected patients, including 64 immune activated (IA) patients, 22 immune tolerant (IT) carriers and 30 healthy controls (HCs). We demonstrated that the numbers and percentages of peripheral and hepatic Vd2 T cells were significantly lower in IA patients than that in HC and IT subjects, and the frequency of Vd2 T cells was reversely correlated with liver damage, but not with HBV DNA load. Vd2 T cells in IA patients exhibited an impaired proliferative capacity, due to abnormalities in the G2/M cell cycle phases and a skewed chemotactic ability, which contributed to the lower number of these cells in the liver. Finally, an in vitro co-culture assay showed that Vd2 T cells significantly suppressed the production of the interleukin-17-producing CD4+ T cells (Th17)-associated cytokines, IL-17 and IL-22, in both cell contact-dependent and interferon (IFN)-γ-dependent mechanisms. In summary, inflammatory microenvironment in IA patients results in decreased numbers of Vd2 T cells in peripheral blood and liver, which in turn impairs the suppressive function of Vd2 T cells to Th17 cells. Therefore, adoptive transfer of Vd2 T cells may provide a novel therapeutic approach for IA patients.

Results: SE monocytes were mainly CD16-negative, whereas 75–80% of RT monocytes were CD16-positive. SE monocytes mainly derived from the classical subset (CD14+/CD16−) and exhibited high phagocytic activity. RT monocytes primarily originated from CD14+/CD16 and CD14+CD16+ monocytes, displayed an immature DC-like phenotype (DC-SIGN+, HLA-DR+, CD80/CD86+) and expressed higher levels of CCR7, CCR8 and C62L, which are involved in migration towards lymphoid tissues. In contrast to SE monocytes, RT monocytes induced a robust Ag-specific and allogeneic T-cell proliferation and T-cell activation indicated by CD69, CD71, CD26, HLA-DR and CD25 expression. Conversely, SE monocytes suppressed TCR-induced T-cell proliferation and dampened T-cell activation. Whereas RT monocytes secreted TNF-α, IL-1β, MIP-1α, MIP-1β and CXCL1 following LPS stimulation, SE monocytes largely failed to release pro-inflammatory cytokines but constitutively secreted MCP-1. Soluble factors derived from activated-liver-myofibroblasts promoted subendothelial retention.

Conclusions: Migratory processes across HSEC shape the subsequent fate of monocyte-derived cells giving rise to immunologically anergic macrophage-like cells as well as immunologically competent pre-DCs. Inflammatory liver-resident cells further modulate the compartmentalization of monocyte-derived cells.

EGR signaling impairs the antiviral activity of interferon-alpha

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Interferon-alpha (IFN-α) exhibits its antiviral activity through STAT signaling and the expression of interferon response genes (IRGs). Viral infection has been shown to result in activation of epidermal growth factor receptor (EGFR) – a host cell entry factor used by several viruses including hepatitis C virus. However, the impact of EGFR activation for cellular antiviral responses is unknown. Here we uncover a crosstalk between EGFR and IFN-α signaling with therapeutic impact for IFN-α based therapies and functional relevance for viral evasion and antiviral resistance. We show that combining IFN-α with EGFR inhibitor erlotinib potentiates the antiviral effect of each compound in a highly synergistic manner. Synergy correlated with reduced STAT3 phosphorylation in the presence of erlotinib, whereas STAT1 phosphorylation was not affected. Indeed, reduced STAT3 phosphorylation correlated with enhanced expression of suppressors of cytokine signaling 3 (SOCS3) in the presence of erlotinib and enhanced expression of the IRGs RSAD2 and Mx1. Moreover, EGFR stimulation reduced STAT1 dimerization but not phosphorylation indicating that EGFR crosstalk to IFN signaling acts on the DNA binding level of STATs.

Conclusions: Our results support a model where inhibition of EGFR signaling impairs STAT3 phosphorylation leading to enhanced IR expression and antiviral activity. These data uncover a novel role of EGFR signaling for the antiviral activity of IFN-α and open a previously undisclosed perspective to improve the efficacy of IFN-α-based antiviral therapies.
Background: Chronic hepatitis B is characterized by sub-optimal T cell responses to viral antigens. A therapeutic vaccine capable of restoring immune responses to HBV could potentially improve S antigen seroconversion rates in the setting of specific antiviral therapy. A yeast-based immunotherapy platform (Tarmogens) was used to develop a clinical vaccine candidate expressing hepatitis B X (HBxAg), S (HBsAg), and core antigen (HBcAg) conserved across all HBV genotypes (X-SCORE). The aim of our current study was to assess whether epitopes associated with acute HBV clearance are efficiently presented to T cells by X-SCORE-pulsed dendritic cells (DCs).

Methods: Mice were subcutaneously immunized with 3 weekly doses of X-SCORE or empty vector yeast (control). One week later, T cell responses were evaluated by lymphocyte proliferation assay (LPA), interferon-γ (IFNγ)/IL-2 ELISpot, intracellular cytokine staining (ICS), and tumor challenge assays. Human T cells specific for HBC18–27 and HBs183–91 were incubated with X-SCORE-pulsed DCs and IFNγ production was measured to evaluate presentation of relevant HBV epitopes to the T cells.

Results: Mice immunized with X-SCORE compared to controls showed induction of T cell responses specific for HBxAg by tumor protection (haz. ratio (hr)=0.27), LPA (3–fold increase vs. control), and IL-2 ELISpot (6–fold increase). Immune responses to HBcAg were also observed in tumor protection (hr=0.48), and responses to HBsAg were shown by the ability to expand HBs190–197 specific T cells ex vivo (13–fold increase vs. baseline by pentamer staining) and by IFNγ production by HBsAg-specific CD8+ T cells (3.6 fold increase vs. control). HBcAg- and HBcAg-specific human T cells produced IFNγ following incubation with X-SCORE-pulsed DCs (4.2 to 23-fold increase vs. control).

Conclusions: The X-SCORE vaccine candidate elicits HBxAg-, HBsAg- and HBcAg-specific T cell responses in murine models. HBsAg- and HBcAg-specific human epitopes associated with clearance of acute HBV infection are efficiently presented to virus-specific T cells by X-SCORE-pulsed DCs. These data suggest that X-SCORE could be used to improve outcomes in chronic HBV patients, through the induction of HBV-specific T cell responses targeting infected hepatocytes.
HBV infected patients (19 HBeAg neg., 1 HBeAg pos.) and compared it to Influenza (Flu)-, CMV- and HCV-specific CD8+ T cells. In addition, the functional relevance of inhibitory receptor blockade (PD-1, 2B4, Tim-3, CTLA-4, CTLA-4) was tested in vitro assays.

**Results:** HBV-specific CD8+ T cells expressed higher amounts of PD-1 and 2B4 compared to CD160, KLRG1 and Tim-3. The PD-1+2B4+ HBV-specific CD8+ T cells were enriched in the liver compared to the peripheral blood. The expression pattern of inhibitory receptors clearly differed between HBV-specific (high PD-1 and 2B4 expression) versus FLU- (high Tim 3 expression) or CMV-specific CD8+ T cells (high 2B4 and KLRG1 expression).

In contrast, the similar high expression of PD-1 and 2B4 was observed on HCV-specific CD8+ T cells, suggesting a common hierarchy of inhibitory receptor expression between CD8+ T cells specific for hepatitis B and C virus. However, coexpression analysis revealed a lower coexpression of inhibitory receptors on HBV-specific CD8+ T cells compared to exhausted HCV-specific CD8+ T cells. The subsequent blockade of inhibitory receptors resulted in individual functional response patterns regarding in vitro proliferation or antiviral efficacy.

**Conclusions:** These results suggest a clear hierarchy of inhibitory receptor expression on HBV-specific CD8+ T cells that is dominated by PD-1 and 2B4. Compared to exhausted HCV-specific CD8+ T cells, HBV-specific CD8+ T cells appear less severely exhausted. These findings have implications for therapeutic interventions targeting exhausted T cells during chronic HBV infection.

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**373 REDUCED HEPATITIS B VIRUS SURFACE ANTIGEN EXPRESSION IN OCCULT HEPATITIS B INFECTION: ALTERATION OF A POST-TRANSKRATIONAL REGULATORY MECHANISM?**

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**Background and Aims:** Occult hepatitis B virus (HBV) infection (OBI) is defined as low plasma level of HBV DNA with undetectable HBV surface antigen (HBsAg) outside the pre-seroconversion window period. OBI has been associated with HBV transmission through transfusion and organ transplantation, and viral reactivation in immuno-compromised individuals. The mechanisms leading to OBI remain largely unknown. Previous work showed that lack of detectable HBsAg could be related to mutations in the HBV S protein affecting detection by current immunoassays. Other studies suggested that S mutations might impair virion and/or S protein excretion and contribute to the OBI phenotype. The potential role of OBI-specific amino acid substitutions in the S protein on HBsAg production and excretion was examined in vitro.

**Methods:** The S gene of HBV genotypes B, C, and D HBsAg+ controls (n = 8) and OBI strains (n = 18) were transfected in HuH7 cells. HBsAg protein was quantified in culture supernatants and cell extracts. HBsAg intracellular distribution was examined by immunofluorescence. Selected mutations were functionally either repaired or introduced in control strains using site-directed mutagenesis.

**Results:** The intracellular (IC)/extracellular (EC) HBsAg production ratio was ~1.0 for the majority of controls. Three IC/EC HBsAg patterns were observed in cloned OBI strains: pattern 1 defined as IC/EC ratio 1.0 in 5/18 OBI clones; pattern 2 with detectable IC HBsAg production but low or undetectable EC HBsAg (IC/EC: 7.0–800) in 6/18 OBI; and pattern 3 with both low or undetectable IC and EC HBsAg in 7/18 clones. In pattern 2, IC HBsAg presented as densely packed fluorescence localized in the perinuclear area. In 4/6 pattern 2 OBI clones, HBsAg excretion deficit was corrected by site-directed mutagenesis of sM75T or sY100S or sP178R substitution and caused a similar excretion deficit when sM75T or sP178R were introduced in a control strain, but not sY100S suggesting possible cis-interaction with other uncharacterized substitutions. The sP178R substitution was present in all clones sequenced of two OBI samples.

**Conclusions:** OBI-specific substitutions at positions s75, s100 and s178 induced S protein intracellular retention. Preponderance of sP178R carriers in the viral quasispecies infecting two individuals is likely responsible for the OBI phenotype.

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**375 CELL CYCLE ARREST AND MODULATION OF HBV REPLICATION IN POST TRANSCRIPTIONAL PROCESS BY miRNA-125b**

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**Background and Aims:** miRNAs are reported to modulate cell growth and regulate the replication of hepatotropic viruses HBV and HCV. Previously, we showed that two miRNAs miR-1 and miR-449a could enhance HBV replication by the upregulation of FXR, a liver-specific transcription factor. However, they are only expressed at a low level in hepatocytes. Here, we asked whether miRNAs
relative high expressed in hepatocytes could also modulate the growth of hepatoma cells and influence HBV replication.

Methods: A number of miRNAs with decreased expression in hepatocellular carcinoma were tested for their ability to control cell cycle and cell proliferation by transfection in established hepatoma cell lines. Their influence on HBV replication were analyzed at different levels including viral transcription, assembly, and viron production.

Results: Among the tested miRNAs, miR-125b could inhibit hepatoma cell proliferation and block the cell cycle at the G1/S phase transition. In addition, miR-125b could enhance HBV replication both in HBV genome stable expression system and in HBV transfection system. However, no seed sequence in HBV genome sequence for miR-125b was found. Using the reporter plasmids containing HBV genome fragment and promoters, it was shown that miR-125b did not regulate the gene expression through direct binding to HBV genome and had only marginal influence on HBV transcription. Further analysis revealed that miR-125b enhanced HBV nucleocapsid formation and progeny secretion in a dose-dependent manner.

Conclusion: This is the first time we identified a specific factor that positively influence HBV replication in the post-transcriptional process. Our results demonstrated that some miRNAs with the ability to arrest the cell cycle at the G1/S transition may preferentially up-regulate HBV replication, and these miRNAs could enhance HBV replication in different processes in a synergistic manner.

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SOLUBLE CD163 IS ASSOCIATED WITH LIVER INFLAMMATION AND FIBROSIS IN CHRONIC HEPATITIS B VIRUS INFECTION
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Background and Aims: Activated macrophages play an important pathophysiologic role in liver inflammation and development of fibrosis in chronic hepatitis B virus infection. Recently, soluble CD163 (sCD163), a sensitive marker for macrophage activation, was found to be associated with the severity of liver cirrhosis and portal hypertension but so far sCD163 serum levels have not been further investigated in patients with HBV. Aim of our study was to investigate whether CD163 serum levels correlate with liver inflammation and fibrosis in patients with chronic hepatitis B infection.

Methods: In a retrospective cohort study sCD163 serum levels were assessed by ELISA (Enzyme-linked immunosorbent assay) together with clinical and laboratory data in 202 patients with chronic hepatitis B infection. The relation between parameters for liver fibrosis and necroinflammation and sCD163 serum levels was assessed by ELISA (Enzyme-linked immunosorbent assay) to assess their specific role in the phenotype.

Results: Among the tested miRNAs, miR-125b could inhibit hepatoma cell proliferation and block the cell cycle at the G1/S phase transition. In addition, miR-125b could enhance HBV replication both in HBV genome stable expression system and in HBV transfection system. However, no seed sequence in HBV genome sequence for miR-125b was found. Using the reporter plasmids containing HBV genome fragment and promoters, it was shown that miR-125b did not regulate the gene expression through direct binding to HBV genome and had only marginal influence on HBV transcription. Further analysis revealed that miR-125b enhanced HBV nucleocapsid formation and progeny secretion in a dose-dependent manner.

Conclusion: This is the first time we identified a specific factor that positively influence HBV replication in the post-transcriptional process. Our results demonstrated that some miRNAs with the ability to arrest the cell cycle at the G1/S transition may preferentially up-regulate HBV replication, and these miRNAs could enhance HBV replication in different processes in a synergistic manner.

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SOLUBLE CD163 IS ASSOCIATED WITH LIVER INFLAMMATION AND FIBROSIS IN CHRONIC HEPATITIS B VIRUS INFECTION
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Background and Aims: Activated macrophages play an important pathophysiologic role in liver inflammation and development of fibrosis in chronic hepatitis B virus infection. Recently, soluble CD163 (sCD163), a sensitive marker for macrophage activation, was found to be associated with the severity of liver cirrhosis and portal hypertension but so far sCD163 serum levels have not been further investigated in patients with HBV. Aim of our study was to investigate whether CD163 serum levels correlate with liver inflammation and fibrosis in patients with chronic hepatitis B infection.

Methods: In a retrospective cohort study sCD163 serum levels were assessed by ELISA (Enzyme-linked immunosorbent assay) together with clinical and laboratory data in 202 patients with chronic hepatitis B infection. The relation between parameters for liver fibrosis and necroinflammation and sCD163 serum levels was analyzed.

Results: Serum levels of sCD163 were significantly higher in patients with liver fibrosis (Ishak score ≥2) in comparison to patients without fibrosis (1709 vs. 1242 ng/l, p=0.022). Furthermore sCD163 was markedly increased in patients with inflammatory activity in liver biopsy (HAI score ≥5 vs. <5: 1752 vs. 1255 ng/l, p=0.029). In line with this, a significant correlation between sCD163 and AST serum levels was observed (r=0.452, p=0.001). No correlation was found between sCD163 and HBsAg or HBV-DNA serum levels.

In patients with elevated liver enzymes and high viral load (>2000IU/ml) with indication for antiviral therapy sCD163 levels were significantly higher than in HBsAg carriers with normal serum liver enzymes and low viral load (1630 vs. 1054 ng/l, p<0.001).

Conclusion: The correlation of sCD163 levels in different phases of chronic Hepatitis B infection with fibrosis and necroinflammation indicates that macrophage activation plays a role in HBV pathogenesis. The sCD163 serum level is a new promising non-invasive marker for liver fibrosis and necroinflammation in patients with chronic hepatitis B infection.

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IMPLICATION OF CORE GENE IN HBV COVALENTLY CLOSED CIRCULAR DNA ACCUMULATION
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Background and Aims: Persistence of HBV cccDNA in infected hepatocytes represents the main mechanism responsible for the chronicity of infection. Upon the study of cccDNA levels in liver biopsies derived from a cohort of 60 HIV–HBV co-infected patients, an individual with a cccDNA level 300 fold higher than the average of cohort was identified.

Our objective was to detect and analyze possible mutations in the viral genome determining the cccDNA accumulation observed in vivo.

Methods: Following intrahepatic DNA extraction, the viral genome originated from the cccDNA-accumulating patient has been cloned in a GFP-expression plasmid under the control of a CMV promoter. After transfection of the construct into the HepG2 cell line, fourteen replication competent clones were identified, sequenced and further characterized for cccDNA and rcDNA levels by a specific qPCR technique. Moreover, expression of viral proteins was analyzed by Western-blotting and secretion of HBs antigens and Dane particles was assessed by immunosassays and Southern-blotting.

Results: The characterization analysis highlighted several clones accumulating a higher amount of cccDNA than the wild-type virus. Sequence analysis and alignment allowed us to identify a pattern of scattered missense mutations all along the viral genome. To determine which of them were functionally relevant, we performed fragment substitutions experiments involving the Spoly, core and X regions of the HBV genome between clones accumulating cccDNA and wild-type virus. Interestingly, substitution of the mutated region encoding the Core protein seemed to be sufficient to convert a wild-type virus into a cccDNA accumulating variant. The different single mutations present in the sequence are currently under evaluation by a site mutagenesis assay to assess their specific role in the phenotype.

Conclusion: Our results underline a previously unknown role of the Core gene in cccDNA accumulation. The precise mechanisms responsible for this phenomenon are currently being studied by genetics and functional approaches. These new findings may open new clinical and therapeutic perspectives.

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HEPATITIS B CORE (HBc) PROTEIN IS A KEY AND VERY EARLY NEGATIVE REGULATOR OF THE INTERFERON RESPONSE
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Background and Aims: HBV has evolved various strategies to evade innate immunity, in particular IFN response. While looking for new strategies, which could explain in particular the precocity of this inhibition, we identified the HBV core protein (HBc) as a master and very early regulator of IFN response.
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Methods: HepaRG and PHH were either infected with HBV or transfected with either purified viral nucleocapsid or recombinant HBc protein. Moreover engineered HepaRG cell lines, which express HBV proteins, were also used to analyse the role of individual proteins. Ligands of PRRs were used to engage innate receptor and analyse the inhibitory effect of HBV proteins. HBV replication was analysed with standard procedures, whereas the effect of viral proteins on the expression of various interferons (α, b, l, ...), interferon-induced (ISG) and pro-inflammatory cytokines genes was analysed by RT-qPCR, WB and ELISA. ChIP experiments were also performed to analyse the binding of HBC to target promoters as well as to analyse the recruitment of epigenome-modifying enzymes to target promoters.

Results: HBV is capable to inhibit dsRNA-mediated interferon response after only few hours of infection. This inhibition occurs also with Dane-depleted and UV-inactivated virus suggesting that neo-synthesis of HBV protein is not mandatory. Using engineered cell lines we demonstrate that HBC is responsible for this very early inhibition. The transfection of purified nucleocapsid or recombinant HBc leads to the same inhibitory phenotype. HBc needs to be located in the nucleus to inhibit the transcription of targeted genes (i.e. IFNs, ISG), as inhibition of its trafficking, revert the phenotype. ChIP analyses with anti-HBc revealed that HBc is capable to bind to target promoters and to recruit epigenome-modifying enzymes to establish a negative mark on target promoters.

Conclusion: Our results demonstrate that HBc is responsible for the very early inhibition of IFN response by HBV. The precocity of this inhibition is instrumental for the establishment of a persistent infection in vitro and is possible because HBC from incoming virions is capable to inhibit gene expression in the nucleus of infected cells by either direct interference with transcriptional machinery or by recruiting epigenome-modifying enzymes leading to repressive marks.

379 ULTRA-DEEP PYROSEQUENCING OF BASIC CORE PROMOTER, PRECORE AND CORE REGIONS OF HEPATITIS B VIRUS TO STUDY VIRAL VARIABILITY AND QUASISPECIES COMPLEXITY

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Introduction: The basic Core promoter (BCP) and preCore (pC) region are crucial for hepatitis B virus (HBV) replication and HBeAg expression, whereas Core gene contains main epitopes. pC and Core are little overlapped and optimal for studying the complexity of the quasispecies.

Aim: To analyze the BCP, pC, and Core regions of HBV by ultra-deep pyrosequencing (UDPS) and evaluate the evolution of quasispecies complexity by Shannon entropy (SE).

Materials and Methods: Thirty samples from 10 patients were analyzed at 3 time points: baseline, before treatment, and 12 months after lamivudine non-response. Two periods were considered, untreated (U) and viral breakthrough (VBK). PCR fragments (494 bp) included the BCP, pC, and codons 1–84 of Core. Sequences at percentages >0.25% were analyzed.

Results: A total of 822,905 sequences were analyzed. Overall variability of BCP and pC regions showed 11 positions with variability >15%, some of them related with HBeAg expression (1762, 1764, and 1896). Patients 5 and 6 presented different HBeAg status during the periods analyzed, and showed the highest number of changes in the BCP and pC regions. The distribution of changes in Core gene in the 30 samples analyzed is shown in Figure 1.

Figure 1. The SE (Figure 2) increased at U and decreased at VBK in 6 cases. In 2 cases, SE was maintained during U and decreased at VBK. In the other 2 patients, complexity decreased at U, but increased at VBK.

Figure 2.

Conclusions: pC and BCP variability was mainly due to HBeAg expression. The significant concentration of changes in immunodominant regions seems to confirm their main role in the immune response during chronic HBV infection. The increased complexity during natural evolution may indicate an effect of the host immune response against this region. In addition, the SE decrease after VBK may be due to selection of variants at the polymerase gene.

Study funded by FIS PS09/00899.

380 RAPAMYCIN MODULATES THE COURSE OF HEPATITIS B VIRUS (HBV) INFECTION IN HYDRODYNAMIC INJECTION MOUSE MODEL: THE ROLE OF Treg IN HBV PERSISTENCE

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Background: Hydrodynamic injection (HI) of pAAV/HBV1.2 in BALB/c mice leads to a transient HBV gene expression and replication in liver, representing a useful system for the study of HBV infection. Rapamycin (RAPA) can select activated Treg in vitro. In this study, the effect of RAPA on HBV replication was investigated in HBV HI mice model, and the possible role of Treg in HBV persistence was analyzed using RAPA treated mice model.

Methods: HI was performed in BALB/c mice with pAAV/HBV1.2. Mice were treated with RAPA or saline (NS) for 10 weeks, respectively. HBsAg, HBsAb and HBV DNA were measured by ECLIA and real-time PCR. CD molecules and cytokine mRNAs in mouse liver tissues were detected by real-time RT-PCR. The frequencies of antigen-specific IFN-γ secreting splenocytes were measured by ELISPOT assay. The frequencies of CD4+CD25+FoxP3+ Treg in PBMC, splenocytes and Liver infiltrating lymphocytes (LILs) were measured by FACS analysis.
Results: In RAPA treated mice, serum HbsAg and HBV DNA persisted for more than 4 months, even at 6 weeks after RAPA withdrawal. While NS treated mice cleared HbsAg and HBV DNA in serum within 4 weeks and developed HBsAb (Fig. 1). The frequency of Treg was lower in PBMC and spleenocytes of RAPA treated mice when compared with those of NS treated mice. The frequencies of Treg were higher in LLls of RAPA treated mice at 10 days and 30 days after HI (Fig. 2). The levels of CD3/CD4/CD8/Perforin/Fas-L/IFN-$\gamma$/TNF-$\alpha$ mRNAs were lower in liver of RAPA treated mice, but those of TGF-$\beta$/FoxP3/IL10 were higher in liver, when compared with those of NS treated mice (Fig. 3). However, the frequencies of antigen-specific IFN-$\gamma$ secreting cells were higher in the RAPA treated mice (Fig. 3).

Conclusion: Rapamycin facilitate HBV persistence in HI mouse model, primarily due to the enhancement of TGF-$\beta$ mediated Treg response. The role of Treg in HBV persistence need further study.

Figure 1. RAPA prolonged HV replication and expression in mice received HI. Mice were treated with saline or immunosuppressive drugs RAPA from week −1 to week 10 and received HI with 10$^{15}$ of pAAV/HBV1.2 at week 0. HbsAg, anti-HBs and anti-HBc antibodies in the sera at indicated time point were detected by commercial ECLIA. The serum HBV DNA concentrations were determined by real time PCR. (A) Detection of HbsAg (line) and HBV DNA (bar) in saline group after HI. The cutoff values of HbsAg and HBV DNA concentrations are 1.0 COI (solid line) and 5×10$^{3}$ copies/ml (dashed line), respectively. (C) Detection of anti-HBs antibodies in mice after HI at week 0, 7, 10, and 26. The cut off value of the anti-HBs antibody assay was 10 IU/L.

Figure 2. The influence of RAPA treatment on Treg frequency. Mice were treated with saline or immunosuppressive drugs RAPA from week −1 to day 30 and received HI with 10$^{15}$ of pAAV/HBV1.2 at week 0. PBMC was isolated at indicated time point from peripheral blood. Mice were sacrificed at the indicated time points and spleen and liver samples were collected. Splenocytes and LLls were isolated. The frequencies of PBMC (A), splenocytes (B) and LLls (C) were detected by FACS analysis. The data was analyzed by unpaired bilateral t test. Value of $p<0.05$ is considered as a significant difference.

Figure 3. The influence of RAPA treatment on HBV-specific T cell response and expression of T-cell affiliated genes and cytokines. Mice were sacrificed at 30 days after HI. Liver and spleen were collected. The mRNA levels of the CD marker (A), CTL related cytokine (B) and negative regulation (C) in liver tissue were analyzed by real time RT-PCR and shown as mean expression levels relative to 10$^{15}$ beta-actin mRNA copies. $^p<0.05$. The data was analyzed by unpaired bilateral t test. (D) Detection of HBV-specific IFN-$\gamma$ producing splenocytes in mice treated with saline or RAPA. Splenocytes were isolated and pulsed with rHbcAg (0.5 μg/ml), rHbsAg (0.5 μg/ml) or CMV peptide (0.5 μg/ml). HBV specific IFN-$\gamma$ producing cells in 2×10$^6$ splenocytes were analyzed by ELISPOT.

381 INTERLEUKIN-33 BREAKS HOST IMMUNE TOLERANCE VIA T FH CELLS IN HEPATITIS B VIRUS INFECTION
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Background: Chronic hepatitis B (CHB) patients are immunotolerant to the degree that they do not elicit an anti-HBV response sufficient to clear the virus or destroy infected cells. Our previous study has reported that IL-33 was an important factor in the pathogenesis and immune response of CHB. But it was still not clear how IL-33 played a role in HBV. The aim of this study was to investigate the effects of IL-33 on HBV in vivo and in vitro.

Method: After stimulation of IL-33 in three different concentrations (0 μg/ml, 1 μg/ml, 100 μg/ml), the HepG2.2.15 RNA was extracted and the gene expression data were analyzed via Illumina’s GenomeStudio Gene Expression Module. Three groups of mice were involved in the in vivo experiment: normal C57BL/6 (control), C57BL/6 HBV transgenic mice (Tg), and IL-33 treated HBV-tg mice by intra-abdominal injection twice a week. The concentrations of serum HBV loads, ALT, AST and hepatic HbsAg, HBeAg, HbsAb and HBeAb in the HBV-tg mice were determined. Liver sections of HBV-tg mice were stained by anti-HBsAg, anti-HBeAg, anti-HBcAb, and examined microscopically. The frequency of CD4+CXCR5+ Tfh cells in the spleen and liver of HBV transgenic (HBV-tg) mice and Wild Type (WT) mice were characterized by flow cytometry analysis.

Results: IFR4, AICDA and PRDM1 gene expressions were progressively increased and BCL-6 gene expression progressively decreased. In addition, CXCR5 and IL-21 gene expressions were increased. The Gene expression data revealed that IL-33 promoted the differentiation of B cells into Plasma cells. Moreover, the study found that IL-33 treatment obviously reduced the concentrations of serum HBV loads in vivo, but increased the concentrations of hepatic HbsAb, HBeAb in HBV-tg mice. It is also found that the frequencies of splenic and hepatic CD4+CXCR5+ Tfh cells in HBV-tg mice treated with PBS were significantly higher than that of WT mice, but reduced significantly after IL-33 treatment. The concentrations of serum ALT were negatively correlated with the percentages of CD4+CXCR5+Tfh cell expression in IL-33 HBV-tg mice.

Conclusions: IL-33 could effectively break the host immune tolerance and induce HBeAb and HbsAb secretion via Tfh cells in HBV-tg mice.

382 SERUM INTERLEUKIN-37 AND LIVER INJURY IN CHRONIC HEPATITIS B PATIENTS WITH HBeAg SEROCONVERSION
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Objective: IL-37 is a new anti-inflammatory cytokine, which plays an important role in protecting against tissue injury during infections via limiting immune and inflammatory reactions. This study aimed to investigate whether IL-37 has a role in the pathogenesis of Chronic Hepatitis B.

Method: 15 healthy controls (HC), 20 HBeAg positive CHB patients were included in the study. After Adefovir Dipivoxil (ADV) treatment, 20 HBeAg positive CHB patients were divided into two groups (10 with HBeAg clearance, another 10 without). The serum level of IL-37 in HC, CHB patients was measured using ELISA. The Th1 cytokines (IFN-$\gamma$, TNF-$\alpha$, IL-2), and Th2 cytokines (IL-4, IL-6, IL-10) were measured before and after ADV therapy using cytometric bead array (CBA). The concentrations of clinical parameters in hepatitis B patients such as serum HbsAg, HBeAg, ALT, AST and HBV loads were also measured.
POSTERS

383 DNA METHYLATION PROFILE OF HBV INFECTION
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Results: It was found that the level of serum IL-37 was significantly higher in CHB patients than the HC. After 48 weeks of ADV treatment, the levels of serum IL-37 decreased in CHB patients with HBeAg clearance compared to the baseline. Before ADV treatment, the levels of Th1 and Th2 cytokines were lower in CHB patients than the HC. But after ADV treatment the concentrations of serum IL-2, IL-6, IFN-γ, and TNF-α in CHB patient with HBeAg clearance were significantly increased than the baseline. In addition, the study found that the levels of serum IL-37 were positively correlated with serum ALT in CHB, while the levels of IL-2 and IL-4 were negatively associated with serum AST.

Conclusions: IL-37 may participate in the immune response of CHB patients with HBeAg seroconversion. And serum levels of IL-37 may be a marker to evaluate the level of liver injury with chronic HBV infection.

384 GENES IN WIDE FUNCTION MORE THAN IMMUNE RESPONSE WERE INVOLVED IN CHRONIC HBV INFECTION BY CHIP-CHIP ANALYSIS OF H3K9 ACETYLATION STATUS IN CD4+ T CELLS
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Background: Histone 3 lysine 9 acetylation (H3K9ac) is an important epigenetic modification, which is associated with active transcription of wide genes in multiple organisms. However, the association between H3K9ac status and diverse progress of chronic HBV infection is unclear.

Methods: Variation of H3K9ac status was analyzed in CD4+ T cells from CHB patients in different clinical stages, 3 CHB in tolerance phase (CHB-T), 3 CHB in active phase (CHB-A), 3 CHB-related liver failure (CLF) and 3 normal control (NC), by using chromatin immunoprecipitation microarray (ChIP-chip) technology with 11,148 distinct probes at transcription initiation sites (Nimblegen Human ChIP 385K RefSeq Promoter Arrays). ChIP-qPCR and real-time PCR were used to validate the microarray results and quantitate the transcriptional activities of target genes.

Result: The number of H3K9ac probes in CHB-A group (2886) was the most (CHB-T, 2431; CLF, 2162; NC, 2141). Compare with NC, CHB-T group showed that 73 genes acetylated while 1 deacetylated in promoter region. These genes were clustered into membrane protein and associated with regulation of protein kinase activity, cell communication and lymphocyte homeostasis by GO function analysis. 3 genes (AKT1, IRAK1, CASP3) were involved in apoptosis pathway by KEGG signal pathway analysis. CHB-A group showed 160 genes acetylated and 2 deacetylated in promoter region, which were clustered into membrane protein, and associated with ion transport. 6 genes (PLCB3, ADCY7, PDE1B, SLC25A6, CACNA1G, MYLK) were participated in calcium signaling pathway. CLF group showed 25 genes acetylated and 3 deacetylated in promoter region, which were mostly cytoskeleton (PKHD1, DYNLL2, NFX2, ACTN2, STAG2) and associated with cytoskeletal protein binding. The result of CHIP qPCR coincided well with the microarray data. Quantitative RT-PCR revealed the correlations closely between the genes mRNA expressions and the H3K9acetylation levels from the data of microarray.

Conclusion: The H3K9ac status level of gene promoter in CD4+ T cells was different in the pathogenic process of CHB at different clinic stages. The genes with aberrant H3K9ac status were involved in wide function such as apoptosis, ion transport and cytoskeleton more than immune response, which could provide a new insight for further investigation the pathogenesis of HBV infection.

385 IMPACT OF A181T AND I233V MUTATIONS IN HEPATITIS B VIRUS POLYMERASE REVERSE-TRANSCRIPTASE ON VIRAL REPLICATION AND DRUG-RESISTANCE
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Background and Aims: Emergence of drug-resistant hepatitis B virus (HBV) against the nucleos(t)ide analogues is a major problem for antiviral treatment in chronic hepatitis B (CHB) patients. We analyzed evolution of drug-resistant mutations and characterized effects of rtaA181T and rtaI233V mutations on viral replication and drug-resistance.

Methods: Full sequences in HBV polymerase reverse-transcriptase (RT) domain was analyzed from serial sera of a CHB patient treated with lamivudine and adefovir. The RT domains of wild type and HBV
mutants isolated from serial sera were amplified and converted into the replication-competent HBV 1.2mer constructs. Each construct was analyzed for in vitro drug susceptibility assay by southern. We made a series of mutant clones and determined the replication ability and resistance to antiviral agents.

**Results:** Conserved mutations in rt204, rt181, rt236, and rt233 were identified during the viral breakthrough. In viro study showed the effect of rtA181T mutation on viral replication and drug resistance is dependent on the mutation in overlapping surface gene. The rtA181T mutant harboring surface stop (rtA181T/sw172*) showed a decrease in viral replication and increase in drug resistance compared to the rtA181T mutant harboring surface mutation (rtA181T/sw172S). Moreover, the rtA181T/sw172* mutant exhibited a secretion defect of viral particles. The rtI233V mutation which emerged during adefovir therapy reduced the viral replication and conferred resistance to adefovir. However, the rtI233V mutation did not affect the viral replication and drug resistance of rtA181T/sw172* mutant.

**Conclusions:** The consequence of rtA181T mutation in viral replication of HBV and drug resistance is dependent on the mutations in overlapping surface gene. The rtI233V mutation affects the replication ability and resistance to adefovir.

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**386 HEPATITIS B RNA SPlicing SUPPORTS VIRAL REPLICATION THROUGH FUNCTIONING AS DEDICATED CORE mRNA**

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**Background and Aims:** Splicing of the hepatitis B pregenomic RNA generates viral particles with defective viral genomes, which was identified in chronically infected patients across all genotypes. Its physiological function remained uncertain. Previous studies demonstrated its capability to enhance HBV replication. We hypothesized that HBV RNA splicing effectively convert pregenomic RNA into dedicated HBV core and X mRNA, which improves the efficiency of virion formation.

**Methods:** HepG2.2.15 cell line (which stably produces HBV DNA and viral particles) was used. The respective expression vectors were constructed using the pcDNA 3 plasmid and transfected into the cells at 2 concentrations (0.5ug and 1ug of DNA), using XtremeGENE transfection reagent. The respective cells were harvested after 48 hours and the level of protein expressions were confirmed by Western blot. The production of viral DNA and RNA in the cells and medium, were determined by real-time PCR (normalized with β-actin levels). HBV surface antigen (HBsAg) was measured with commercial ELISA assay.

**Results:** There was a dose-dependent enhancement of HBV DNA production with increased co-transfection of SP1 construct (the most common HBV splice variant), up to three-folds compared with controls. There was also a similar increase in the intracellular HBVDNA with additional core peptide expressed by transfected plasmids, but not with X expression vector. There was also an increase in the excreted viral particles in the medium as determined by HBV DNA measurement. There was no effect in the HBsAg levels in the culture medium.

**Conclusions:** The common splice variant, SP1, enhanced the full-length HBV replication, which may contribute to chronicity and viral persistence. This effect was similar when core peptide alone was added, which support the hypothesis that it was an important rate-limiting factor of viral replication, and was enriched by HBV RNA splicing. This was not seen with X protein-enrichment.

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**387 ALPHA-INTERFERON INDUCES METHYLATION OF HEPATITIS B VIRUS DNA IN HUMAN HEPATOMA CELL LINE**

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**Background and Aims:** Recently, methylation of hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) is known to occur in liver tissues of chronic hepatitis B patients, and cccDNA methylation is associated with down-regulated transcriptional activity of HBV. However, the mechanisms underlying HBV cccDNA methylation is largely elusive. Alpha-interferon (IFN-α) is an antiviral agent for chronic hepatitis B with superior sustained viral response compared to oral nucleoside(1)ide analogues. In this study, we aimed to demonstrate the possible relationship between the cccDNA methylation and the antiviral efficacy of interferon-α in a cell culture model.

**Methods:** HepG2 cell line were transfected with pH92 plasmid which contains 1.1x-length HBV genome. Replication of HBV was confirmed by detection of HBV DNA from culture supernatant. Plasmid-transfected cells were treated with or without IFN-α (1000 U/mL) for 5 days, then HBV cccDNA was isolated. Cytosine methylation of HBV DNA was assessed by bisulfite modification, followed by methylation-specific PCR (MSP) or bisulfite sequencing PCR analysis. Expression of DNMT was assessed by real-time qPCR. Interaction between HBV cccDNA and methyltransferases (DNMTs) was assessed by chromatin immunoprecipitation (ChIP) assay. Promoter activity of DNMT was measured by luciferase reporter assay.

**Results:** MSP showed methylation of HBV cccDNA in IFN-treated cells, whereas no methylation was found in control cells. BSP showed increased ratio of methylation-positive clones in IFN-treated cells compared to control cells. Expression of DNMT3A and 3B mRNA was upregulated by IFN. ChIP assay revealed binding of DNMT3A and DNMT3B on HBV cccDNA. Reporter assay confirmed that IFN transactivated promoter activity of DNMT3A and DNMT3B in HepG2 cells.

**Conclusions:** IFN-α induces CpG methylation of HBV cccDNA. IFN-mediated methylation may be ascribed to transcriptional up-regulation of DNMT3.

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**388 HEPATITIS B VIRUS X PROTEIN INDUCES TRANSCRIPTION OF HUMAN TNF-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) GENE DEPENDS ON sp-1**

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**Background and Aim:** It is well known that TNF-related apoptosis-inducing ligand and virus infection are strongly associated with hepatocyte injury during hepatitis and hepatocellular carcinoma (HCC). The aim of our study was to define the transcription factor(s) and upstream signal transduction pathways involved in the transcription of human TRAIL (hTRAIL) in response to hepatitis B (HB) virus.

**Material and Methods:** Expression plasmids of hepatitis B core (Hbc), hepatitis B virus S protein (HBs), and hepatitis B virus X protein (HBx) protein were cotransfected with TRAIL promoter luciferase reporter construct into HCC cell line (HepG2) respectively. The expression and location of sp1 in HepG2 cells were detected by confocal microscopy. EMSAs were performed using biotin-labeled oligonucleotides of the putative binding sites and nuclear extracts from HBx transfected HepG2 cells. 13 patients with biochemical, histological, and clinical evidence of severe chronic
viral hepatitis B, 11 patients with mild chronic viral hepatitis B, and 11 HBsAg carriers were studied for sp1 expression in PBMC.

**Results:** It showed that HBx protein, not HBs or Hbc increased hTRAIL expression in HepG2 cell. A strong regulatory region from −89 to −29 (relative to the transcriptional starting site) was shown to be responsible for gene transcription in response to HBx protein. Moreover, Electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (ChIP) and confocal Microscopy revealed sp1 translocated to the nucleus and enhanced hTRAIL expression in response to HBx proteins through two clusters of Sp1-binding sites within the hTRAIL proximal promoter. Short hairpinRNA (shRNA) interference plasmid of sp1 was constructed and shown to inhibit hTRAIL gene transcription. Furthermore, sp1 protein was highly expressed in peripheral blood mononuclear cells from severe chronic hepatitis B (CHB) patients in contrast to that in mild CHB patients.

**Conclusion:** Taken together, this study demonstrates HBx porotein induces hTRAIL gene transcription and this provides new insight into the molecular mechanisms of viral hepatitis and HCC.

**ENHANCING VIRUS-SPECIFIC IMMUNITY IN VIVO BY COMBINING THERAPEUTIC VACCINATION AND PD1/PD-L1 BLOCKADE IN CHRONIC HEPADNAVIRAL INFECTION**

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**Background and Aims:** Previously, we demonstrated that combination of antiviral treatment and therapeutic vaccination was able to induce specific immune responses and transiently suppress woodchuck hepatitis virus (WHV) replication in the woodchuck model. Recently, we have also demonstrated that in vitro blockade of the woodchuck PD-1/PD-L1 pathway could restore the T cell functions in chronic WHV infection. In this study, we examined whether in vivo blockade of the PD-1 pathway in combination with antiviral nucleoside analogue treatment and therapeutic vaccination could enhance CD8 T cell immunity and resolution of a chronic WHV infection.

**Methods:** The woodchuck PD-1 was cloned and characterized, and the expression patterns of PD-1 in woodchucks with acute or chronic WHV infection were investigated. Woodchucks chronically infected with WHV were treated with entecavir and received repeated DNA vaccines. During the vaccination, woodchuck PD-L1 antibody was intravenously applied. The restoration of T cell function by this combination therapy was examined by lymphoproliferation assay and CD107a degranulation assay. The antiviral effect was evaluated through monitoring viremia and viral replication in the livers of treated woodchucks.

**Results:** Characterization of woodchuck PD-1 reveals a high similarity to the counterpart of mammalian species. PD-1 expression on CD8 T cells correlates with WHV viral load during WHV infection. Antiviral entecavir treatment decreases PD-1 expression on CD8 T cells in chronic carriers. In vivo blocking PD-1/PD-L1 pathway in combination with antiviral treatment and therapeutic vaccination synergistically enhances the function of virus specific CD8 T cell, and improves viral control in woodchucks chronically infected with WHV.

**Conclusions:** This new approach of triple therapy (combination of nucleotide analogue, DNA vaccine and PD-1 blockade) may be useful for the design of immunotherapeutic strategies in patients with chronic hepatitis B.
PRE-CLINICAL PROOF-OF-CONCEPT STUDIES EXPLORING SCHEDULES OF ADMINISTRATION OF AN ADENOVIRUS-BASED HBV IMMUNOTHERAPEUTIC SHOW WIDE POTENTIAL FOR INDUCTION OF ROBOTIC AND LONG-LASTING T-CELL RESPONSES

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Background and Aim: In the context of chronic Hepatitis B Virus infection, active immunotherapies aiming at inducing robust T cells are attracting as a tight correlation was established between host’s effective T cell responses and viral control. It is anticipated that an immunotherapeutic product will need to be administered several times to sustain the induced HBV-specific T cell responses and allow the cure of HBV infected hepatocytes along time. We explored using an adenovirus-based prototype HBV immunotherapeutic both conventional or unconventional (highly intensive) homologous prime/boost administration schedules with respect to their capacity to induce specific T cell responses.

Methods: The prototype immunotherapeutic is based on a human Adenovirus serotype 5 encoding 2 HBV antigens (Core and Polymerase). Conventional schedules of immunization (1, 2 or 3 injections at 2 or 4 months interval) and unconventional schedules of immunization (1, 3 or 6 times at one week apart) were evaluated in HLA-A2 transgenic mice and/or HBV transgenic mice. Induced T cell responses were monitored at 2 weeks or 2 months after last injection using Elispot IFN-gamma and ICS assays. Memory and activation markers displayed by induced T cells were also explored.

Results: Robust and multispecific T cell responses were detected whatever the tested schedule at 2 weeks after last injection. Following conventional schedules, HBV-specific IFN-gamma CD8+ T cells were mainly effector memory cells (CD44+/CD62L−), some of them expressing CD27+ and CD43+ activation markers. Following 6 injections performed one week apart, a robust HBV-specific IFN-gamma T cell response was also detected (spot means/10⁶ cells/epitope ranging from 544 to 1126) in spite of robust humoral and cellular immune responses targeting the adenovirus vector. Two months after the 6 injections, HBV-specific T cells are still clearly detectable. One third of the detected cells are positive for KLRG1 while two third are KLRG1-cells, 1/3 of them displaying the activation markers CD27+/CD43+.

Conclusions: These data show that a variety of immunization schedules can be contemplated for an Ad5-based HBV immunotherapeutic that result in robust and multispecific T-cells displaying attractive features in the context of a therapeutic application to HBV chronic carriers.

HIGHER SERUM CONCENTRATION OF IL-17F IS ASSOCIATED WITH IMMUNE CONTROL OF HBV INFECTION

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Background and Aims: Recent reports have proven the impact of Th17 lymphocytes on inflammatory reactions in the liver disease and control of HBV infection. This study aims to assess serum interleukin 17A (IL-17A) and interleukin 17F (IL-17F) concentrations in patients chronically HBV-infected with regard to the phase of infection.

Methods: Group of 143 patients with chronic HBeAg negative HBV infection, were enrolled in this study. Serum IL-17A and IL-17F were assessed by ELISA at baseline. The results were analysed with regard to viral replication and liver function tests. Three groups were distinguished: CHB-detectable HBV DNA and elevated ALT (n = 30), LR-serum HBV DNA <2000 U/mL and normal ALT (n = 41), RE-serum HBV DNA> 2000 U/mL and normal ALT (n = 58). After 3 months of follow-up HBV DNA was assessed in samples available from 129 patients to determine the type of control of HBV infection. Four patterns of infection were identified (LR-LR, LR-RE, RE-LR, RE-RE). Control group consisted of 30 healthy volunteers.

Results: Serum concentration of IL-17F was significantly lower in chronic HBV infection (median: 3.30 pg/mL vs. 9.03 pg/mL, p = 0.00001) compared to controls. Patients with stable low replication (LR-LR, n = 16) had higher IL-17F compared to the group with continuous HBV replication (RE-RE, n = 33), (4.59 pg/mL vs. 3.2 pg/mL, p = 0.02). Moreover patients with significantly elevated HBV-DNA levels (>2000 IU/mL) at baseline that decreased in the follow-up (RE-RE, n = 23) demonstrated higher IL-17F as compared to the RE-RE group (3.78 pg/mL vs. 3.2 pg/mL, p = 0.04). Serum IL-17A concentrations were comparable in CHB and control groups, as well as across respective follow-up groups. However, serum
IL-17A concentration inversely correlated with serum ALT activity ($R_t = -0.2$, $p = 0.01$) and HBsAg concentration ($R_t = -0.68$, $p = 0.01$).

**Conclusions:** The obtained results might suggest that higher serum concentration of IL-17F is associated with immune control of HBV infection which may result in further decline of HBV-DNA.

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**Background and Aims:** The selective oral TLR7 agonist GS-9620 is being developed for the treatment of chronic viral hepatitis by safely inducing long-term immune control after finite therapy. The mechanism of GS-9620-mediated anti-viral effects involves secretion of type I interferons, e.g. IFN-$\alpha$. The goal of GS-9620 therapy is to induce a local hepatic anti-viral response in the absence of systemic IFN-$\alpha$ to avoid interferon-mediated adverse effects. In this in vivo study we investigated whether a low oral dose of GS-9620 can induce a hepatic anti-viral gene expression signature in the absence of detectable serum levels of IFN-$\alpha$.

**Methods:** Nine weeks old male CD-1 mice received vehicle or a dose of 0.3 mg/kg GS-9620 via oral gavage and were sacrificed at 2, 4, 8, 12, and 24 h post-dose (n = 5 per time point). Serum levels of IFN-$\alpha$ were assessed together with the liver expression of antiviral genes MX1, OAS1, and ISG15.

**Results:** GS-9620 induced transient expression of hepatic OAS1, MX1, and ISG15 in all treated animals. Undetectable serum IFN-$\alpha$ (<8 pg/ml) was observed in 2/5 to 5/5 animals at each terminal time point. At 2 to 12 h post-dose, all animals with undetectable serum IFN-$\alpha$ had concurrent two-fold or higher elevation of the hepatic expression of OAS1, MX1, or ISG15 (Figure 1). At 24 h, the hepatic anti-viral gene expression was comparable to vehicle-treated and naïve control animals (not shown).

**Conclusions:** Low dose (0.3 mg/kg) treatment of mice with GS-9620 induced hepatic expression of MX1, OAS1, and ISG15 in animals with undetectable IFN-$\alpha$ in serum. These results suggest that a low oral dose of selective TLR7 agonist can achieve immune activation in the liver in the absence of high systemic levels of IFN-$\alpha$.

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**Background and Aims:** To investigate whether Flavocoxid (containing the natural flavonoids, baicalin and catechin) exerts any antiviral activity against HBV and, if used in combination with Entecavir (ETV), it may act synergistically to completely inhibit HBV activities.

**Methods:** HepG2 cells were transfected with linear wild-type HBV genomes. HBV replicating cells were treated with different dosages of Flavocoxid to determine the drug inhibitory concentrations (IC$_{50}$). Treatment with Flavocoxid or ETV or with drugs combination started 3 hours after transfection and was renewed every other day for 5 days. Total HBV replicative intermediates, viral transcripts and cccDNA levels were evaluated in untreated and treated HepG2 cells by quantitative real-time PCR, Southern and Northern blots experiments. To analyse the epigenetic modulation of HBV cccDNA the cccDNA-ChIP assay was applied to untreated and treated cells.

**Results:** The analysis of HBV replicative intermediates synthesized in the presence or absence of Flavocoxid enabled to determine that IC$_{50}$ of this drug was 75 µg/mL. Five days of Flavocoxid treatment reduced viral RNA levels by 70% than in untreated cells. The ratio between the pregenome/C-mRNA and the preS/S mRNAs did not change during treatment, indicating that all these transcripts were similarly affected by Flavocoxid. This effect was paralleled by a decrease of HBsAg amounts in the supernatants. HBV replicative intermediates decreased to more than 80% of the initial level at 5 days post-treatment. Of note, a 30% reduction of cccDNA amounts was also observed. In cells treated with Flavocoxid and ETV combination therapy, the reduction of the levels of HBV replicative intermediates, transcripts and HBsAg was of about 85%, 90% and 75%, respectively, compared to control cells. In addition, HBV cccDNA was highly hypoacetylated in cells treated with combination therapy.

**Conclusions:** The results of our study demonstrate for the first time that Flavocoxid:

- a. is capable to inhibit HBV replication,
- b. exerts its antiviral activity against HBV at multiple levels and
- c. acts synergistically with ETV in a cell-based HBV replication system.

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**Aim:** To verify the possible occurrence of liver HBV reinfection in HBsAg-positive and HBV/HDV coinfected patients as well as de novo infection in HBsAg-negative liver transplant recipients in the course of LT.
Methods: Explanted and transplanted liver from 21 patients (5 with HBV-related, 6 with HBV/HDV-related and 10 with HCV-related chronic liver disease) were analysed. All but one donor were HBsAg negative, 6 were anti-Hbc positive, 14 were negative for all serum markers. From transplanted livers, biopsy specimens obtained before LT, in post-perfusion period and at end of LT were available. HBV DNA was tested in all liver tissue specimens by nested-PCR amplifications specific for 4 different HBV genomic regions. Total HBV DNA, HBV cccDNA and HDV RNA were quantified by specific real-time-PCR approaches.

Results: At the end of surgery, two of the 5 HBsAg-positive patients (both donors were anti-Hbc positive), 1 of the 6 HBV/HDV co-infected patients (the donor was HBsAg-positive) and 2 of the 10 HCV-infected patients (one donor was anti-Hbc positive and the other anti-Hbc negative) showed the presence of quantifiable amounts of HBV DNA in the liver (range: 6x10^{-4}-1x10^{-3} copies/cells). Sequencing analysis of the isolated HBV genomes showed that they were donor viral strains. HBV cccDNA could be detected and quantified (range: 2x10^{-5}-2x10^{-3} copies/cell) in all the HBV-positive patients. None of the HBV/HDV-infected patients showed HDV RNA in the transplanted liver.

Conclusions: 1. HBV genomes infecting the transplanted liver cause the re-infection of the recipients in the course of LT; 2. HDV does not re-infect the transplanted liver at the time of LT; 3. occult HBV infection (namely, presence of intrahepatic HBV DNA in HBsAg negative individuals) may be present in transplanted liver and may persist during LT independently of the anti-Hbc status of the donor.

397 THE PRO-INFLAMMATORY RESPONSE INDUCED BY TOLL-LIKE RECEPTOR ACTIVATION IN VIVO IS NOT IMPAIRED IN THE LIVER OF HBV-TRANSGENIC MICE LACKING HBsAg


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Introduction: Chronic infection with the hepatitis B virus (HBV) is a major cause of liver-related morbidity and mortality worldwide. Previously, it has been shown that chronicity of infection may be facilitated by an attenuation of innate and adaptive immune responses through HBsAg. Here, we have studied the immunological effects of HBV replication in a transgenic mouse model, lacking the HBsAg (tg1,4HBV-S-mut). We also characterized siRNA-mediated HBV suppression in this system.

Methods: Different nanolipid-formulated siRNAs specific to the HBxAg mRNA, preferentially targeting hepatocytes were injected intravenously into HBV-S-mut mice. HBV negative livers were used as controls. After 48h and 10d tissue (liver, heart, spleen, kidney) was prepared and expression of HBV-RNA, HBV core protein, IFN-beta (IFN-β), interferon sensitive gene 15 (ISG15), interferon-induced protein with tetratricopeptide repeats 1 (IFI-T1), IL-6 and TNF-alpha (TNF-α). ELISAs and cytokine arrays were performed to detect HBcAg and cytokine levels in serum.

Results: Single injection of siRNAs against HBV led to suppression of HBV DNA after 48h which was sustained for more than 10 days. The HBeAg serum levels were also reduced about 90% after treatment with HBxAg-specific siRNA. The expression of IFN-β, IFN-T1 and ISG15 was up-regulated in HBV positive animals compared to control animals, which could be normalized by treatment with HBxAg-specific siRNAs. Transcriptome analysis after siRNA mediated knockdown exhibited different up-regulated and down-regulated genes in HBV-S-mut mice compared to livers, which could be returned to basal expression after siRNA-mediated suppression of HBV. In HBV mice microRNA analysis identified regulated miRNAs, which are associated with HBV infection and progression of hepatocellular carcinoma.

Conclusions: In contrast to human liver from HBV patients, in HBV transgenic mouse model lacking HBsAg viral replication is associated with expression of IFNs and ISGs. Thus, we hypothesize that HBsAg is suppressing the IFN induction and ISG response in vivo. This hypothesis will be challenged in HBsAg transgenic mice.
Background and Aims: Steatosis is a common histopathological feature of chronic hepatitis B (CHB), and has been associated with the severity of liver disease. Recently, the rs738409 I148M PNPLA3 polymorphism has been demonstrated to influence steatosis susceptibility in a large series of patients with biopsy proven CHB.

Aim: To evaluate whether PNPLA3 I148M influences steatosis susceptibility in a large series of patients with biopsy proven CHB.

Methods: 235 treatment-naïve CHB patients consecutively examined by percutaneous liver biopsy were enrolled. In ≥2 cm long liver tissue cores, steatosis and fibrosis were staged by Kleiner and METAVIR scores, respectively. The I148M polymorphism was determined by Taqman assays.

Results: Steatosis was present in 146 (62%) patients, of whom 22 (10%) had severe steatosis, i.e. involving >33% of hepatocytes. Steatosis was independently associated with age (OR 2.67, 1.50–4.92 for age≥50 years), BMI (kg/m²); OR 2.84, 1.30–6.76 for BMI ≥27.5), diabetes or impaired fasting glucose (OR 4.45, 1.10–30.0), and PNPLA3 148M allele (OR 1.62, 1.00–7.0 for each 148M allele). Independent predictors of severe steatosis were BMI (OR 3.60, 1.39–9.22 for BMI ≥27.5 kg/m²), and PNPLA3 148M allele (OR 6.03, 1.23–5.0 for each 148M allele). PNPLA3 148M alleles were associated with a progressive increase in severe steatosis in patients with, but not in those without, acquired cofactors such severe overweight and a positive history of alcohol intake (p=0.005).

Conclusions: In CHB patients, the PNPLA3 I148M polymorphism influences the susceptibility to steatosis, and, in particular when associated with severe overweight and positive alcohol intake, to severe steatosis.

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NUCLEOSIDE ANALOGUES ALONE OR COMBINED WITH VACCINATION PREVENT WHV INFECTION AND INDUCE PROTECTIVE IMMUNITY: NEW STRATEGY FOR HBV POST-EXPOSURE PROPHYLAXIS

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Background: Nucleotide analogues (NA) are successfully used in HIV post-exposure prophylaxis (PEP), but whether NA can be used in Hepatitis B virus (HBV) PEP is unknown. In this study, we analyzed the possibility of using NA in HBV PEP in a Chinese woodchuck model.

Methods: Chinese woodchucks were inoculated intravenously with woodchuck hepatitis virus (WHV) stock and then treated with pWHcIm (Group B), Entacavir (ETV, Group C), or ETV plus pWHcIm (Group D). Six untreated Chinese woodchucks were served as Control (Group A). Twenty weeks later, they were re-challenged with WHV stock. Two Chinese woodchucks were served as re-challenge control (Group E). Sera WHsAg, WHcAb, WHsAb and WHV DNA were measured by ELISA and real-time PCR, respectively.

Results: Chinese woodchucks treated with ETV plus pWHcIm were protected from WHV infection and developed anti-WHc antibodies (Fig. 1). They were also protected from WHV re-challenge (Fig. 2). Meanwhile, Chinese woodchucks treated with ETV alone were protected from WHV infection, but only four woodchucks developed anti-WHc antibodies and were protected from WHV re-challenge (Fig. 1 and Fig. 2). The two animals (1121 and 1123) that did not develop anti-WHc antibodies were not protected from re-challenge (Fig. 2).

Figure 1. The kinetics of WHV DNA, WHsAg, WHcAb and WHsAb following the primary WHV inoculation in Chinese woodchucks. After primary inoculation with a WHV stock at the dose of 108 GE, Chinese woodchucks received pWHcIm vaccine (group B), ETV (group C) and ETV combined with pWHcIm vaccine (group D). Group A was left untreated and used as a control. Serum samples were collected at different time points. WHV DNA, WHsAg, WHcAb and WHsAb were measured by real-time PCR or specific ELISAs. The cut-off value is presented by a dotted horizontal line.

Figure 2. The kinetics of WHV DNA, WHsAg, WHcAb and WHsAb following WHV re-challenge in Chinese woodchucks. Twenty weeks after primary viral inoculation, 2 animals from each group A and B, and all animals from groups C and D were re-challenged with WHV stock at the dose of 109 GE. Two Chinese woodchucks were inoculated with the same amount of WHV and served as the re-challenge control (group E). The serum was collected at different time points. WHV DNA, WHsAg, WHcAb and WHsAb were measured by real-time PCR or specific ELISAs. The cut-off value is presented by a dotted horizontal line.
Conclusion: ETV alone or in combination with DNA vaccine can completely protect Chinese woodchucks from hepadnaviral infection and induce partial or complete protective immunity, respectively. It appears likely that nucleoside analogues alone or in combination with vaccine may be useful in HBV PEP.

401 PERSISTENCE OF CHIMERIC HEPATITIS B VIRUSES IN THE HYDRODYNAMIC INJECTION MOUSE MODEL

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Background and Aims: Viral and host factors may contribute to the persistence of hepatitis B virus (HBV). In the mouse model, the HBV-related virus, woodchuck hepatitis virus (WHV), was able to persistently replicate after hydrodynamic injection while HBV was usually cleared. We generated HBV and WHV chimeras to determine the WHV genome region responsible for persistence.

Methods: HBV genome regions were replaced by the corresponding WHV genome regions and cloned as 1.3 fold overlength genome. The HBV-WHV chimeric genomes were hydrodynamically injected into BALB/c mice. The replication of chimeric genomes in mice was examined.

Results: Eight chimeric genomes were constructed and were found to be able to express surface and nucleocapsid proteins in vitro and in vivo. The replication in mice was confirmed by detection of viral surface protein and DNA. The HBV-WHV chimeric genomes containing the part of WHV core region were able to persist. The presence of this WHV genome region was associated with low levels of viral DNA, indicating a reduced replication activity.

Conclusion: These results are consistent with findings in other studies that the hepadnaviral nucleocapsid protein is an important determinant for viral persistence vs. clearance.

402 REGULATION OF HBV REPLICATION BY miRNA-449a THROUGH TARGETING CREB5 IN HEPATOMA CELLS

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Background and Aims: MicroRNAs (miRNAs) are a class of short non-coding RNAs involved in posttranscriptional gene regulation of multiple pathways. Recent studies have highlighted the potential role of miRNAs in chronic HBV infection and HCC development. Previously, we reported that miR-1 could enhance HBV replication by targeting HDAC4 and E2F5. Here, we addressed the question whether other HCC-related miRNAs regulate HBV replication by targeting host factors.

Methods: miRNA mimics were transfected into hepatoma cell lines stably transfected with a replication-competent HBV genome. HBV replication and gene expression were examined by Southern blot and Northern blot. Microarray analysis was performed to determine the effect of miRNAs on the cellular gene expression profiles. The phenotype of hepatoma cells was characterized by proliferation assay, cell cycle FACS analysis, and real time RT-PCR.

Results: By screening 10 reported HCC-related miRNAs in HepG2.2.15 cells, miR-449a was found to strongly enhance HBV replication, transcription, progeny virions secretion, and antigen expression in a dose-dependent manner. Ectopic expression of miR-449a resulted in G1/S cell cycle arrest and upregulation of a number of hepatocyte-specific genes, including core promoter binding transcription factor farnesoid X receptor α (FXRA). Microarray analysis of the global cellular gene expression profile revealed that 156 candidate targets were down-regulated by miR-449a. Among these candidate genes, cAMP responsive element binding protein 5 (CREB5) was identified as a target of miR-449a with specific binding sites within its 3′ UTR. Knock down of CREB5 expression increased HBV replication and FXRA expression while CREB5 over-expression inhibited HBV replication.

Conclusions: These results indicated that miR-449a targets CREB5 to increase FXRA expression, thereby promotes HBV replication and gene expression. Likely miR-1, it could also inhibit cell cycle transition and promote hepatic cell differentiation. Our findings provide a new concept to understand the role of miRNAs in HBV replication.

403 MESENCHYMAL STEM CELLS EXPRESSING IFITM3 CAN CAUSE THE INHIBITION OF HEPATITIS B VIRUS REPLICATION IN VITRO

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Background: Amniotic-fluid-derived mesenchymal stem cells (AF-MSCs) may be an important way in cell treatment in liver diseases. Hepatitis B virus (HBV) infection can influence the hepatic differentiation and regeneration of engrafted MSCs in liver with HBV-infection. MSCs could resist to HBV infection and suppress of HBV replication. The interferon-induced transmembrane protein (IFITM) mediate cellular resistance to varied virus. The MSCs expressed high levels of IFITM3 when were co-cultured with HepG2 cells transfected transiently with play pHBV1.31. And the mechanism of inhibition HBV replication by MSCs is not yet clear.

Objectives: The present study was to investigate the IFITM3 mediate cellular resistance of MSCs to HBV in vitro. And to ascertain whether IFITM3 play a key role in the mechanism of inhibition HBV replication by MSCs in vitro.

Methods: In present study, firstly the difference of IFITM3-expression was detected between MSCs co-cultured with HepG2.2.15 cells and MSCs. The HepG2.2.15 cell were co-cultured with the genetically modified IFITM3-expressing AF-MSCs, the genetically RNAsilencing IFITM3 and MSCs, respectively. The IFITM3 expression, HBVDNA replication and expression including HBV surface antigen (HBsAg), core antigen (HBeAg) and e antigen (HBeAg) were assessed after cells co-culture in vitro.

Result: Our results showed that the levels of IFITM3 expression were significantly higher in AF-MSCs co-cultured with HepG2.2.15 cells than AF-hMSCs. The high levels of IFITM3 expression observed in genetically modified AF-MSCs are consistent with the time-line for peak expression after lentiviral transduction in vitro. And extreme low levels of IFITM3 expression in RNA interferenced AF-MSCs are consistent with the time-line for bottom expression. There were no inhibition of hepatitis B virus replication in HepG2.2.15 cells co-cultured with RNA interferenced AF-MSCs, and over-expressing IFITM3 could effectively inhibit HBV replication and antigen expression in vitro, the inhibitory effects were maintained for at least three weeks.

Conclusion: Our study suggest that IFITM3 may be a key factor in the mechanism of inhibition HBV replication by MSCs in vitro and AF-MSCs genetically modified to over-express IFITM3 could effectively inhibit HBV replication and may be a novel therapeutic approach in the treatment of HBV-infected end-stage liver diseases.
Background and Aims: A close relationship exists between HBV infection and renal dysfunction. This study was undertaken to evaluate the degree of renal dysfunction in HBV positive patients in a university hospital in Mumbai and factors predicting it.

Methods: We retrospectively analyzed 663 HBV positive patients presenting to our clinic from January 2010-June 2012 for renal infection and renal dysfunction. This study was undertaken to evaluate the degree of renal dysfunction in HBV positive patients in a university hospital in Mumbai and factors predicting it.

Results: Of 584 patients, 24 had acute hepatitis, 209 non-cirrhotic Chronic Hepatitis B (CHB), 214 were inactive carriers, 108 had CHB with cirrhosis, 22 were in immunotolerant phase, 6 had acute-on-chronic liver failure, 1 patient post liver transplant. Majority of patients were HBeAg negative (74.82%). 141 patients (24.1%) had comorbidities (diabetes mellitus and/or systemic hypertension). Serum creatinine was elevated (>1.5 mg/ml) in 20 patients (2.7%) while eGFR (MDRD) was below normal (<90 ml/min/1.72m²) in 291 patients (49.8%). Patients were grouped by baseline renal function:

1. eGFR <60 ml/min/1.72m², 42 patients,
2. eGFR 60–90 ml/min/1.72m², 249 patients,
3. eGFR >90 ml/min/1.72m², 293 patients.

Based on HBV disease status, renal function was different: In acute group (acute hepatitis, immunotolerant), 26.53% patients had mild renal dysfunction (eGFR 60–90 ml/min/1.72m²) versus chronic disease group (inactive carrier status, CHB, cirrhosis, others) with 44.49% (p = 0.008). In multivariate analysis, age, gender, hypertension and chronic disease stage had significant association with reduced eGFR. 1 year follow-up eGFR values were available for 321 patients, of which 79 (25%) showed worsening of renal function by >20% while 58 patients (15%) showed >20% improvement. Within the cirrhotic population, Diabetes was significantly more prevalent than in other populations (33% of patients). 62/108 (57%) of cirrhotic patients had reduced renal function.

Conclusion: HBV infection is associated with significant degree of reduced renal function, reaching up to 50% in the chronic population of our clinic. Comorbidities (25%) or antiviral treatment might further increase renal risk, therefore regular monitoring of renal function is recommended. MDRD formula was shown to be more sensitive than serum creatinine for renal function evaluation.

405 HARPE STUDY: PREVALENCE OF RENAL ABNORMALITIES IN CHRONIC HBV INFECTION

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Introduction: Few data are available on renal abnormalities (RA) prevalence in chronic hepatitis B virus infected patients. The HARPE study (Hepatitis And Renal Parameters Evaluation), multicentric, prospective, evaluated the prevalence of renal impairment, and RA: electrolytes, urinary sediment in chronic HBs antigen-positive (HBsAg+) patients, with active or inactive infection.

Patients and Methods: Included patients were adult, mono-infected and oral anti-HBV treatment (AT)-naïve: 268 patients were included over 2 years by 8 liver units. Univariate tests and multiple linear regressions comparing the RA between inactive and chronic active carriers, between untreated and about to start AT patients, and between patients with normal transaminase level and abnormal one, were performed with the SAS software, version 8.02 (SAS, Inc., Cary, NC).

Results: The mean age of patients (58% males) was 42±14 years, mean time since HBV diagnosis was 8.7 years: 59.6% (155/260) were inactive carriers, 35.0% (91/260) had an active infection including 58.2% (53/91) with abnormal transaminase levels, 3.8% (10/260) were immune tolerant and 1.5% (4/260) were unclassified with fluctuating transaminase levels. 47 patients were about to start an AT: 47.2% (43/91) of the active patients, 1.9% (3/155) of the inactive carriers and 10% (1/10) of the immune tolerant patients. Prevalence of proteinuria, hematuria, glycosuria, leukocyturia were 37.4% (58/155), 20.8% (32/154), 3.9% (6/153) and 11.7% (18/154), respectively. 55.8% of patients had at least one RA and 40.7% of patients had at least an abnormal glomerular filtration rate under
90 ml/min/1.73m². According to the international (KDOQI/KDIGO) CKD (chronic kidney disease) definition 27% of patients had CKD stage 1 to 3 and none had stage 4 or 5. Diabetes, hypertension and dyslipidemia were observed respectively in 4.6%, 9.2% and 38.8% patients. There were no significant differences in RA prevalence between the 3 groups compared.

**Conclusion:** RA are present in 1/2 and CKD in 1/4 of HBsAg(+) patients, regardless of the immune stage and before any AT, evidencing the need for:
1. a renal evaluation in all;
2. a regular renal monitoring before and during AT to diagnose and manage renal impairment and adjust dose of AT to the renal function.

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**FULL GENOME ULTRA-DEEP PYROSEQUENCING INDICATES AN IMPORTANT ROLE OF HYPERMUTATION IN THE NATURAL PROGRESSION OF CHRONIC HEPATITIS B**

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**Background and Aims:** Human APOBEC3 (A3) cytosine deaminases are antiviral restriction factors capable of editing the genome of the hepatitis B virus (HBV). Despite the importance of the human A3 protein family for the innate immune response little is known about the clinical relevance for HBV. The aim of this study was to utilize ultra-deep pyrosequencing (UDPS) data to analyze the phenomenon of G-to-A hypermutation of the HBV genome and to relate it to fundamental patient characteristics of chronic hepatitis B.

**Methods:** Ultra-deep pyrosequencing was performed to analyze the viral population of 47 HBeAg-positive and 33 HBeAg-negative treatment naïve patients. Seven overlapping amplicons were used to cover the whole genome of HBV. The G-to-A preference exchanges were used to identify hypermutated sequences. Each read was classified as normal or hypermutated based on the combination of these two statistics with individual cutoffs.

**Results:** Median alanine-aminotransferase levels did not differ significantly between HBeAg-positive and HBeAg-negative patients. Median HBV DNA level were 10.1 log copies per mL and 8.0 log copies per mL for HBeAg-positive and HBeAg-negative, respectively (P < 0.001). Our data indicates that for HBeAg-positive and HBeAg-negative hepatitis G-to-A hypermutation focused in a region between nucleotide positions 1100 and 1600. This part of the genome is single-stranded in mature HBV particles. Hypermutation rates for HBeAg-negative patients were more than 10-fold higher compared to HBeAg-positive patients. For HBeAg-positive chronic hepatitis G-to-A hypermutation rates were significantly associated with the relative prevalence of the G1764A mutation (P = 0.0002), which is related to HBeAg seroconversion. Additionally, we found that for HBeAg-negative patients higher hypermutation rates were significantly associated with the higher degrees of fibrosis obtained by histology (P = 0.004).

**Conclusions:** The G-to-A hypermutations were unequally distributed across the genome. Additionally, the frequency of hypermutated genomes significantly depended on the patients’ HBeAg status and the degree of fibrosis. In total our data suggest an important role of G-to-A hypermutation in the natural progression of chronic hepatitis B and especially in the development of HBeAg-negative hepatitis.
NA therapy in comparison to Peg-IFN is lacking and might provide useful information into viral immune control.

**Aims:** To compare the kinetics of HBsAg, IP-10 and HBV DNA levels before and during therapy with Peg-IFN and then in Peg-IFN non-responders (NR) after at least 6 months break (median 15 months) during consequent NA therapy in the same cohort of CHB patients.

**Patients:** 44 monoinfected CHB patients (68% HBeAg+, 20% cirrhotic, median age 37y) were treated with Peg-IFN for 48 weeks. 19 Peg-IFN non-responders were consequently treated with NA (9 entecavir and 10 tenofovir) for at least 12 months.

**Methods:** Plasma levels of HBsAg were measured by Abbott ARCHITECT® assay, HBV DNA by real-time PCR [both log10 IU/ml] and IP-10 levels by ELISA [pg/ml] at baseline, month 6 (M6), M12 and follow-up month 6 (FUM6) for Peg-IFN. All results are presented as medians.

**Results:** During Peg-IFN therapy, baseline HBsAg, HBV DNA and IP-10 were similar. HBsAg decline was steeper in responders (n=17) vs. non-responders (n=27) during therapy and follow up (M6: −1.03 vs. −0.26; M12: −1.47 vs. −0.39 and FUM6: −0.27 vs. −1.63, all p<0.01), but IP-10 levels were similar. During NA therapy in 19 Peg-IFN NR, HBsAg decline was similar between Peg-IFN and NA therapy (M6: −0.23 vs. −0.13 and M12: −0.31 vs. −0.28), while IP-10 levels kinetic was different during Peg-IFN and NA therapy in same patients (baseline: 162 vs. 288; M6: 331 vs. 124 and M12: 279 vs. 129, both p<0.01).

**Conclusions:** Response to Peg-IFN was linked with sharper HBsAg levels decline on treatment. Plasma HBsAg kinetics were similar during Peg-IFN and NA therapy in Peg-IFN non-responders in contrast to differences in IP-10 kinetics during Peg-IFN and consecutive NA therapy likely due to disparity in viral control.

**409 INDICATIONS FOR LIVER BIOPSY IN HBeAg-NEGATIVE CHRONIC HEPATITIS B VIRUS INFECTION WITH PERSISTENTLY NORMAL ALT. REAL LIFE STUDY**

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**Background and Aims:** According to the recent EASL guidelines, patients with hepatitis B antigen (HBeAg) negative chronic hepatitis B with persistently normal ALT levels require liver biopsy or therapeutic intervention only if serum hepatitis B virus (HBV) DNA is more than 20,000 IU/ml. The aim of this study was to evaluate the severity of the histological lesions among patients with 20000 IU/ml < HBV DNA < 20,000 IU/ml to identify the eventual frequency of those requiring treatment.

**Methods:** Among a total of 80 Liver biopsies performed in patients with HBeAg negative chronic HBV infection, between January 2007 and April 2012 in our department, 38 patients were included in this study. All patients had positive hepatitis B surface antigen (HBsAg), negative HBeAg for at least 6 months and persistently normal ALT activity. The serum HBV DNA cut off was 2000 IU/mL. Patients with hepatitis delta virus, human immunodeficiency virus or hepatitis C virus coinfection were excluded, as well as those with cirrhosis at presentation. Patients were considered to have persistently normal ALT activity if they had ALT values below 40 IU/ml during the total follow-up period, which was at least 1 year. The same team analyzed histological lesions according to METAVIR classification. Grading score g2 and/or stage S2 was used for treatment indication.

**Results:** Metavir grading score g2 and/or stage S2 was found in 36.8% of patients (14/38). Forty percent of patients with HBV DNA between 2000–20,000 IU/ml (10/25) had histological indication for treatment, whereas 30.7% (4/13) of chronic hepatitis B patients with HBV DNA >20,000IU/ml.

**Conclusion:** In our study, we demonstrated that HBeAg negative chronic HBV patients with persistently normal ALT and HBV DNA between 2000 and 20,000IU/ml had indication of treatment in 40% of cases, and could be missed when enforcing the new EASL guidelines. These results could be explained by a long evolution of the chronic hepatitis B in our patients. Finally, other large studies are necessary to confirm and analyze these results.

**410 HBSAg LOSS IS CORRELATED WITH LESS BASELINE VIRAL DIVERSITY IN GENOTYPE A, HBeAg+ CHRONIC HEPATITIS B (CHB) SUBJECTS TREATED WITH TENOFOVIR DF (TDF) LONG-TERM**

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**Background and Aims:** Previous analyses demonstrated lower genetic distance within HBV polymerase/reverse transcriptase (pol/RT) and HBsAg in HBeAg+ genotype D (gtD) CHB subjects who lost HBsAg compared to control subjects who maintained high HBsAg levels during 192 weeks of TDF treatment. This study focuses on comparable groups of 14 genotype A (gtA) subjects to explore genotype-specific differences in the mean and variance in genetic distance.

**Methods:** Study GS-US-174–0103 subjects were randomized 2:1 to receive TDF or adefovir dipivoxil for 48 weeks followed by open-label TDF. After 4 years, 23/266 experienced HBsAg loss, including 14 gtA subjects. 17 gtA subjects that maintained high HBsAg levels with similar baseline HBV DNA and ALT were selected as controls. Population sequencing was performed on baseline samples with MUSCLE alignment used to calculate viral diversity and PhyML to create a pair-wise genetic distance matrix for regions within HBsAg and pol/RT genes. Non-parametric Levene test for homogeneity of variances in control and S-loss groups was performed for each region, and equality of mean genetic distances within regions was evaluated using the Mann–Whitney-U test. The Hochberg procedure was used to control for multiple testing.

**Results:** The mean pair-wise genetic distance was significantly lower in 5 of 8 regions in HBsAg loss subjects compared to controls. Variance was significantly lower in the same five regions. Differences in the mean and variance in genetic distance were localized to the pol/RT gene, encompassing the small HBsAg gene. No significant differences were observed in the preS1/preS2 regions. These findings on lower mean genetic distance are consistent with previous results in gtD HBsAg loss subjects. The data also reveal differences, including significantly lower variance in gtA HBsAg loss subjects compared to controls, which was not seen in gtD, and greater mean genetic distance within analyzed regions in gtD compared to gtA subjects.

**Conclusions:** In this cohort, lower levels of mean and variance in genetic distance in small HBsAg and pol/RT genes were observed in gtA, HBeAg+ CHB subjects who lost HBsAg compared to controls who maintained high HBsAg levels on TDF treatment. Collectively, these findings point to immune-mediated pressure and genotype-specific mechanisms underlying HBsAg loss.

**411 A NEW HBSAg/ANTI-HBs IMMUNE COMPLEX ASSAY PREDICTS HBeAg LOSS IN CHRONIC HEPATITIS B PATIENTS**

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**Background and Aim:** We studied whether HBsAg/anti-HBs immune complex levels in chronic hepatitis B (CHB) patients...
receiving anti-viral therapy could be used as a response marker at baseline (BL), or early during treatment to predict treatment outcome.

**Methods:** A prototype array-based assay served (IMPACT – Immunological Multi-Parameter Chip Technology, Roche Diagnostics) to determine HBsAg, anti-HBs and complex levels. We tested a panel of serum samples of 40 HBeAg-positive and 44 HBeAg negative patients who received pegylated interferon and adefovir for 48 weeks and were followed subsequently for 2 years.

**Results:** HBsAg loss occurred in 4 of 40 HBeAg positive and 7 of 44 HBeAg negative patients. Sixteen of 40 HBeAg positive patients lost HBeAg and 13 of them formed anti-HBe. At BL complexes were present in 83 (95%) patients, whereas free anti-HBs levels were only detectable in 5 patients. Complex levels at BL and WK 12 were higher in HBeAg positive patients with HBeAg loss, compared to patients who retained HBeAg (p=0.0046 and p=0.026 respectively). ROC analysis for HBeAg loss in HBeAg positive patients at BL and WK 12 showed AUC 0.77 (p=0.004) and AUC 0.73 (p=0.026) for complex levels and AUC 0.57 (non significant) and AUC 0.61 (non significant) for HBsAg levels. We saw no correlation in either HBeAg-positive or -negative patients between complex levels and HBsAg loss. Nor did we find any correlation between complex and HBsAg or anti-HBs levels.

**Discussion:** We demonstrated for the first time that before and during treatment HBsAg/anti-HBs immune complex levels can predict HBeAg loss in HBeAg positive CHB patients treated with peg-interferon and adefovir. Complexes were present in almost all patients at BL and were higher in patients that lost HBeAg. In conclusion, determining HBsAg/anti-HBs immune complex levels before and early during treatment could select CHB patients with an optimal chance to achieve HBeAg loss.

412 PREVALENCE, VIROLOGICAL AND CLINICAL CHARACTERISTICS OF CHRONIC HEPATITIS DELTA VIRUS INFECTION (CHD) IN ROMANIA


**Background:** Chronic hepatitis delta (CHD) is associated with accelerated progression of fibrosis, early occurrence of hepatic decompensation and an increased risk for hepato cellular carcinoma. In Romania, the real prevalence of HDV infection is not known. The aim of our study was to assess the prevalence, virological, clinical and epidemiological features of HDV infection in Romanian patients.

**Methods:** We conducted a national, multicenter, prospective study on 2761 HBsAg(+) patients in 10 centers. Sociodemographic characteristics and potential risk factors were collected using a dedicated questionnaire. Virological markers of HBV and HDV infection, biochemical and clinical features of liver disease were evaluated. Qualitative or quantitative variables were analyzed using nonparametric tests, the Chi-square test, Fisher’s exact test or the Mann–Whitney test, as appropriate. Data have been analyzed using STATA/SE 11 package.

**Results:** Study population comprises 2761 HBsAg(+) patients, 84.78% HBeAg negative, 55.7% males, with a mean age of 43.8±13.8 years, predominantly from urban area (78.9%). Liver cirrhosis was detected in 22.4% of patients, while 68.5% had chronic hepatitis; in 9.1% the type of liver disease was not recorded. The prevalence of IgG anti-HDV(+) patients was 23.14%, out of which 16.44% were HDV RNA positive. The prevalence of IgG anti-HDV and HDV RNA was higher in females than in males (24.63% vs. 21.96%, p=0.243 and 17.02% vs. 15.98%, p=0.345, respectively). By age groups, the highest prevalence of HDV infection was encountered in patients aged 50–59 years (29.8%), followed by patients aged 60–69 (24.85%), and 40–49 years (21.93%) (p=0.0001). Seroprevalence of delta hepatitis was significantly higher in AgHBs(+) cirrhotics vs. noncirrhotics (54.79% vs 19.78%, p=0.0001). A reduced proportion of IgG anti-HDV & HDV RNA positive patients with chronic delta hepatitis, respectively cirrhosis had HBV DNA levels above 2000UI/ml (5.97%, p=0.0001), or >10000UI/ml (25%, p=0.001), respectively.

**Conclusions:** HBsAg(+) population in Romania is characterized by increased prevalence of HBeAg(−) HBV infection and high prevalence of HDV coinfection (23.14%). Such as in HCV and HBV mono infections, a cohort phenomenon can be observed, with the highest prevalence in age groups 50–59 and 60–69. Many of these patients had advanced liver disease (22.4% cirrhotics) and low HBV viral load.

413 AGE AND THE INACTIVE HBsAg CARRIER STATE ARE PREDICTIVE OF HBsAg LOSS IN A “REAL-LIFE” COHORT OF PATIENTS WITH CHRONIC HBV INFECTION

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**Background and Aims:** In chronic hepatitis B virus (HBV) infection, HBsAg loss is the main indicator of recovery. Incidence and predictive factors of HBsAg clearance were described in cohorts of selected patients. We aimed to assess factors associated with HBsAg loss and its incidence in a “real-life” cohort of non-selected patients.

**Methods:** Patients with HBsAg(+) for at least 6 months, negative for HIV, HCV and HDV, without liver transplantation and hepatocellular carcinoma were prospectively included. Epidemiological, clinical, biological and therapeutic parameters were collected from the first visit until the loss of HBsAg or the end of follow-up. To determine the factors linked to HBsAg loss, univariate and multivariate statistical analyses were performed.

**Results:** This study included 315 patients followed over a mean period of 5.7 years ± 3.9. One hundred and nine patients were inactive HBsAg carriers, 69 patients had HBeAg(+) chronic active hepatitis (CAH) of whom 14 were untreated and 55 were treated, 135 had HBeAg(−) CAH of whom 62 were untreated and 73 were treated, and finally 2 were immune tolerant carriers. By univariate analyses, HBsAg loss was associated with older age (P=0.001, HR=1.050, CI=1.021–1.080). By multivariate analysis, older age (P=0.001, HR=1.054, CI=1.022–1.088) and the inactive HBsAg carrier state (P=0.019, HR=2.653, CI=1.172–6.024) predicted
HBsAg clearance. Gender, race, BMI, alcohol consumption, HBV DNA and ALT serum levels, HBeAg status and anti-HBV therapy were not associated with HBsAg loss. Inactive carriers had an annual incidence of HBsAg clearance of 23.4 cases per 1,000 persons-years which was superior to those observed in CAH groups: untreated HBeAg (+) (19.1), treated HBeAg (+) (20.7), untreated HBeAg (−) (8.1), treated HBeAg (−) (10.1).

Conclusions: The results of this study in a non-selected “real-life” population of HBV carriers show that older age and the inactive HBsAg carrier state are independent predictive factors of HBsAg loss. Administration of treatments in HBeAg (+) or (−) CAH groups increased only slightly the annual incidence of HBsAg loss. Host-related factors seem to be important for HBsAg clearance.

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THE SEROSTATUS OF HEPATITIS B AFTER 50 MONTHS FOLLOW-UP AMONG 313 HEPATITIS B SERONEGATIVE YOUNG ADULTS BORN AFTER NEONATAL HEPATITIS B VACCINATION ERA
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Introduction: This study aims to investigate the serostatus of those who were hepatitis B seronegative at the freshmen age receiving hepatitis B booster or not after 50 months follow-up in Taiwan.

Materials and Methods: Young adults born after July 1986 in 2011 master program entry health check-up at one medical center in Taiwan were invited to participate. Three hepatitis B viral markers including hepatitis B surface antigen (HBsAg), antibody to hepatitis B core protein (anti-HBc), and antibody to hepatitis B surface antigen (anti-HBs) were tested by ELISA. Their hepatitis B viral markers examined in bachelor program entry health check-up were linked by chart review. The university hepatitis B vaccination records were linked through University health center. This study was approved by Research Ethics Committee at National Taiwan University Hospital.

Results: A total of 313 young adults (58%) who were all seronegative for three hepatitis B seromarkers were enrolled. 215 (68.7%) of them were male. Their mean age was 22.7 ± 0.8 years. The mean follow-up period was 50.1 ± 7.6 months. No one had become HBsAg positive or anti-HBc positive. 173 cases (58.5%) had either hepatitis B vaccination records or they self reported hepatitis B vaccination during their university life. 128 (74.0% of 173 cases) became seroprotective for hepatitis B (anti-HBs > 10 mIU/mL). The seroprotection rate for hepatitis B vaccines doses among the vaccinees was 56.7% for one dose, 81.6% for two doses, and 89.4% for at least three doses, respectively. It was worth noting that 45 cases (26%) still remained seronegative for hepatitis B after receiving at least one dose of hepatitis B vaccine. Six of 48 cases (12.5%) who had received at least 3 doses of hepatitis B booster vaccines failed to be seroprotective.

Conclusions: No breakthrough infection of hepatitis B was noted after 50.1 months follow-up for 313 hepatitis B seronegative young adults born after Neonatal Hepatitis B vaccination era. At least two doses of hepatitis B vaccines booster was suggested to get higher seroprotection rate. The Re-vaccination failure rate for these young adults was estimated to be 12.5%.

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SNP rs12356193 AND PLASMA CARNITINE LEVELS ARE ASSOCIATED WITH HBsAg LOSS AFTER COMBINATION TREATMENT WITH PEGINFERON AND ADEFOVIR
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Background and Aim: Genome wide association studies (GWAS) can identify genetic factors that influence therapy response, e.g. the IL28B polymorphism in hepatitis C. We performed a GWAS in chronic hepatitis B (CHB) patients to find associations between single nucleotide polymorphisms (SNPs) and treatment outcome.

Patients and Methods: 84 CHB patients (40 HBeAg positive; 44 HBeAg negative) received Peg IFN αα-2a and Adefovir for 48 weeks, followed by a treatment-free follow-up. Ethnic background was: Caucasian (26), African (27) and Asian (31). Primary clinical outcome – undetectable HBsAg at week 96 (Abbott AxSYM) – was achieved in 9 patients (11%). All patients with HBsAg loss developed anti-HBs antibodies. Patients were genotyped with the Illumina Human Omni1-Quad BeadChip. Associations were analyzed in R Statistical Software with GenABEL, including the Eigenstrat algorithm for population stratification. In total 777,443 SNPs passed quality control. After Bonferroni correction, associations with p < 6.43 x 10–8 (Cochrane-Armitage’s trend test), were considered genome wide significant. Plasma carnitine levels were measured by tandem mass spectrometry.

Results: One SNP, rs12356193 located in the SLC16A9 gene, was significantly associated with HBsAg loss at week 96 (p = 2.61e-09). This allele is rare in people of Asian ancestry. HBsAg loss distribution in non-Asians was: AA 0% (n=37), AG 43% (n=14), GG 100% (n=2). This SNP was strongly associated with carnitine levels in other GWAS studies. Mean baseline carnitine levels in non-Asian patients was 35.3 µmol/L (SD 7.4). All non-Asian patients with HBsAg loss were male (n=8). In non-Asian males (n=44), patients with genotype AG/GG had lower carnitine levels (32.7 vs 38.5 µmol/L, p=0.008). In addition, carnitine levels were lower in non-Asian males with HBsAg loss (28.4 vs 38.3 µmol/L, p<0.001).

Conclusion: In a GWAS we identified a SNP (rs12356193) in the SLC16A9 gene associated with HBsAg loss in non-Asian CHB patients. In non-Asian patients, baseline carnitine levels were lower in patients with the favourable allele, as well as in patients with HBsAg loss. Besides a role in prediction of treatment outcome, these novel associations may provide new insight into the host immune response to HBV infection.

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PREDICTION OF ACTIVE HBV-CARRIER STATUS IN A COHORT OF EUROPEAN UNTREATED HBsAg NEGATIVE PATIENTS: A PROSPECTIVE LONGITUDINAL STUDY
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Background and Aims: Former studies from Asia could demonstrate the impact of single point HBV-DNA levels at baseline...
for prediction of development of disease progression during natural course of HBV infection.

The aim of the present study was to prospectively assess predictive factors associated with the outcome during long-term follow-up of a cohort of European untreated HBeAg negative HBs Antigen carriers.

Methods: 244 treatment-naive patients from 5 different German centers for liver diseases with persistent HBeAg-negative HBV infection were prospectively followed (mean 2.7 years; range 2–35 months). All patients were considered not to be candidates for antiviral treatment at study inclusion based on international guidelines (ALT <2 ULN and/or HBV-DNA <2000 U/ml). Biochemical and virological parameters as well as non-invasive fibrosis tests were performed at baseline and at least once per year during follow-up. Treatment decision was made by the presence of active carrier status (HBV-DNA>2000U/ml, repeated elevated transaminase levels and/or evidence of advanced fibrosis/cirrhosis).

Results: Patients were classified according to their virolocal and biochemical profile: nALTNHBV (normal ALT, HBV-DNA ≤2000U/ml), nALTEHBV (normal ALT, HBV-DNA>2000U/ml), eALTNHBV (elevated ALT, HBV-DNA ≤2000 U/ml) and eALTEHBV (elevated ALT, HBV-DNA >2000U/ml). At baseline classification of nALTNHBV, nALTEHBV, eALTNHBV and eALTEHBV was observed in 62.5%, 21.4%, 11.6% and 4.5% of our patients compared to 60.7%, 20.5%, 15.2% and 3.6% at follow-up. Antiviral treatment was started in 4/244 patients after 1 (n=2) and 2 years (n=2) of follow-up. HBsAg concentration (p<0.04), ALT (p<0.04), AST (p<0.035) and HBV DNA>2000U/ml (p<0.049) at baseline were significantly associated with development of active carrier status. The combined single point quantification of HBsAg (>2000 U/ml), HBV-DNA (>2000U/ml) and elevated transaminases at baseline identified active hepatitis B carriers with 87% diagnostic accuracy, 75% sensitivity, 99% specificity, 75% PPV as well as 99% NPV. No patient developed HCC or cirrhosis during 2 years follow-up.

Conclusion: Higher baseline levels of HBsAg, HBV-DNA and transaminases turned out to be strong predictors of active HBV carrier status during prospective follow-up. Further analyses with long-time monitoring and larger number of patients are required to evaluate dynamics of HBV infection leading to disease progression.

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IL28B POLYMORPHISM CORRELATES WITH ACTIVE HEPATITIS IN PATIENTS WITH HBeAg-NEGATIVE CHRONIC HEPATITIS B

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Background and Aims: The clinical relevance of single nucleotide polymorphisms (SNPs) near the IL28B gene is controversial in patients with hepatitis B virus (HBV) infection. This study aimed to investigate the role of viral and host factors, including IL28B genotypes, in the natural course of chronic hepatitis B (CHB).

Methods: The study enrolled consecutive 115 treatment naïve CHB patients. HBV viral loads, genotypes, precore and basal core promoter mutations, serum hepatitis B surface antigen (HBsAg) and interferon-gamma inducible protein 10 (IP-10) levels as well as four SNPs of IL28B were determined. Serial alanine transaminase (ALT) levels in the previous one year before enrollment at an interval of three months were recorded. Factors associated with active hepatitis, defined as persistent ALT >2 x upper limit of normal (ULN) or a peak ALT level >5 x ULN, were evaluated.

Results: The prevalence of rs8105790 TT, rs12979860 CC, rs8099917 TT, and rs10853728 CC genotypes were 88.3%, 87.4%, 88.4% and 70.9%, respectively. In HBeAg-positive patients (n=48), HBV viral load correlated with active hepatitis, while in HBeAg-negative patients (n=67), rs10853728 CC genotype (p=0.032) and a trend of higher IP-10 levels (p=0.092) were associated with active hepatitis. In multivariate analysis, high viral load (HBV DNA >10^8 IU/ml, p=0.042, odds ratio=3.946) was significantly associated with HBeAg-positive hepatitis, whereas rs10853728 CC genotype (p=0.019, odds ratio=3.927) was the only independent factor associated with active hepatitis in HBeAg-negative population.

Conclusions: HBV viral load and IL28B rs10853728 CC genotype correlated with hepatitis activity in HBeAg-positive and HBeAg-negative CHB, respectively. Both viral and host factors play roles in disease activity during different phases of CHB.

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ASSOCIATION OF POLYMORPHISM IN microRNA 219–1 WITH CLEARANCE OF HEPATITIS B VIRUS INFECTION

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Background: Polymorphisms in the primary microRNA region may be associated with natural course of hepatitis B virus (HBV) infection. In the present study, we evaluated if the microRNA 219–1 (miR-219–1) polymorphism can influence the susceptibility towards persistence of HBV infection and the progression to hepatocellular carcinoma (HCC) in patients with chronic HBV infection.

Methods: A total of 1,439 individuals having either past or present evidence of HBV infection were enrolled for the study. The subjects were divided into 4 groups; (1) spontaneous recovery from HBV infection (n=404), (2) chronic HBV carrier without cirrhosis (n=313), (3) chronic HBV carrier with cirrhosis (n=305), and (4) hepatocellular carcinoma (n=417). We genotyped three polymorphic variants (rs421446, rs107822 and rs213210) in the pri-miRNA region of miR-219–1.

Results: The rs421446 T allele was found to be strongly associated with HBV clearance (OR = 0.73, P=0.0005 in a codominant model and OR = 0.67, P=0.0009 in a dominant model, OR = 0.69, P=0.04 in a recessive model, respectively). The rs107822 G allele was also found to be associated with HBV clearance (OR = 0.79, P=0.008 in a codominant model and OR = 0.72, P=0.01 in a dominant model, respectively). In haplotype analysis, ht2 (T-G-T) and ht1 (C-A-C) were found to be in significant association with the clearance of HBV. However, no significant association was observed between miR-219–1 polymorphism and the risk of HCC occurrence in chronic HBV carrier.

Conclusion: Our result suggests that polymorphisms in the pri-miRNA region of miR-219–1 might be a genetic factor for HBV clearance after infection.
419  QUANTITATIVE SERUM HEPATITIS B SURFACE ANTIGEN IMPROVES PREDICTABILITY OF HBV DNA SEROCLEARANCE BUT NOT HBeAg SEROCLEARANCE IN CHRONIC HEPATITIS B PATIENTS

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Background and Aims: HBeAg and HBV DNA seroclearance are important milestones in the natural history and treatment of chronic hepatitis B. This study aims to investigate whether the addition of serum HBsAg levels can improve the current predictability of both HBeAg and HBV DNA seroclearance.

Methods: 2139 HBeAg seropositive treatment naive individuals with detectable HBV DNA who were anti-HCV seronegative and free of liver cirrhosis were enrolled during 1991–1992 and followed-up until June 30, 2004. A subset of 431 HBeAg seropositive individuals was used to examine HBeAg seroclearance. Cox proportional hazards models were used to construct regression models for seroclearance, and predictive accuracy was assessed with time-dependent ROC curves. Baseline HBsAg levels were quantified using the Roche Elecsys HBsAg II Quant assay.

Results: Serum HBsAg levels did not play a significant role in HBeAg seroclearance. Compared to those with serum HBsAg levels <10,000 IU/ml, those with levels ≥10,000 IU/ml had an adjusted rate ratio of HBeAg seroclearance of 0.98 (0.58–1.64). HBV DNA levels remained the most important predictor of HBeAg seroclearance. However, serum HBsAg levels were the most important predictor of HBV DNA seroclearance, even after adjustment for HBV DNA levels, whose effect greatly decreased when HBsAg levels were added. Compared to individuals with baseline serum HBsAg levels ≥10,000 IU/ml, the multivariate adjusted rate ratio (95% CI) of HBV DNA seroclearance was 1.68 (1.02–2.77), 3.20 (1.93–5.30), and 9.32 (5.57–15.62) for those with serum HBsAg levels of 1000–9999, 100–999, and <100 IU/ml, respectively. The corresponding rate ratios among HBeAg seronegative individuals were 1.10 (0.57–2.13), 2.39 (1.25–4.56), and 6.56 (3.43–12.56). Adding serum HBsAg levels into regression models improved AUROC’s from 0.60–0.77, and from 0.57–0.69 for the five- and ten-year prediction of HBV DNA seroclearance (P < 0.001).

Conclusion: The addition of serum HBsAg levels to current HBV DNA-based models can significantly improve the predictability of HBV DNA seroclearance among chronically infected genotype B and/or C carriers.

420  A PAUCITY OF LIVER DISEASE IN CANADIAN INUIT WITH CHRONIC HEPATITIS B VIRUS, SUBGENOTYPE B6 INFECTION

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Background: Clinical impressions suggest that chronic hepatitis B virus (HBV) infections in the Canadian Inuit are less associated with serious adverse outcomes than has been described in other HBV infected patient populations.

Objective: To document the clinical and biochemical features, liver-related morbidity and all cause mortality in Canadian Inuit with chronic HBV infections.

Methods: Administrative databases were reviewed for individuals identified as hepatitis B surface antigen (HBsAg) positive during a 1983–85 seroepidemiologic survey of viral hepatitis in Baffin Island, Canada. An equal number of age and gender matched HBsAg negative individuals from the same communities served as controls. Baseline HBV viral loads, genotypes and specific mutations were compared in HBsAg positive survivors and non-survivors. A subset of surviving HBsAg positive carriers were reassessed 25–30 years following their initial diagnosis for evidence of advanced liver disease and changes to their serologic/virologic findings.

Results: 144 HBsAg positive individuals were identified. All were Canadian Inuit. The mean age at diagnosis was 38±17 years and 69 (61%) were male. Median follow-up was 23 years (range: 2–28 years). Viral quantitation from stored sera could be performed in 70 infected individuals. The median viral load was 4.3 log 10 IU/ml (range: 2.3–8.8 log 10 IU/ml) and all were genotype B, subgenotype B6. Liver biochemistry, morbidity and all cause mortality rates were similar in HBsAg positive carriers and controls. Following multivariate analyses, only age at diagnosis predicted mortality in HBsAg carriers. In a subset of 34 HBsAg positive survivors who underwent follow-up assessments, clinical, biochemical and radiologic examinations of the liver were essentially normal. 27/34 (79%) remained HBsAg positive and 15/19 (79%) HBV-DNA positive. Viral loads, genotype distribution and prevalence of genomic mutations in this cohort remained largely unchanged.

Conclusions: The results of this study suggest that chronic HBV infections in the Canadian Inuit are infrequently associated with serious adverse outcomes. Whether this finding reflects unique features of the host, presence or absence of external factors that influence the course of HBV and/or intrinsic properties of the HBV B6 subgenotype remains to be determined.
none on nonBnonC group. Occult HBV infection was detected in 12 CHC cases (20.7%) and 8 nonBnonC cases (30.8%). There were no significant differences on positive rate between tumor and non-tumor tissues on any legions.

II. HBsAb was positive in 2 case in group O (10%) and 22 cases in control (26.2%) (n.s.). HBeAb was positive in 4 case in group O (20%) and 26 cases in control (40.6%) (n.s.). No case was HBeAb positive in group O and 8 positive cases in control (12.5%) (n.s.). There were no significant difference on hepatic function markers, and nor on tumor sizes, tumor numbers, AFP and DCP.

Conclusions: Detecting with high sensitive detection system, occult HBV infection rate among HCC patient was 23.8% in Japan. Occult HBV infection cannot be detected with serum study.

422 THE IMPACT OF DECREASING LEVELS OF SERUM HBsAg ON HBsAg SEROCLEARANCE IN CHRONIC HEPATITIS B
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Background and Aims: Decreasing levels of serum HBsAg preceeding HBsAg seroclearance has not been investigated sufficiently. The aim of this study was to investigate the association between decreasing levels of HBsAg and HBsAg seroclearance.

Methods: We studied 99 Japanese chronic hepatitis B patients who had been followed for longer than 10 years in our institute. HBsAg levels were measured annually using a HISCL HBsAg assay based on CLEIA developed by Sysmex corporation (Köbe, Japan). The assay had a quantitative range of −1.52 to 3.39 log IU/mL. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range. We tested 1,469 samples.

Results: In a mean follow-up of 15 years, 21 patients achieved HBsAg seocclearance with an incidence rate of 1.4% per year. The median age of patients was 39 years (range 32.3–48.8 years). The median HBsAg and ALT levels were 3.6 log IU/mL and 44.5 IU/l, respectively. Multivariate analysis revealed that HBV-DNA level at baseline was the only predictive factor for HBsAg and HBsAg seroclearance. The measurement of both HBsAg and HBcrAg level is useful for discriminating e-IC and evaluating the risk of HCC.

423 THE COMBINATION OF HBsAg AND HBV CORE-RELATED ANTIGEN (HBcrAg) MEASUREMENT IS USEFUL FOR DISCRIMINATING HBeAg NEGATIVE INACTIVE CARRIER AND EVALUATING THE RISK OF HCC
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Background and Aims: Patients with chronic hepatitis B virus (HBV) infection have a high risk for developing hepatocellular carcinoma (HCC). Patients with higher serum HBVDNA levels have an increased risk of developing cirrhosis and hepatocellular carcinoma (HCC). However, little is known about the association between serum levels of HBV related proteins and the risk of developing HCC.

Methods. A total of 1014 patients followed for at least one year at our institution (407 HBeAg positive chronic hepatitis (e+CH), 257 HBsAg negative chronic hepatitis (e-CH), 350 HBsAg negative inactive carrier (e-IC)) were studied. Serum levels of HBVDNA, HBsAg, and HBV core-related antigen (HBcrAg) [1] measured at presentation and their impact for the prediction of HCC development and the diagnosis of e-IC was determined.

Results: For the diagnosis of e-IC in patients with HBsAg negative and HBV DNA levels of <4.0 log copies/ml, the area under the ROC was 0.674 for HBcrAg level and 0.549 for HBsAg level. Sensitivity and specificity for the diagnosis of e-IC was 89% and 62% for HBcrAg (Cut off 3.0 log IU/mL), and 36% and 70% for HBsAg (Cut off 2.0 log IU/mL). Seventy eight percent of patients categorized in HBcrAg levels of <3.0 log IU/mL and HBsAg levels of <2.0 log IU/mL at baseline were e-IC carriers. The risk of developing HCC was higher in patients with higher HBV DNA level (≥5.0 log IU/mL), HBsAg level (≥2.0 log IU/mL) and HBcrAg level (≥3.0 log IU/mL). In patients with HBcrAg ≥3.0 log IU/mL and HBsAg≥2.0 log IU/mL, cumulative incidence of HCC was 2.0% at 5 years, 3.8% at 10 years, and 8.3% at 15 years. However no HCC developed in patients with HBcrAg levels of <3.0 log IU/mL and HBsAg levels of <2.0 log IU/mL.

Conclusion: The measurement of both HBsAg and HBcrAg level is useful for discriminating e-IC and evaluating the risk of HCC.

Reference(s)

424 OPTIMIZED HBsAg TITER MONITORING IMPROVES INTERFERON THERAPY IN PATIENTS WITH CHRONIC HEPATITIS DELTA
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Background: Hepatitis delta virus (HDV) is a defective virus requiring the simultaneous presence of hepatitis B virus (HBV). Interferon alpha (conventional or pegylated) is the only drug effective on HDV replication. However, the optimal duration of interferon (IFN) therapy is not well defined. The efficacy of IFN therapy can be assessed by measuring HDV RNA levels, but HDV RNA assays are not standardized and not widely available. Treatment with interferon for 48 weeks, which is considered as the treatment of choice for HDV infection, resulted in sustained HDV RNA clearance in about one quarter of patients.

Objective: The aim of our study was to prospectively monitor HBs Ag titers during interferon therapy for chronic HDV and to adapt the duration of the therapy based on the titer.

Methods and Patients: We analyzed HBsAg levels of 4 patients who received peg interferon alpha2a at 180 ìg per week as a time-individualized therapy according to the evolution of HBsAg titer.

Patients were male; age was 43 to 46 years. Metavir score was F4 for one patient and F3 for the others. However, the optimal duration of interferon therapy was different for each patient: 4000 IU, 8000 IU, 12000 IU, and 16000 IU. Pretherapeutic Ag HBs levels were 7900, 4200, 3500, and 1320 IU/ml.

Results: We prospectively assessed the HBs Ag titers using the Quantitative Architect Abbott Method every three months. The treatment was stopped when HBs Ag levels reached negative values (<0.5 IU/mL). During treatment, HBs titers decreased in all patients and reached a negative value (<0.5 IU/ml) after seven months, two years, three years, and four years of therapy. This negative HBs Ag value was stable (normal ALT and loss of HBs Ag) in all patients 12 months after the end of therapy.

Conclusion: Even with a limited number of patients, this study demonstrates for the first time that adapting interferon treatment duration through HBsAg titer monitoring provides a loss of HBs Ag and the cure of chronic HDV.
TO CLINICAL OUTCOME IN PATIENTS WITH CHRONIC HDV INFECTION

Patients and Methods: 136 consecutive anti-HDV positive patients (93 males, mean age 56 yrs) with chronic hepatitis, followed for a mean of 20.6 yrs (2–41). 93 patients (68%) had a previous history of treatment with standard IFN for a mean of 20 months (range 6–94). Mean follow-up after IFN treatment was 14 yrs (4–21). IL28B genotype was obtained by Taqman SNP genotyping assay on fresh blood samples.

Results: 50 patients (37%) had genotype CC, 68 (50%) had genotype CT and 18 (13%) had genotype TT. Among IFN treated patients, the distribution of genotypes was CC in 32 (34%), CT 48 (52%), TT 13 (14%). There were no differences among genotypes in terms of age, gender, prevalence of cirrhosis at baseline, number of treated patients and treatment duration. Moreover, there were no differences in terms of biochemical response (22%, 25% and 15%, respectively; p=ns), virological response (12%, 10%, 7%, respectively; p=ns) and sustained virological response (19%, 25%, 31%, respectively; p=ns). When IL28B genotypes were analyzed according to clinical outcome, TT appeared to be significantly and independently associated to the risk of unfavourable outcome (i.e, progression to cirrhosis, HCC, liver decompensation and liver related death). Only HDV-RNA levels at presentation were significantly and independently associated to the risk of unfavourable outcome (p=0.001; OR 1.89; 95% C.I. 1.292–2.765). When the outcomes were individually analyzed, again neither HBsAg or HBV-DNA levels were associated to the occurrence of any unfavourable event, while HDV-RNA levels were significantly and independently associated to the risk of progression to cirrhosis and HCC development either by univariate analysis (p<0.001; OR 1.57; 95% C.I. 1.20–2.05 and p=0.033; OR 1.66; 95% C.I. 1.04–2.65, respectively) and multivariate analysis (p=0.007; OR 1.60; 95% C.I. 1.20–2.12 and p=0.019; OR 1.88; 95% C.I. 1.11–3.19, respectively).

Conclusions: Serum levels of HBsAg and HBV-DNA do not correlate with clinical outcome in chronic HDV infection. HDV-RNA levels correlate with the risk of progression to cirrhosis and HCC development.

427 HIGH RATES OF HBsAg “a DETERMINANT” VARIABILITY AND IMMUNE ESCAPE MUTATIONS ARE ASSOCIATED WITH HBV REACTIVATION UPON IMMUNOSUPPRESSIVE THERAPY

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Background: HBV- Reactivation is defined as an abrupt reappearance or rise of serum HBV-DNA in patients with inactive or resolved HBV-infection (Hooftnagle, 2009). The role of immune-escape HBsAg-mutations in this phenomenon is still anecdotic. Here, we investigated the burden and the patterns of immune-escape HBsAg-mutations correlated with HBV-reactivation.

Methods: This study included 114 patients: 14 patients (12 genotype-D, 2 genotype-A) experiencing HBV-reactivation triggered by immune-suppressive therapy [9 (64.3%) received rituximab and 5 (35.7%) corticosteroids] and 100 HBV chronically-infected drug-naive patients as control. The association of HBsAg-mutations with HBV-reactivation was assessed by Fisher-test. HBsAg genetic divergence was assessed by PAML-software. Immune-escape mutations analyzed were retrieved from in-vivo and/or in-vitro published studies.

Results: At the time of HBV-reactivation, patients had higher median (IQR) log serum HBV-DNA and ALT than steady-state controls [6.2 (4.8–7.7) IU/ml versus 3.9 (3.2–5.2) IU/ml, P=0.002; and 105 (50–499) IU/ml versus 42 (29–73) IU/ml, P=0.001, respectively]. Eight patients experienced HBV-reactivation during (N=7) or 6-months after the end of (N=1) lamivudine-prophylaxis;
4/8 (50%) rapidly developed lamivudine-resistance mutations. Patients with HBV-reactivation were characterized by a degree of HBsAg-variability higher than that in stably-HBV-infected patients, particularly in a-determinant critical for modulating HBV-infectivity and evasion from neutralizing-antibodies (mean genetic-divergence: 0.058 versus 0.017, P < 0.001). Furthermore, both the percentage of patients with at least 1 immune-escape mutation, and the median number of immune-escape mutations in a-determinant, were significantly higher in patients with HBV-reactivation than in controls (64.3% [9/14] versus 27% [27/100], P = 0.008; and 1 [IQR: 0–2] versus 0 [IQR: 0–2], P = 0.002, respectively). Moreover, ≥1 specific HBsAg-mutation (M103I-T123N-S143L-D144E-S154L-V190A) was present in 8/14 (57.1%) patients with HBV-reactivation status (independently from the presence of drug-resistance mutations), while in controls they were absent (0/100 for M103I-T123N-D144E-S154L), or nearly absent (1/100 for S143L and 2/100 for V190A). All the identified mutations (except for V190A, situated in the membrane-embedded C-terminal domain) are localized in a-determinant, and are known to be associated with decreased HBsAg-antigenicity. T123N introduces an N-linked glycosylation-site, thus potentially reducing HBsAg immunogenic-surface.

Conclusions: HBV-reactivation correlates with an increased HBV ability to evade the pressure imposed by humoral immune-reserve. This highlights the need of a careful monitoring, and of establishing an adequate, high-genetic-barrier therapy (if required) in order to prevent HBV-reactivation and consequent liver damage in the setting of immune-suppression.

428 VERTICAL HEPATITIS B VIRUS TRANSMISSION DESPITE SEROVACCINATION OF THE NEWBORN IN HIGHLY VIRAEMIC MONO-INFECTED MOTHERS FROM VARIOUS ETHNIC ORIGINS: A RETROSPECTIVE STUDY IN PARIS, FRANCE

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Background and Aims: Data concerning vertical hepatitis B virus (HBV) transmission come mainly from Asia. Conditions regarding highly viraemic mother ethnicities, subtypes, childbirth, serovaccination of newborns differ in Europe from Asia. We studied HBV transmission to newborns who got serovaccinated, from highly viraemic mothers of various ethnicities carrying different HBV subtypes.

Methods: Retrospective monocenter analysis of mothers with HBV-DNA >10^5 IU/ml who gave birth from 1 January 2004 to 31 December 2011 in Obstetrics Department; demographic and clinical data about delivery were collected from medical records, data concerning serovaccination in health record of the children; HBV subtypes were assessed by sequences analysis; HBV status of the children was defined.

Results: 11,417 women were followed, giving birth to 18,372 living children, 9390 male, 8982 female, 2296.5 births/year, 1.6 children/woman. Four hundred thirty-seven (4%) mothers were HBsAg positive, 97/437 (22%), 109/437 (25%), 231/437 cases (53%) originating from Europe, Asia, sub-Saharan Africa respectively. HBV-DNA <2000, between 2000 and 10^4 IU/ml, and not determined (ND) during pregnancy was retrieved in 72/97 (74%), 21/97 (22%), 1/97 (1%), 3/97 (3%) in women originating from Europe respectively, 51/109 (47%), 12/109 (11%), 41/109 (38%), 5/109 (4%) in women originating from Asia respectively, 162/231 (70%), 48/231 (21%), 10/231 (4%), 11/231 (5%) in women originating from sub-Saharan Africa respectively. Among the 36/52 women with HBV-DNA >10^4 IU/ml who were not given treatment during pregnancy, and whose childbirth occurred more than 9 months ago, 28 (78%) could be assessed for vertical transmission, representing 41 childbirths. Eleven/41 (27%) children were chronically infected, mostly showing an immunotolerant profile; fourteen/41 (34%) had been transiently infected, sixteen/41 (39%) had been protected by serovaccination. The risk of vertical transmission did not differ according to maternal HBV-DNA, child sex, ethnic origin, HBV subtype, child birth rank.

Conclusions: Serovaccination of newborns does not protect them from HBV transmission when the mother is highly viraemic, whatever her ethnicity or HBV subtype. Antiviral treatment during the last trimester could be helpful.

429 LIVER DISEASE IN HBV–HIV CO-INFECTION: SIGNIFICANT FIBROSIS IS PRESENT DESPITE HBV SUPPRESSION WITH ANTI-RETROVIRAL THERAPY

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Background: Despite the widespread use of anti-retroviral therapy (ART) active against both HIV and HBV, liver-related mortality remains high among HIV infected persons. Liver biopsy is rarely performed in the setting of suppressed HBV-DNA, yet co-infected individuals were at risk of developing liver injury for many years prior to treatment initiation. We sought to better define the spectrum of histologic liver disease, and correlates of liver disease, in HBV–HIV co-infection.

Methods: A retrospective analysis of liver histology in HBV–HIV patients from 5 centers was performed. Those with decompensation, HCV or other liver disease were excluded. Liver biopsy results and demographic and clinical data obtained at the time of the biopsy were recorded. Predictors of fibrosis (comparing those with bridging fibrosis/cirrhosis to those with stage 0–2 fibrosis) were assessed.

Results: Fifty-three patients were included. The mean age was 42, 96% were male, 30% were African American, 66% were on ART and the vast majority (95%) had undetectable HIV RNA. 62% were eAg+, 35% had HBV-DNA <1000 U/L and 30% had an undetectable HBV-DNA. 6% of those tested for anti-HDV (n=30) were positive. Mean laboratories were AST 52 U/L, ALT 57 U/L, and CD4 420. Liver histology showed advanced fibrosis in 31 (58%). Compared to those with mild disease, those with advanced histology were more likely to be on ART (p=0.02), to have a lower CD4 nadir (p=0.04) and higher AST (p=0.08). There was no difference between groups with respect to HBeAg status or presence of HDV. In a subset with available data (n=46), HBV-DNA <1000 IU/ml was not associated with disease severity. FIB-4 performed slightly better than APRI in differentiating advanced fibrosis from mild disease [AUROCs 0.78 (95% CI 0.71–0.85) and 0.68 (95% CI 0.48–0.89), respectively].

Conclusions: A high proportion of HBV–HIV co-infected patients have advanced fibrosis despite HBV suppression. Those on ART appear to have more advanced liver disease, and this may have masked any benefit associated with HBV treatment. Larger, well-designed studies of liver histology and its correlates are needed to better define the spectrum of liver disease in this understudied population.
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KEY GENETIC SIGNATURES IN THE WHOLE pre-S1/pre-S2/S GENE CORRELATE WITH HBV-INDUCED CARCINogenesis BY AFFECTING HBsAg SECRETION AND RELEASE


12.5% to 37.5%), while these mutations are absent (0/48 for N40IS) in vivo. 19/19 patients with HCC carry ≥1 of them (range-prevalence: 12.5% to 37.5%). This couple of mutations drastically decreases the retention and secretion in an in-vitro model.

Methods: Sixty-seven HBV chronically-infected patients (74.6% genotype-D; 20.9% genotype-A; and 4.5% others), 19 of them with HCC and 48 asymptomatic patients (as control), were analyzed. Association of mutations with HCC was assessed by Fisher-test with Benjamini-Hochberg for multiple-comparison correction. Mutants were constructed by site-directed mutagenesis and expressed in Huh7-hepatocytes using the expression-vectors pCHD9 with full-length HBV-genome (for mutations S98T51–P203QS–S210RS) and pCHsAgD with pre-S1/pre-S2/S-gene (for N40I–K141N51). After 72h quantitative-HBsAg was tested in cell-supernatants and in cell-lysates using Abbott-Architect assay.

Results: Novel mutations in pre-S1 (S98T51), and S region (N40I–K141N51–P203QS–S210RS) correlate with HCC (P = 10^-3 to 10^-4) in vivo. 19/19 patients with HCC carry ≥1 of them (range-prevalence: 12.5% to 37.5%), while these mutations are absent (0/48 for N40I) or occurring with low frequency (1.9% for K141N–P203QS, 4.7% for S98T51, 7.5% for S210RS) in non-HCC patients. Strong correlations are observed for P203QS+S210RS ( phi=0.83) and N40I+K141N ( phi=0.56).

S98T51 corresponds to the nucleotide codon 3175–3177 of HBV-genome localized in the promoter of the middle S-protein. In in-vitro experiments, its presence determines a 3.71 fold-increase in HBsAg-levels released in supernatants (S98T51: 127.47±3.53 IU/mL; WT: 34.40±13.28 IU/mL, P = 0.00016).

An opposite scenario is observed for P203QS+S210RS, localized in the membrane-embedded C-terminal domain critical for HBsAg-secretion. This couple of mutations drastically decreases supernantant HBsAg-levels compared to P203Q and S210R alone, and to wild-type (P203Q+S210R: 9.42±1.35 IU/mL; P203Q: 26.85±13.01 IU/mL; S210R: 25.95±8.82 IU/mL; WT: 34.40±13.28 IU/mL, P < 0.01). P203Q+S210R also decrease the ratio of supernantant to lysate HBsAg compared to wild-type (0.61 for P203Q+S210R versus 1.20 for wild-type), supporting their ability in inducing HBsAg intracellular-retention.

Similarly, K141N determines a 52%-reduction of supernantant HBsAg-levels compared to wild-type (K141N: 13.43±9.68 IU/mL; WT: 28.25±13.12 IU/mL. This reduction is greater when K141N is co-present with N40I (K141N+N40I: 4.67±2.31 IU/mL, P = 0.012).

K141N introduces a new N-linked glycosylation-site, while N40I resides in the cytosolic-loop involved in HBsAg-secretion.

Conclusions: Key genetic-elements in pre-S1/pre-S2/S-gene correlate with HCC by inducing intracellular HBsAg-retention and/or by potentially altering the production of mRNAs for S-gene. Their detection may help identifying patients at higher HCC-risk.

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PRE-EMPITIVE OR PROPHYLACTIC THERAPY FOR REACTIVATION OF HEPATITIS B VIRUS IN PATIENTS WHO RECEIVED IMMUNOSUPPRESSIVE OR CYTOTOXIC THERAPY: EVALUATION OF BOTH HBsAg-POSITIVE AND HBsAg-NEGATIVE COHORTS


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Aim: The present study was preformed to evaluate the effect of pre-emptive or prophylactic therapy with entecavir for HBV reactivation in rheumatoid arthritis (RA) patients, patient with malignant lymphoma (ML), patients with hematopoietic stem cell transplantation for hematological disorder (HSCT), and patients with renal transplantation.

Patients and Methods: We enrolled 138 patients with antibodies against hepatitis B core antigen (anti-HBc) consisted of 87 RA patients (14 with HBsAg), 26 ML patients (4 with HBsAg), 14 patients with HSCT (one with HBsAg), and 11 patients with renal transplantation (3 with HBsAg). RA patient was started treatment with anti-rheumatic drugs or additionally received anti-TNF-a. ML patients were treated with rituximab combination therapy. HBV-DNA levels were measured every month to 3 months by a real-time PCR method. Anti-HBs was examined in patients with anti-HBs. Entecavir was administrated to patients with HBV-DNA levels ≥2.1 log/mL.

Results: The mean observation period was 36 months (range: 8–51 months). Entecavir was administrated to all of 20 patients with HBsAg and more than 2.1 log/mL of HBV-DNA at the enrollment. In HBsAg-negative patients, HBV was reactivated in 3 (14%) of 22 ML patients treated with rituximab combination therapy, and 3 (23%) of 13 patients with HSCT. All of 6 patients with HBV reactivation had less than 100 IU/mL of anti-HBs at the enrollment. In RA patients, HBV reactivation occurred in all of 2 patients with HBsAg and HBV-DNA levels <2.1 log/mL and in 1 (14%) of 73 patients without HBsAg. In patients who received anti-TNF-a therapy, antibodies against HBsAg decreased significantly. There was no HBV reactivation in HBsAg-negative patients with renal transplantation. Entecavir inhibited HBV amplification and prevented HBV-associated hepatic failure in patients with HBV reactivation.

Conclusions: ML patients treated with rituximab combination therapy and patients with HSCT had high risk for HBV reactivation, in particular patients with less than 100 IU/mL of anti-HBs. HBV DNA monitoring and pre-emptive or prophylactic therapy with entecavir were effective means of preventing HBV-associated hepatic failure in patients with HBsAg as well as those with only anti-HBc who received immunosuppressive or cytotoxic therapy.

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TRANSFER OF HEPATITIS B SURFACE MARKERS AND HBV DNA FROM HEPATITIS B SURFACE ANTIGEN POSITIVE MOTHERS TO THEIR OFFSPRING

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Background: HBsAg, a soluble antigen, can pass through placenta to mother and infant but it is still unclear whether other hepatitis B surface markers or HBV DNA can pass through human placenta and have an impact on infants.
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Background and Aims: The European Association for the Study of the Liver (EASL) defines inactive hepatitis B virus (HBV) carriers based on HBVDNA and alanine aminotransferase (ALT) levels. This study aimed to evaluate the risk of disease progression in such patients.

Methods: 417 patients with negative hepatitis B e antigen (HBeAg), HBVDNA <20,000 IU/ml and normal ALT at baseline underwent liver stiffness measurement (LSM) by Fibroscan in 2006–2008 and again in 2010–2012. Fibrosis progression was defined as increase in LSM by 30% or more and an absolute LSM suggestive of advanced fibrosis at the second assessment.

Results: At baseline, the mean age was 49±11 years, 53% were males, ALT was 29±11 IU/l, HBVDNA was 2.7±0.9 log IU/ml, hepatitis B surface antigen (HBSAg) was 2.5±1.4 log IU/ml, and LSM was 6.4±3.2 kPa. 56 (13.4%) patients had advanced fibrosis at baseline. At an interval of 44±7 months, 10 of 361 (2.8%) patients without advanced fibrosis at baseline developed fibrosis progression and 49 (13.6%) required antiviral therapy. Among 244 patients with baseline HBVDNA <2,000 IU/ml, 11.9% had HBVDNA ≥20,000 IU/ml during follow-up, 8.2% required antiviral therapy and 2.9% had fibrosis progression. Corresponding figures in 117 patients with baseline HBVDNA 2,000–20,000 IU/ml were 16.4%, 24.8% and 2.6%, respectively (P<0.001, <0.001 and =0.76, respectively). The addition of HBSAg level to HBVDNA did not improve the prediction of outcomes.

Conclusions: Among patients with negative HBeAg and normal ALT, those with HBVDNA 2,000–20,000 IU/ml had similarly low risk of fibrosis progression but were more likely to develop indications for treatment than those with HBVDNA <2,000 IU/ml.

Acknowledgements: This study was supported by the Research Fund for the Control of Infectious Diseases of the Hong Kong SAR Government (Ref 11100372).

Results: All patients showed declines of total HBSAg at the 2nd time-point to 40±27% of the baseline level. HBSAg measured with the mAb against the S-domain of all surface proteins correlated with Abbott-Vector values at both time-points (r = 0.95, p < 0.0001). At baseline, L- and MHBs accounted for only 43±16% and 48±5.3% of total HBSAg. The relative decline of L- and MHBs was more pronounced compared to SHBs in 5/7 patients. At the second time-point the L- and MHBs ratio decreased to 2.9±1.7% and 3.7±4.5%, respectively whereas the relative SHBs-fraction increased from 90.9±6.1% to 93.4±6.1%. Control patients with CHB had significantly higher ratios of L- and MHBs and lower ratios of SHBs (p<0.01).

Conclusion: This is the first study investigating the ratio of L-, M-, SHBs in patients with AHB. Kinetics of L- and MHBs, which contain B- and T-cell epitopes within their specific preS-domains, may reflect the immune response towards HBV more accurately compared to total HBSAg.
AntihDV-IgM as a marker of disease activity in hepatitis delta


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Introduction: Hepatitis delta frequently leads to liver cirrhosis and hepatic decompensation. As treatment options are limited, there is a need for biomarkers to determine disease activity and to predict the risk of disease progression. Anti-HDV-IgM levels may correlate with histological and biochemical activity in HDV infection. However, the exact mechanisms behind this association are unclear.

Methods: We first investigated baseline samples of 120 HDV-infected patients recruited in the HDIT-2 trial that enrolled patients in Germany, Greece, Turkey and Romania (Yurdadaydin et al., AASLD 2012). Evaluation of liver biopsies was performed by a central pathologist. HDV-RNA, HBsAg and HBV-DNA levels were determined in one laboratory. Anti-HDV-IgM-testing was performed using the ETI-DELTA-IGMK-2 assay (DiaSorin). In addition, fifty-four cytokines, chemokines and angiogenetic factors were measured in sera using multiplex technology (Bio-Plex System).

In a second step, we studied an independent cohort of 78 patients for the development of liver-related clinical endpoints (decompensation, HCC, liver transplantation or death; median follow up of 3.0 years, range 0.6–12).

Results: Anti-HDV-IgM serum levels were negative in 18 (15%), low (OD: 0.1–5) in 76 (63%), and high in 26 (22%) patients. Anti-HDV-IgM correlated with histological inflammatory activity (p < 0.01) and biochemical disease activity (ALT and AST p < 0.01). HDV replication was independent from anti-HDV-IgM, however, a negative correlation was observed between anti-HDV-IgM and HBV-DNA (p < 0.01). Various inflammatory serum cytokines were significantly associated with anti-HDV-IgM including IP-10, MIG, IL-8, IL-17, MCP-1, IL-16, TNF-β and the interleukin-2-receptor-alpha. Principal-component-analysis including all clinical, virological and immunological parameters available confirmed the importance of anti-HDV-IgM to distinguish patients with different grades of liver disease.

The association between anti-HDV-IgM and ALT, AST, HBV-DNA was confirmed in the second independent cohort. Moreover, liver-related clinical endpoints developed only in anti-HDV-IgM-positive but not in anti-HDV-IgM-negative patients (39% vs. 0%, p = 0.05).

Conclusions: Serum anti-HDV-IgM is a robust, easy-to-apply and relatively cheap marker to determine disease activity in hepatitis delta which has prognostic implications. High anti-HDV-IgM levels may indicate an activated immune system which leads to a suppression of HBV-DNA but not of HDV-RNA.
stiffness measurements (LSM-KPa) by TE (10 valid measurements, success rate >60% and IQR/LSM <30%). All cases were followed for ≥ 24 months (34±8 months, range 24–44). Thirty two patients started NA treatment at entry and FibroScan was done almost annually (9±3.1 months) while 23 patients had already started NA treatment (3–5 years ago).

Results: The slope of changes in LSMs over time in years is shown in Figure 1. LSMs changes over time (expressed in kPa/month) showed a slight faster pattern of fibrosis regression at the beginning (first two years) and a slow steady pattern later during a follow-up period of four years. Improvement in fibrosis was seen in 33/55 patients (60%) (drop of −2.06±1.6 in kPa). Importantly, regression of cirrhosis (initial LSM >12kPa) to less than 6.1kPa was observed in 3/55 (5.4%) patients. Worsening of LSM was observed in 3 cases due to other liver disease cofactors. The baseline median LSM was 9.2±3.1 kPa. LSMs significantly decreased during the follow-up period after the start of NA treatment (8.4±0.9, 7.8±1.3, 7.3±1.9 and 6.8±1.6 kPa at years 1, 2, 3 and 4, respectively).

Conclusions: LSMs changes annually showed an early faster and a later steady pattern of fibrosis regression over time as measured by transient elastography for chronic HBeAg (−) hepatitis treated with NA. Longer follow-up of these patients by TE is necessary to assess fibrosis regression in chronic hepatitis B patients under antiviral therapy.

Baseline and Week 24 HBeAg Levels Are Associated with Telbivudine Treatment Response in the 2410 Roadmap Study in Chronic Hepatitis B

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Introduction: Precore (PC) and basal core promoter (BCP) mutations are the most prevalent naturally occurring variations of HBV and impair HBeAg production. We assessed the predictive use of PC/BCP mutations and HBsAg/HBeAg in the LDT600A2410 Roadmap study with telbivudine (LDT) monotherapy and tenofovir (TDF) add-on until week 104 (EoT).

Methods: mITT population was 100 HBeAg-positive CHB patients. Quantitative HBsAg/HBeAg was analyzed at screening, W24, W52, W76 and EoT and correlated with serological, combined (normal ALT, undetectable HBV-DNA) and complete (normal ALT, undetectable HBV-DNA, HBeAg loss) EoT response (Fig. 1). PC/BCP mutation analysis was available from 64 patients at screening.

Results: At screening, significantly lower HBeAg (375.1 vs. 698.2 PEIU/mL) and HBV-DNA but higher ALT levels were seen for patients achieving W24 undetectable HBV-DNA. PC/BCP results were available from 37/55 patients with undetectable HBV-DNA at W24. Of those, 29.7% patients had PC/BCP mutations, 70.3% had wildtype. In patients with HBeAg loss pre-treatment HBeAg was lower (161.9 vs. 213.3 PEIU/l), number of patients with PC/BCP mutants was lower (27.0% vs. 50%) but ALT was higher. Patients with combined and complete EoT response showed lower screening HBeAg levels (176.5 and 156.9 PEIU/l vs. 638.6 and 213.3 PEIU/l). The 8 patients with HBsAg loss had higher screening HBsAg (5.1 vs. 4.2log10IU/l) and HBV-DNA (9.76 vs. 9.11log10IU/ml) levels but lower ALT (92 vs. 111U/l) but similar HBeAg levels. 3 patients had PC/BCP mutations. W24 predictive factors for combined and complete EoT response were undetectable HBV-DNA (positive predictive value (PPV) 87% and 64%, negative predictive value (NPV) 24% and 73%) and W24 HBeAg <10PEIU/l (n = 54, PPV 85% and 67%, NPV 22% and 76%).

Conclusions: High HBeAg and HBsAg response was shown in telbivudine roadmap study 2410. Lower pre- and on-treatment HBeAg levels were associated with improved outcomes at 2 years. The presence of PC/BCP mutations negatively influenced HBeAg loss rates.
POSTERS

08b. VIRAL HEPATITIS C: CLINICAL (EXCEPT THERAPY)

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1148M PNPLA3 VARIANT PROMOTES STEATOSIS ACCORDING TO VIRAL AND IL28B GENOTYPE BUT DOES NOT AFFECT SUSTAINED VIRAL RESPONSE IN PATIENTS WITH HEPATITIS C

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Background and Aims: To assess the impact of PNPLA3 polymorphisms on steatosis in patients with hepatitis C.

Methods: We included 445 patients with hepatitis C, from 6 Spanish hospitals. 63.6% (283/445) males and 36.4% (162/445) females; viral genotype 1 68.1% (303/445); steatosis was detected in 44.3% (197/445) vs non-steatosis 55.7% (248/445). PNPLA3 GG/GC genotype 50.1% (222/445) and PNPLA3 CC genotype 50.1% (223/445); steatosis was detected in 47.4% (113/249) and IL28B-CT/TT genotype 45.4% (196/430); seve

Results:

- Variables associated with steatosis in multivariate analysis were: G allele of PNPLA3 [O.R. 1.82 (IC95%: 1.21–2.73); p = 0.004], viral genotype [O.R. 0.8 (IC95%: 0.66–0.98); p = 0.018] and BMI [O.R. 0.93 (IC95%: 0.89–0.98); p = 0.003]. G allele of rs738409 of PNPLA3 was associated with steatosis (50.9%; 113/222) vs non-G (37.7%; 84/223) [p = 0.005]. In viral genotype 1, G allele was related to steatosis (47.4%; 73/154 vs 32.2%; 48/149; p = 0.007), but not in viral genotype 3 (60.5%; 23/38 vs 63.6%; 28/44; p = 0.772).
- Polymorphisms of IL28B gene were not linked with steatosis (IL28B-CC 45.4%; 113/249) and IL28B-CT/TT (45.6%; 233/511) [p = 0.955]. However, G allele of PNPLA3 showed a close association with steatosis in patients with IL28B-CT/TT genotype (56.2%; 77/137 vs 35%; 49/140; p = 0.0001), but not in IL28B-CC (41.7%; 35/84 vs 42%; 34/81) (p = 0.968). In contrast, G allele did not influence sustained viral response (54.5%; 121/222 vs 56.1%; 125/223; p = 0.742).
- G allele of polymorphism rs738409 of PNPLA3 gene modulates the development of steatosis, particularly in patients with viral genotype 1 and IL28B-CT/TT genotype, but was not associated with sustained viral response. Metabolic steatosis seems to be PNPLA3 regulated but viral steatosis not.

Conclusions: KLF12 polymorphism rs9543524 predicts anemia in patients with chronic hepatitis C treated with peginterferon and ribavirin.

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KLF12 POLYMORPHISM rs9543524 PREDICTS ANEMIA IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH PEGINTEROFON AND RIBAVIRIN

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Background and Aims: To analyze the role of genetic disorders in the prediction of developing anemia during peginterferon plus ribavirin treatment.

Methods: DNA was extracted from patients with viral hepatitis C treated with peginterferon plus ribavirin. KLF-12 polymorphism (rs9543524) was selected from 92 SNPs showing p values <10−3 associated with SVR and anemia, by Taqman probe (Applied Byosistems, Barcelona, Spain). This SNP was validated in 565 patients with hepatitis C: mean age was 45±10 years old; 60% (337/565) males and 40% (228/565) females; viral genotype 1 70% (398/565), genotype 2 3% (17/565), genotype 3 17% (96/565), genotype 4 10% (54/565). IL28B-CC genotype was 36% (202/559) and IL28B-CT/TT genotype 64% (357/559). Severe fibrosis (F3–F4) was found in 18% (70/401) and mild fibrosis (F0–F2) 82% (331/401). Anemia was defined as hemoglobin (Hb) ≤10g/dL, which was present in 23.9% (135/565). Anemia was not related to SVR (53.7%; 73/155 vs 55%; 236/430).

Results: KLF12 was the most relevant polymorphism associated with anemia. Patients with KLF12-CT were 65% (369/565) and CT/CC 72/135 vs 55%; 236/430).

Conclusions: The presence of KLF12-CT genotype in patients with hepatitis C, treated with peginterferon and ribavirin was associated with the development of anemia. Krüppel-like family of transcription factors (KLFs) is a key gene in the regulation of β chain of hemoglobin and erythropoiesis.

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TREATMENT FAILURE MAY LEAD TO ACCELERATED FIBROSIS PROGRESSION IN PATIENTS WITH CHRONIC HEPATITIS C

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Background: Chronic hepatitis C (CHC) patients with treatment failure remain at risk for continuing fibrosis progression and complications of liver disease. However it has not been investigated whether there is an increased risk for accelerated fibrosis progression after failed interferon-based therapy. We aimed to investigate long-term influence of treatment failure on fibrosis progression compared to untreated patients with CHC.
Methods: We studied 157 patients in accordance with the inclusion criteria, who underwent paired liver biopsies from 1994 to 2012. Ninety-three patients had treatment failure (TF) and 64 patients were treatment-naïve (TN). Annual fibrosis progression rate (FPR) was calculated and significant fibrosis progression (SFP) was defined as ≥2 stage increase in fibrosis during follow-up. Because of the differences in baseline biochemical and histological features, multiple regression analyses with backward elimination were performed to find out independent predictors of SFP and FPR.

Results: Demographic characteristics and duration between paired liver biopsies were similar in TF and TN-groups. Baseline ALT and GGT levels were significantly higher in TF-group compared to TN-group (p < 0.001). According to regression analyses strongest independent predictor of fibrosis progression was GGT level (OR: 1.04, 95%CI 1.02–1.06, p < 0.001). Treatment experience (OR: 1.3, 95% CI 1.2–1.5, p = 0.002) and follow-up ALT levels (OR 95%CI p value) were treatment-naïve (TN). Annual fibrosis progression rate (FPR) and follow-up ALT levels (95%CI p value) were treatment-naïve (TN).

Conclusion: Failed interferon-based treatment against CHC may lead to accelerated FPR in long-term compared to natural course.

Table 1. Logistic regression analysis of predictors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR (95%CI)</th>
<th>p value</th>
<th>Multivariate OR (95%CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment experience</td>
<td>5.80 (3.05–11.3)</td>
<td>&lt;0.001</td>
<td>4.55 (1.6–9.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5.86 (2.09–16.5)</td>
<td>&lt;0.001</td>
<td>4.09 (1.02–16.5)</td>
<td>0.047</td>
</tr>
<tr>
<td>ALT≥60 IU/l</td>
<td>5.21 (2.03–13.4)</td>
<td>&lt;0.001</td>
<td>1.51 (0.4–5.7)</td>
<td>0.60</td>
</tr>
<tr>
<td>Follow-up ALT≥60 IU/l</td>
<td>4.40 (1.83–10.6)</td>
<td>&lt;0.001</td>
<td>4.20 (1.41–12.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>1.03 (1.02–1.05)</td>
<td>&lt;0.001</td>
<td>1.03 (1.01–1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrosis at baseline biopsy</td>
<td>3.06 (1.53–6.13)</td>
<td>&lt;0.001</td>
<td>3.06 (1.53–6.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interface hepatitis</td>
<td>2.81 (1.07–7.37)</td>
<td>0.036</td>
<td>2.81 (1.07–7.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Steatosis</td>
<td>4.06 (1.69–9.75)</td>
<td>&lt;0.001</td>
<td>4.06 (1.69–9.75)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

442 A DIAGNOSTIC SCORE FOR THE PREDICTION OF SPONTANEOUS RESOLUTION OF ACUTE HEPATITIS C VIRUS INFECTION

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Background and Aims: Outcome of acute hepatitis C (AHC) depends on the ability of the host to mount a strong immune response, influenced by patient’s immuno-genetic background. In this respect killer-cell immunoglobulin-like receptor (KIR) 2DL3:HLA-C1/C1, via affecting NK-cell function, as well as presence of HLA-B27 were addressed to influence treatment response in chronic HCV as well as spontaneous clearance (SC) of AHC. Aim of this study was to investigate the role of KIR2DL3:C1/C1 and HLA-B27 in patients with acute presentation of HCV.

Patients and Methods: 100 Caucasian patients (m/f: 55/45; SC: 61; mean age at infection: 35±15 years [mean±SD]; VC: 74; GT1/4/6: 59, GT2/3:28) were investigated. The presence of KIR-genes was determined by SSP (sequence specific primer) typing as described before (Ludajic K. et al, 2009); HLA-determination by SSOP (sequence specific oligonucleotide probes) typing, using a commercial kit (HistoSpot, BAG, Lich, Germany) according to manufacturer’s instructions.

Results: HLA-C status was: C1/C1 in 36, C2/C2 in 18 and C1/C2 in 46 patients. KIR2DL3:positivity was examined in 82 patients. KIR2DL3:HLA-C1/C1 was neither associated with spontaneous...
clearance (SC: 32.8% vs. no-SC: 25.6%; p = 0.5) nor with favorable IL28B C/C-genotype (C/C: 28.1% vs. non-C/C: 33.3%; p = 0.7). HLA-B27 was detected in only 5 AHC patients and again no significant association with spontaneous resolution of AHC or IL28B C/C-genotype could be found (SC: N = 3 vs. none SC: N = 2; p = 1.0; C/C: 6.2% vs. non-C/C: 2.8%; p = 0.7). In addition other factors, known to be predictive for spontaneous resolution of AHC, like peak-bilirubin (5.3 vs. 4.9 mg/dl; p = 0.7); peak-GPT (1041 vs. 1155 U/l; p = 0.6), HCV-RNA at baseline (5.0 vs. 4.9 log10 IU/ml; p = 0.6); HCV-RNA decline till week 4 after first presentation (1.8 vs. 1.1 log10 IU/ml; p = 0.2); IP-10 (3.0 vs. 2.9 log10 pg/ml; p = 0.4) or detectability of HCV-CD4+Th1-cells (23% vs. 27%; p = 1.0) were not different in KIR2DL3:HLA-C1/C1 positive patients compared to those without this combination.

Conclusion: Neither KIR2DL3:HLA-C1/C1 nor HLA-B27 were predictive for spontaneous resolution of AHC. Determination of KIR2DL3:HLA-C1/C1 and HLA-B27 doesn’t seem to provide additional information in the management of patients with acute hepatitis C virus infection.


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Summary: The HCV infection is associated with insulin resistance and Diabetes Mellitus type II. To date the interaction between immune and inflammatory markers and cardiovascular risk doesn’t be analyzed.

Objective: To analyze some inflammatory and immune markers and their relation to vascular risk in hepatitis C patients in early and advance stages of fibrosis.

Methods: We performed a prospective case–control study including 69 healthy subjects and 96 patients with HCV genotype 1 and 50 healthy subjects matched by age and sex. The HCV group was divided in order to the stage of the fibrosis (non significant fibrosis, F0–F2 and significant fibrosis, F3–F4). None of the patients had signs of infection or acute liver failure at the time of inclusion. The stage of fibrosis were evaluated by Fibroscan. The renal function and the following markers C-reactive protein, serum amyloid A component (SAA), cystatin C (CYSC), plasma pentraxin 3 (PTX3), homocysteine and lipoprotein A [Lp (a)] were measured in all patients. Statistical analysis was performed using data from the IBM SPSS Statistic 19.

Results: Plasma levels of PTX3, SAA, CRP, Lp (a) and homocistein were significantly higher in patients with HCV than in the control patients (Table 1). We found a strong relation between the advance stage of fibrosis and PTX3 levels and Lp (a). However, the differences weren't significant in SAA, CRP and homocistein levels in the significant fibrosis vs non significant fibrosis.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Healthy controls</th>
<th>Chronic hepatitis C</th>
<th>Non significant fibrosis (F0–F2)</th>
<th>Significant fibrosis (F3–F4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>69</td>
<td>96</td>
<td>41</td>
<td>53</td>
</tr>
<tr>
<td>sCreatinine (mg/dl)</td>
<td>0.91+0.04</td>
<td>0.92+0.12</td>
<td>ns</td>
<td>0.84+0.15</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>1.40+0.21</td>
<td>1.59+3.18</td>
<td>0.05</td>
<td>1.52+1.1</td>
</tr>
<tr>
<td>Homocysteine (umol/L)</td>
<td>9.0+3.4</td>
<td>11.6+0.0</td>
<td>0.03</td>
<td>12.2+8.2</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>3.4+3.1</td>
<td>12.1+5.2</td>
<td>0.001</td>
<td>12.3+14.0</td>
</tr>
<tr>
<td>SAA (mg/dL)</td>
<td>3.1+1.2</td>
<td>5.04+6.02</td>
<td>0.001</td>
<td>3.29+1.04</td>
</tr>
<tr>
<td>PTX3 (ng/mL)</td>
<td>0.54+0.15</td>
<td>11.11</td>
<td>0.001</td>
<td>0.77+0.5</td>
</tr>
</tbody>
</table>

Conclusions: This is the first study to demonstrate a marked elevation of proinflammatory markers in chronic HCV infection and the relation with the stage of fibrosis. It supports the possibility of an increased cardiovascular risk in these patients.

445 rs4374383 SINGLE NUCLEOTIDE POLYMORPHISM OF MERTK GENE INFLUENCES THE PROGRESSION OF LIVER FIBROSIS IN THALASSEMIA MAJOR PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION V. Calvaruso1, F. Bronte2, S. Grimaudo2, A. Di Cristina2, V. Di Marco2, Sicilian Group for the Study of Hepatitis C in Thalassemia. 1Gastroenterology and Hepatology Unit, Di.B.M.I.S., 2Gastroenterology and Hepatology, University of Palermo, Palermo, Italy

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Background and Aim: Genome-wide association (GWA) studies identified a single nucleotide polymorphism (SNP), located in chromosome 2, associated with the liver fibrosis progression related to HCV infection. We studied the rs4374383 SNP of the MERTK gene, in a large, ethnically homogeneous cohort of transfused thalassemia major (TM) patients with a liver biopsy during the follow-up.

Methods: We evaluated 211 TM patients (median age 19.0, IQR: 13.0–28.0 years, 51.4% men) underwent liver biopsy between 1993 and 2010. Eighty patients (37.9%) were HCV-RNA negative and 131 (62.1%) had a chronic HCV infection (104 genotype 1 or 4 and 27 genotype 2 or 3) acquired during the first 3 year of the life by blood transfusion. Logistic regression analysis was performed to evaluated the correlation between demographic features (gender and age at time of liver biopsy) alleles (AA vs AG/GG) of rs4374383 SNP, viral genotype (1 or 4 vs 2 or 3), histological features (grading of liver inflammation and liver iron concentration) and staging of liver fibrosis evaluated by Scheuer’s score.

Results: In patients without HCV infection, age (OR 1.08; 95% CI 1.01–1.15; p = 0.032) and histological grading (OR 11.73; 95% CI 2.03–67.73; p = 0.006) were independently associated with severe fibrosis (F3–F4). No significant differences regarding gender, liver iron concentration (LIC), ALT values and rs4374383 alleles of MERTK gene between patients with and without severe liver fibrosis have been found. Among patients with chronic HCV infection, age (OR 1.05; 95% CI 1.01–1.10; p = 0.023), HCV genotype 1 or 4 (OR: 3.73; 95% CI 1.22–11.44; p = 0.021), AG/GG alleles of rs4374383 SNP (OR: 4.16; 95% CI 1.08–15.97; p = 0.038) and grading A2–A4 of liver inflammation (OR 3.35; 95% CI 1.39–8.06; p = 0.007) were independently associated with F3–F4 liver fibrosis by logistic regression analysis. Gender, LIC and ALT values were not related with severe fibrosis also in this group.

Conclusion: Our study confirm that the genetic variations in MERTK gene and the duration of infection effect the progression of liver fibrosis in TM patients with chronic HCV hepatitis.

446 ACUTE EXACERBATION OF CHRONIC HEPATITIS C: CLINICAL PRESENTATION, OUTCOME AND RESPONSE TO ANTI-HCV TREATMENT N. Coppola1, M. Pisaturo1, M. Stanzione1, V. Messina2, C. Sagnelli1, L. Alessio1, M. Starace1, G. Pasquale1, E. Sagnelli1. 1Second University of Naples, Naples, 2Division of Infectious and Tropical Diseases, AORN Sant’Anna e San Sebastiano di Caserta, Caserta, Italy

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Aim: To improve the knowledge on acute exacerbation of chronic hepatitis C (ae-CHC).

Methods: We prospectively investigated 82 consecutive, symptomatic ae-CHC patients (Cases) and 82 subjects without reactivation over a decade (Controls), pair matched by age, sex and HCV genotype. The patients in the Case group had a mean age of 53 years (23–87), males predominated (76%) and the most frequent HCV genotypes were genotype 2 (46.4%) and genotype 1 (43.9%) HCV genotype 2 was found in 46.4% and genotype 1 in 43.9%; the patients in the Control group showed the same characteristics, reflecting their selection criteria.
**Table 1.**

<table>
<thead>
<tr>
<th>HBV group</th>
<th>CB2 RR</th>
<th>CB2 QR</th>
<th>CB2 QQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>46 (58.7)</td>
<td>105 (62.5)</td>
<td>46 (27.2)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>52 (22-74)</td>
<td>52 (38-69)</td>
<td>25 (22-73)</td>
</tr>
<tr>
<td>ALT (M + SD)</td>
<td>1.1 ± 1.2</td>
<td>1.3 ± 1.4</td>
<td>1.0 ± 1.1</td>
</tr>
</tbody>
</table>

**Discussion:** The CB2 63 QQ variant in HCV patients was associated with more severe inflammation and hepatocellular necrosis.

**Background:** Recently, an association between IL28B genotype and insulin-resistance (IR), known predictors of outcome to Pegylinterferon (PegIFN) and Ribavirin (Rbv) treatment for chronic hepatitis C, has been hypothesized.

**Aim:** To investigate the association of IR and IL28B genotype in two cohorts of well-characterized HCV patients.

**Methods:** A total of 480 non-diabetic HCV patients were analyzed: 391 non-diabetic patients from the MIST study, and 89 from a previous study conducted at the Metabolic Center of Liver Diseases. All patients were tested for IL28B rs12979860 SNP by RT-PCR and had IR measured by HOMA-IR. Staging of liver disease through liver biopsy was available for all patients.

**Results:** Of the 480 patients included in the study, 164 (34%) were IL28B CC, 316 (66%) were CT/TT. Mean HOMA-IR values did not differ according to IL28B genotype, being respectively 1.14 ± 0.78 in CC vs 1.14 ± 0.78 in CT/TT in the first cohort and 2.4 ± 1.0 vs 2.5 ± 1.0 in the second cohort. HOMA-IR >2 was not associated with IL28B genotype, as it was found in 18/132 CC vs 37/259 CT/TT (p = 1.0) in the first cohort, and 17/32 vs 40/57 (p = 0.12) in the second. In the MIST cohort HOMA-IR >2 did not influence treatment outcome, as sustained virological response (SVR) rates were 28/47 (60%) in HOMA-IR >2 vs 214/344 (62%) in HOMA-IR <2 (p = 0.8). On the contrary, IL28B genotype was a strong predictor of SVR: 84% (111/132) in CC vs 51% (131/259) in CT/TT patients (p < 0.0001).

**Conclusions:** In two large cohorts of non-diabetic HCV patients where IL28B genotype predicted treatment outcome, we found no association between IL28B genotype and HOMA-IR.
immune genes, activation and recruitment of NK cells and T cells to the liver.

**Methods:** We performed longitudinal quantification of the levels of IL28B by ELISA on plasma samples collected from a cohort of injection drug users (n = 30) during acute HCV infection with different outcomes. In addition, we used qRT-PCR to monitor expression of seven genes previously associated with response to interferon following HCV infection in *in vitro* models (IFN6, IFIT1, Mx1, USP18, IP-10) and NK cell activity (NCR3, KLRD1).

**Results:** We observed that patients homozygous for the favourable IL28B rs12979860 C allele (CC) expressed higher levels of IL28B during early acute infection as compared to patients bearing the non-favourable T allele (CT/TT) (p = 0.0205). This observation became more significant when only acute infected patients with chronic evolution were analysed (p = 0.0047). IL28B plasma levels did not correlate with induction of genes examined in PBMCs except for IP-10, KLRD1 and Mx1. Higher IL28B plasma levels correlated with decreased expression of IP-10 (p = 0.0016) and KLRD1 (CD94) (p = 0.0002) and increased expression of Mx1 (p = 0.0283) only in patients with chronic evolution despite having the favourable genotype.

**Conclusions:** Our results suggest that IL28B polymorphism may modulate the recruitment of immune cells to the liver and the NK cell activity by affecting the expression of the interferon-stimulated chemokine IP-10 and antiviral protein Mx1, and the NK cell inhibitory receptor KLRD1.

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**A NOVEL PREDICTION MODEL FOR LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS USING FIBROSCAN AND ROUTINE LABORATORY DATA**

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**Background:** Data mining analysis explores data to discover hidden patterns, trends and enables the development of model to assess liver fibrosis utilizing liver stiffness measured by fibro-scan and simple laboratory data.

**Aim:** To develop a novel model to predict the stage of fibrosis in chronic HCV patients using fibro-scan and routine clinical and laboratory data.

**Methods:** Decision tree learning algorithm was applied to routine laboratory data of 296 chronic HCV Egyptian patients for model building using 20 attributes. Internal cross-validation was performed with 10-folds, and confidence (0.01). Liver histopathology (Metavir scoring) was used to assess accuracy for the model. Transient elastography measurement was used.

**Results:** Fibrosis was classified into three groups F0–F1 (minimal), F2–F3 (moderate), and F4 (severe). The correctly classified instances were 218/296 (74%). Decision tree was able to diagnose F0–F1, F2–F3, and F4 with sensitivity 59% and specificity 36% using only routine data, and with sensitivity 70% and specificity 68.5% using fibroscan. At stiffness 7.25 fibroscan was able to diagnose liver fibrosis F0–F1 with sensitivity 84% and specificity 85% and F2 F3 F4 with sensitivity 78% and specificity 76%. Out of 20 attributes the decision tree models showed that liver stiffness was selected as the variable of initial split (most decisive), with optimal cut-off value of <7.1 the possibility of being F0–F1 167/18 was 89.2%. At liver stiffness from 7.1–13.6 the possibility of being F2–F3 76/33 was 56.7%. Patients with liver stiffness >13.6, hepatic texture was the second important splitting attribute, other attributes as albumin, and AFP have less decisive role for prediction of fibrosis. As shown in fig (1). These results were confirmed statistically using univariate logistic regression analysis with P value <0.1. The reproducibility of the model was confirmed by external validation set on 249 at cut off value <7.1 patients with correctly classified instances 90/15 (83.3%). Liver stiffness from 7.1–13.6 correctly classified instances is 76/20 (73.6).

**Conclusion:** The model tree using fibro-scan and routine clinical and laboratory data can predict degree of hepatic fibrosis in chronic HCV patients with high accuracy and reproducibility.

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**EVALUATION OF PHARMACOKINETIC DRUG–DRUG INTERACTION (DDI) BETWEEN BMS-791325, AN NS5B NON-NUCLEOTIDE POLYMERASE INHIBITOR, DACLATASVIR AND ASUNAPREVI IN TRIPLE COMBINATION IN HCV GENOTYPE 1-INFECTED PATIENTS**

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**Background and Aims:** Interferon and ribavirin free treatments for HCV may be achieved by combinations of direct-acting antivirals. DAClatavir (DCV), an NS5A replication complex inhibitor, and asunaprevir (ASV), an NS3 protease inhibitor were combined with BMS-791235, a potent, selective non-nucleoside inhibitor of the NS5B polymerase, to treat HCV genotype (GT) 1-infected patients. All three drugs are P-glycoprotein substrates, CYP3A4 substrates, OATP1B1 inhibitors and P-glycoprotein inhibitors in vitro and/or in vivo. ASV induces CYP3A4, and is an OATP1B1 substrate. BMS-791235 appears to induce CYP3A4. BMS-794712 is the active metabolite of BMS-791325. No clinically meaningful DDI occurred previously between DCV and ASV. In this study, potential DDIs of the triple combination were assessed in a subset of patients.

**Methods:** Study AI443014 is a phase 2a open-label, multiple-dose study combining DCV (60 mg QD), ASV (200 mg BID, tablet), and BMS-791325 at two doses (75 mg BID or 150 mg BID) in 32 treatment-naive, HCV GT 1-infected, non-cirrhotic patients for 12 or 24 weeks. Non-compartmental pharmacokinetic parameters were derived on Day 14. The pharmacokinetics of all analytes in each regimen (N = 12 for 75 mg BID and N = 18 for 150 mg BID), including BMS-794712, were explored versus historical data graphically and using descriptive statistics.

**Results:** See Table. Day 14 exposures: DCV and BMS-791325 were comparable to historical data. ASV exposures appeared to be reduced by ~30%; variability was high. Metabolic ratio for BMS-794712 was ~25%.

**Conclusion:** No clinically meaningful interaction was observed by addition of BMS-791325 to DCV and ASV. Additional study of both doses of BMS-791325 in this triple combination is warranted to confirm the most appropriate dose in broader patient populations.

| **Table: AUC(TAU) (ng·h/mL), Geometric Mean (CV%) on Day 14** |
|-----------------|-----------------|-----------------|
| **This study**  | **Historical values** |
| Daclatasvir     | 11248 (36)       | 10700 (30.7) N = 11 |
| Asunaprevir     | 1065 (78)        | 1528 (106) N = 12   |
| BMS-791325      | 9554 (65)        | 9170 (34)          |
| BMS-794712      | 2364 (48)        | 2150 (35)          |

DCV 60 mg QD+ASV 200 mg BID+BMS-791325 150 mg BID; ASV 9170 (34)§ 2150 (35)§ 451

Daclatasvir (DCV), an NS5A replication complex inhibitor, and asunaprevir (ASV), an NS3 protease inhibitor were combined with BMS-791235, a potent, selective non-nucleoside inhibitor of the NS5B polymerase, to treat HCV genotype (GT) 1-infected patients. All three drugs are P-glycoprotein substrates, CYP3A4 substrates, OATP1B1 inhibitors and P-glycoprotein inhibitors in vitro and/or in vivo. ASV induces CYP3A4, and is an OATP1B1 substrate. BMS-791235 appears to induce CYP3A4. BMS-794712 is the active metabolite of BMS-791325. No clinically meaningful DDI occurred previously between DCV and ASV. In this study, potential DDIs of the triple combination were assessed in a subset of patients.

**Methods:** Study AI443014 is a phase 2a open-label, multiple-dose study combining DCV (60 mg QD), ASV (200 mg BID, tablet), and BMS-791325 at two doses (75 mg BID or 150 mg BID) in 32 treatment-naive, HCV GT 1-infected, non-cirrhotic patients for 12 or 24 weeks. Non-compartmental pharmacokinetic parameters were derived on Day 14. The pharmacokinetics of all analytes in each regimen (N = 12 for 75 mg BID and N = 18 for 150 mg BID), including BMS-794712, were explored versus historical data graphically and using descriptive statistics.

**Results:** See Table. Day 14 exposures: DCV and BMS-791325 were comparable to historical data. ASV exposures appeared to be reduced by ~30%; variability was high. Metabolic ratio for BMS-794712 was ~25%.

**Conclusion:** No clinically meaningful interaction was observed by addition of BMS-791325 to DCV and ASV. Additional study of both doses of BMS-791325 in this triple combination is warranted to confirm the most appropriate dose in broader patient populations.
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COMPARISON BETWEEN DIFFUSION-WEIGHTED MRI, FIBROSCAN AND HISTOPATHOLOGY FOR ASSESSMENT OF LIVER FIBROSIS IN CHRONIC HCV PATIENTS

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Introduction: Chronic hepatitis C virus (HCV) infection is responsible for liver fibrosis. Number of imaging based methods including transient elastography (fibroscan) and diffusion weighted MRI have been proposed for non-invasive diagnosis and staging of hepatic fibrosis.

Aim of the work: To assess the accuracy of diffusion-weighted MRI and/or fibroscan in the diagnosis of liver fibrosis as compared to histopathology of liver.

Patients and Methods: Abdominal ultrasound, transient elastography (fibroscan) (that measures liver stiffness in kilopascals (KPa)), Diffusion-Weighted MRI of the liver (that measures the apparent diffusion coefficient (ADC)) and needle liver biopsy were done for 52 chronic HCV patients for assessment of liver fibrosis. The used classification for the liver stiffness measured by fibroscan was that described by De L´edinghen and Vergniol (2008) who used stiffness ≥9.5 kPa for diagnosing fibrosis stages ≥F3 (advanced fibrosis).

Results: There was a significant difference between ADC values of F0 versus F1, F3 and F4 (P=0.008, 0.033 and 0.015) respectively, however no significant differences were seen in the ADC values between the other different fibrosis stages.

As regard the liver stiffness values, there was a significant difference between F1&F3 (P =0.001), F1&F4 (P =0.024) and between F2&F3 (P =0.014).

The concordance between fibroscan reading and histopathology was statistically significant (p <0.001, Kappa =0.470).

There was no significant difference in the ADC values between (F0, F1, F2) on one hand and (F3, F4) on the other hand (P=0.387), while there was a highly significant difference in the liver stiffness values between both groups (p <0.001).

Conclusions:
- Diffusion Weighted MRI can be used to distinguish non-fibrotic liver (F0) from liver with advanced fibrosis (F3&F4) but cannot be used to distinguish between the intermediate stages of fibrosis.
- Fibroscan can differentiate between (F0, F1, F2) on one hand and (F3, F4) on the other hand with significant agreement between fibroscan reading and histopathology.

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PREDICTORS OF MORTALITY AMONG UNITED STATES VETERANS WITH HEPATITIS C VIRUS INFECTION

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Background: Hepatitis C virus (HCV) infection is associated with significantly increased risk of mortality. Understanding the predictors of mortality in this subset can be useful for targeting interventions to improve their outcome.

Objective: To determine predictors of mortality among Veterans with HCV infection.

Methods: A retrospective cohort study using the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES). HCV infection was ascertained using antibody testing at baseline. All-cause mortality data was obtained using record linkage. We compared the results with that of age- and sex-matched HCV negative Veteran controls in the ERCHIVES database. Survival analyses were performed using Cox Proportional Hazard models. Multivariate Cox-regression models were used to determine the association of several baseline characteristics with risk of all-cause mortality.

Results: We analyzed data on 195,585 HCV positive and 202,739 HCV negative Veterans who had complete information on all covariates assessed. The all-cause mortality rate among Veterans with HCV infection was 43.9 (43.4–44.3) per 1000 person-years (PY). The corresponding figure for Veterans without HCV infection was 24.0 (23.7–24.4) per 1000 PY. Among Veterans with HCV infection, decompensated liver disease (hazard ratio [95% CI] 3.05 [2.97–3.14]), anemia (2.03 [1.98–2.08]), cancer (1.72 [1.67–1.77]), chronic kidney disease (1.42 [1.38–1.46]) and COPD (1.40 [1.35–1.44]) were the strongest predictors of higher risk of mortality, while HCV treatment was associated with significantly lower risk of mortality (0.43 [0.41–0.46]). The findings were generally comparable with those for HCV negative Veterans.

Conclusion: Decompensated liver disease, anemia and cancer were the strongest predictors of mortality in Veterans with HCV infection. HCV treatment was associated with over 50% reduction in mortality in this group.

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MICRORNA PROFILE MODIFICATIONS IN HEPATITIS C VIRUS-RELATED MIXED CRYOGLOBULINEMIA

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HCV chronic infection is closely related to the development of lymphoproliferative disorders (LPDs), mainly mixed cryoglobulinemia (MC) and some types of lymphoma. The pathogenesis of HCV-LPDs is still largely unknown. Modification of the expression levels of specific microRNAs (miRNAs) has been associated with different autoimmune and/or LPDs. Scarce data exist about the modifications in miRNA expression levels in HCV-related LPDs. The aim of this study was to analyze the expression levels of a panel of miRNAs previously associated with autoimmune/LPDs in a large population of HCV patients with and without MC or non-Hodgkin’s lymphoma (NHL), to identify potential markers of evolution of HCV infection.

PBMC expression levels of miR-17d, miR-16, miR-21, miR-26b, miR-146a and miR-155 were evaluated by Real Time PCR in 167 HCV patients (75 with MC [HCV-MC], 11 with HCV-related NHL) and in 35 healthy blood donors (HS). Synthentic C. Elegans miR-39 was added as external control.

Similar expression levels for miR-146a were observed in all studied groups. A significant increase of expression levels was detected in PBMCs from only NHL patients whereas a significant decrease of miR-26b was detected in both MC and NHL subjects when compared to HS and HCV groups. A restoration of miR-26b levels was observed in the post-treatment PBMC of 35 HCV-MC patients experiencing complete virological and clinical response following antiviral therapy.

This study, for the first time, shows that specific microRNAs in PBMC from HCV patients who developed MC and/or NHL are modulated differently. Results obtained for miR-21, miR-16 and miR-155 are consistent with the upregulation previously observed in other lymphatic malignancies. The specific, reversible downregulation of miR-26b strongly suggests the key role it plays in the pathogenesis
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of HCV-related LPDs and its usefulness as a biomarker of the evolution of HCV infection to these disorders.

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APOLIPOPROTEIN H IS STRONGLY ASSOCIATED WITH IL28B SNP GENOTYPE AND PREDICTS VIRAL CLEARANCE IN BOTH ACUTE AND CHRONIC HEPATITIS C VIRUS INFECTION

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Background and Aims: Hepatitis C viral clearance is been associated with polymorphisms in IL28B, which encodes type III interferon, IFN-13. No functional basis for this relationship has been characterized. HCV requires host lipid metabolism for replication and apolipoproteins have also been implicated in the response to IFN treatment. We aimed to investigate protein quantitative Trait Loci (pQTL) associations between IL28B genotype and apolipoprotein levels in multiple patient cohorts.

Methods: The rs12979860 single nucleotide polymorphism (SNP) in IL28B and plasma apolipoprotein levels were determined and associations with viral clearance were examined in three cohorts of patients with symptomatic acute hepatitis C (aHCV, n = 33), treatment of chronic hepatitis C (cHCV, n = 141), treatment of chronic hepatitis C in HIV/HCV co-infected (n = 42) and in 100 healthy donors. Univariate and multivariate regression analyses were utilized to examine the impact of IL28B genetic variability and apolipoprotein levels (medians) on HCV clearance.

Results: Plasma apolipoprotein H (apoH) levels were significantly higher in “CC” IL28B patients in: (1) the aHCV cohort, 175 vs. 136.5 mg/ml in non-CC (p = 0.03); (2) the HC patients, 249.5 vs. 182 mg/ml in non-CC (p < 0.0001); and (3) the HIV/HCV co-infected 286 vs. 197 mg/ml in non-CC (p = 0.05). ApoH levels were strongly associated with HCV clearance with an OR = 4.58 (95%CI: 1.46–14.3) in aHCV, 191 (95%CI: 1.32–2.76) in cHCV and 9.85 (95%CI: 2.0–68.7) in HIV/HCV co-infection. In multivariate analyses, the effect of apoH levels on HCV clearance remained significant when adjusted for IL28B SNP status, while the positive correlation of IL28B with viral clearance was lost when corrected for apoH levels. Healthy individuals demonstrated no correlation between IL28B genotype and plasma apoH levels.

Conclusions: We have identified a trans-pQTL relationship between the CC IL28B allele and plasma apolipoprotein H, an apolipoprotein independently associated with viral clearance during acute and chronic HCV infection. This IL28B-apoH association was not observed in healthy individuals, suggesting that early post-infection events trigger differential apoH expression in an IL28B allele dependent manner. This relationship identifies apoH as the first protein quantitative trait loci described for IL28B, as well as characterizes a novel host factor implicated in HCV clearance.

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CC GENOTYPE OF rs12979860 IL28B MODERATES PROGRESSION OF LIVER FIBROSIS IN HCV-INFECTED PATIENTS

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Introduction: The rs12979860 polymorphism in IL28B is associated with spontaneous and treatment-induced clearance of HCV. The variations in regional genotype distribution explain different response to treatment. The influence of IL28B genotypes on the natural course of the disease, namely progression of liver fibrosis, has not been established unequivocally yet. The aim of our study was to assess the frequency of IL28B genotypes in HCV-infected patients and healthy controls in the Czech Republic and evaluate their impact on the fibrosis progression in HCV.

Patients’ characteristics: In total, 466 HCV-infected patients from 3 hepatological centers were examined. Sixty nine patients underwent liver transplantation for cirrhosis C. Healthy age and sex matched controls were chosen from the cohort of the MONICA study (WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease Project).

Methods: The IL28B rs12979860 locus was genotyped in all 932 specimens by PCR Thal-RFLP analysis. χ2-test was used to assess the significance of the differences between allelic frequencies.

Results: In the group of HCV liver transplant patients (n = 69), the numbers of carriers and frequency of the CC, CT and TT genotypes were 12 (17.4%), 37 (53.6%) and 20 (29.0%), respectively. In the group of patients with chronic HCV infection (n = 397), the CC genotype was found in 129 (32.5%) patients, CT in 197 (49.6%) patients and TT in 71 (17.9%) patients. In the group of the healthy paired population controls (n = 466), the following numbers the genotype carriers were observed: CC 206 (44.2%), CT 201 (43.1%), TT 99 (20.7%).

The CC genotype was significantly less frequent in the group of the HCV-infected patients, compared to healthy population controls (p < 0.0001). Concurrently, in the group of HCV liver transplant patients, the frequency of the CC genotype was significantly lower than in the group of non-transplanted HCV patients (p = 0.012). The presence of the T allele increased the risk of being listed for liver transplantation in the allelic model (OR 2.29, CI 1.19–4.41).

Conclusions: The differences between the CC genotype frequency recorded in the examined groups indicate that the CC genotype may moderate the progression of liver fibrosis caused by HCV infection.

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THE QUALITY OF LIVER SPECIMEN USING TWO DIFFERENT LARGE NEEDLE BIOPSY DEVICES: A PROSPECTIVE RANDOMIZED-CONTROLLED STUDY

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Background and Aims: Current guidelines recommend a 16-gauge (1.6 mm diameter) large needle to obtain an optimal sized liver biopsy (LB), which should be at least 2 cm in length and contain ≥11 complete portal tracts (CPT). Despite this, in real life clinical practice many operators use large needles with inner caliber smaller than 16G. The aim of this study was to prospectively assess
the diagnostic accuracy of two different types of LB needles with inner caliber <16G.

Methods: We randomized 241 consecutive patients referred for diffuse liver disease to undergo ultrasound-guided percutaneous LB with either a 17G (1.4 mm) or a 18G (1.2 mm) Menghini modified needle. To assess the adequacy and quality criteria for biopsy specimen, data were collected on length of liver specimen and number of CPT. LB requiring >3 passages and samples <2 cm were excluded.

Results: Main indication for LB were grading and staging for HCV (37%) and abnormal liver function tests (18%). Only one minor complication requiring no intervention occurred (intrahepatic hematoma). The mean macroscopic length of the specimen was 3.38±1.19 cm for 17G and 3.44±1.24 cm for 18G, while the mean length after formalin embedding (microscopic length) was 2.79±0.98 cm for 17G and 2.69±1.02 cm for 18G. The mean number of CPT was 10.5±5.6 for 17G and 8.8±5.8 for 18G (p=0.041). Among the 17G group, 78% of specimens had 8–10 or more CPT and in 49% of cases it had at least 11 CPT (“optimal fragment”); in the 18G group, 68% of specimens had 8–10 or more CPT and in 41% of cases it had at least 11 CPT (p=0.054). The correlation between length and CPT showed that to obtain an adequate sample a macroscopic length of 2.9 cm is needed, while a length of 2.6 cm is needed after formalin embedding.

Conclusions: Percutaneous LB can obtain adequate histological material also when using a needle thinner than the recommended 16G provided that the specimen shows a minimum macroscopic length of 3 cm.

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UTILITY OF SERUM SAMPLES FOR IL28B GENOTYPING:
A VALUABLE SOURCE OF GENOMIC DNA
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Background and Aims: Single nucleotide polymorphism (SNP) rs12979860 upstream of the IL28B coding region is strongly associated with hepatitis C virus clearance and is now used in many settings to tailor treatment decisions. Large amounts of high-quality genomic DNA can be obtained from whole-blood. However, in many cases the available amount of blood is limited or only plasma/serum is available. The aim of this study was to assess whether IL28B (rs12979860) genotyping from serum-extracted genomic DNA is a reliable alternative to whole-blood-based determination.

Methods: We determined the IL28B genotype from 146 stored serum samples (~90°C), using three different PCR-based methods (TaqMan® assay and direct sequencing of conventional PCR or SYBR Green-based PCR products). An automated extraction protocol with QIAamp DNA Blood Mini kit (QIAGEN) was used in a subset of 75 samples with sufficient volumes. The results were compared with those obtained from whole-blood extracted DNA in the same individuals (used as the reference IL28B genotype).

Results: Successful genomic DNA extraction was achieved in 143 out of 146 serum samples, using the automated extraction protocol. TaqMan® assay on serum DNA achieved call-rates (genotype assignment) for a SNP other than unknown) in 100% of the samples, while the direct sequencing methods of conventional and SYBR Green PCR amplicons demonstrated lower call-rates, 95.8% and 97.9%, respectively. In comparison with the reference genotypes from whole blood samples, the concordance for TaqMan® assay on serum samples was 100%, versus 98.5% and 98.6% for the two direct sequencing methods, respectively. Using the manual extraction protocol, the call-rates for the three genotyping assays were lower (96%, 90.7%, and 81.3%, respectively), and concordance was less good (95.8%, 98.5%, and 98.3%, respectively) than with automated extraction. The variation in performance could be attributed to different quantities and qualities of DNA obtained with the different extraction methods and to the characteristics of the genotyping method used.

Conclusion: This study demonstrates that stored serum samples represent a reliable source of genomic DNA for SNP genotyping, such as IL28B rs12979860, provided that optimized, standardized techniques are used.

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INSULIN RESISTANCE AND HIGH CHOLESTEROL LEVELS ARE ASSOCIATED WITH VITAMIN D DEFICIENCY IN HCV, HIV AND HIV/HCV COINFECTED PATIENTS
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Background and Aims: Vitamin D plays a role in metabolic syndrome and has also been suggested as an immunomodulator. Lower levels are correlated with severe fibrosis in HCV and HIV/HCV coinfected patients and predict lower response to treatment in those individuals. The aim is to evaluate levels of 25(OH)vitamin D among a population of HCV, HIV and HIV/HCV coinfected patients and describe associated factors.

Patients and Methods: We collected 25(OH)vitamin D samples, demographic data, clinical information and laboratory tests including liver function and metabolic assessment of four groups of patients: 1 – HCV monoinfected, 2 – HIV monoinfected, 3 – HIV/HCV coinfected, followed at reference centres of São Paulo-Brazil and 4 – Healthy Volunteers Control Group.

Results: 422 patients were included for analysis, (129) Group 1, (118) Group 2, (53) Group 3 and (122) Group 4. Mean levels of Vitamin D were similarly insufficient in all groups (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean (ng/mL)</th>
<th>St. D.</th>
<th>St. E.</th>
<th>Median (ng/mL)</th>
<th>IQR (ng/mL)</th>
<th>Min (ng/mL)</th>
<th>Max (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – HCV</td>
<td>129</td>
<td>23.4</td>
<td>10.1</td>
<td>0.89</td>
<td>23</td>
<td>13</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>2 – HIV</td>
<td>118</td>
<td>19.5</td>
<td>9.2</td>
<td>0.85</td>
<td>18</td>
<td>12</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>3 – HIV/HCV</td>
<td>53</td>
<td>24.1</td>
<td>12.9</td>
<td>1.77</td>
<td>22</td>
<td>15</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>4 – Control</td>
<td>122</td>
<td>17.1</td>
<td>5.9</td>
<td>0.54</td>
<td>17</td>
<td>8.75</td>
<td>6</td>
<td>32</td>
</tr>
</tbody>
</table>

In an overall analysis, Vitamin D deficiency (serum levels <20 ng/ml) was associated with higher HOMA index (Graph 1 – p = 0.02 Fisher test) and total cholesterol levels >200 (p = 0.004 Fisher test). When analyzed by Groups, Vitamin D deficiency was associated with:

1. Higher HOMA levels in HCV patients (Graph 2 – p = 0.004 Fisher test).
2. Use of Efavirenz both in HIV (Graph 3 – p = 0.03 OR = 6.69 95%CI: 1.17–38.3) and Coinfected Patients (p = 0.04 OR = 15.0 95%CI: 1.22–184).

Conclusion: This study found high prevalence of vitamin D deficiency, even in healthy volunteers. The association between Insulin Resistance (IR) and Vitamin D deficiency has been demonstrated in other populations, but not previously described in HCV patients. This finding is relevant because both IR and Vitamin D deficiency are related to poor treatment outcomes of Interferon-based regimens.
Background and Aims: The natural course of serum HCVRNA levels during chronic infection remains unclear. We longitudinally evaluated HCVRNA levels following HCV seroconversion to identify factors associated with differences in RNA levels.

Methods: The Amsterdam Cohort Study among drug users (DU) is an open, prospective cohort study initiated in 1985 and is still ongoing. We retrospectively tested for anti-HCV using serum from all participants and identified 106 DU with a known HCV seroconversion interval. HCVRNA levels were measured by bDNA (VERSANT 3.0, lower limit, 615 IU/mL) at yearly intervals. Chronic cases with detectable HCVRNA for more than two years were included. To examine the HCV viral load patterns we used a latent class linear mixed model. We studied the effects of interleukin 28B (IL28B) (rs12979860) genotype, gender and HIV coinfection on HCVRNA levels.

Results: Out of 106 DU, 54 chronic HCV cases were included, of whom 33/54 (61%) were male. Median age at HCV seroconversion was 28 years (IQR 26–35). Median follow-up time was 10.8 years (IQR 6.5–14.9). At acute HCV infection 12/54 (15%) cases were HIV positive and 10 cases acquired HIV during follow-up. We defined two distinct classes of viral load patterns, see figure 1. In multivariable analyses, HCVRNA levels were $0.36 \log_{10}$ IU/mL (95% CI 0.06–0.67) higher for males as compared to females. Individuals with the favorable IL28B CC genotype tended to have higher HCVRNA levels than individuals with Il28B CT/TT genotypes (difference $0.28 \log_{10}$ IU/mL, 95% CI $0.02–0.57$). HIV coinfected individuals had a $0.26 \log_{10}$ IU/mL (95% CI 0.01–0.51) higher HCVRNA level than HCV monoinfected individuals.

Conclusion: To our knowledge, this is the first study to examine the HCVRNA pattern, over up to 17 years, in a unique cohort of HCV seroconverters. Male sex, IL28B CC genotype and HIV coinfection correspond with higher HCVRNA levels than in both classes of HCVRNA patterns. As these factors are associated with treatment outcome, further research on differential effects of HCVRNA levels on the natural history is of great interest.
SPONTANEOUS CLEARANCE OF ACUTE HCV INFECTION IS ASSOCIATED WITH FEMALE SEX, IL28B GENOTYPE AND HCV GENOTYPE 1 INFECTION

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Aims: Although 20–40% of persons with acute HCV infection demonstrate spontaneous clearance, the time-course and host and viral factors associated with clearance remains poorly understood. The aim of this study was to investigate time to spontaneous clearance and associated factors among participants with acute HCV.

Methods: Data for this analysis were drawn from an international collaboration of nine prospective cohorts evaluating HCV infection risk and outcomes among people who inject drugs (InC3 Study). Individuals with incident HCV were identified (seroconversion within two years or symptomatic infection with seroconversion illness), including a subset of individuals with well-defined early acute HCV infection (HCV antibody negative and RNA positive at the time of acute detection). Time to spontaneous clearance was assessed and associated factors were identified using Cox proportional hazards analyses.

Results: Among 669 with incident HCV (35% female, mean age 29, 82% Caucasian), 49% had favorable IL28B genotype (rs12979860 CC), 47% were HCV genotype 1 (15% unknown) and 28% had well-defined acute HCV. Spontaneous clearance was observed in 26% (95% CI: 23–29%) overall and 23% (95% CI 17-30%) among those with well-defined acute HCV. Spontaneous clearance was higher in females (33% vs. 17% males, P = 0.009), those with favorable CC IL28B genotype (36% vs. 17% CT/TT, P = 0.008) and HCV genotype 1 (28% vs. 11% non-1 genotype, P = 0.008). Among females with favorable CC IL28B genotype, spontaneous clearance was 57% (female-CT/TT=20%, male-CC=21% and male-CT/TT=16%) and time to clearance was significantly shorter (P < 0.001, Figure).

In multivariate Cox proportional hazards analysis of time to spontaneous clearance in those with well-defined acute HCV (n = 187), a significant interaction between female gender and IL28B genotype was observed (P = 0.027); with the shortest time to clearance among females with the favorable CC IL28B genotype vs. male-CT/TT, adjusted hazards ratio (AHR) 11.88, 95% CI: 4.40, 32.08; P < 0.001 and HCV genotype 1 vs. non-1, AHR 4.21, 95% CI: 1.79, 9.95, P = 0.001).

Conclusions: This study shows that female sex, favorable IL28B genotype and HCV genotype 1 are associated with spontaneous clearance with a potential synergistic effect between female sex and favorable IL28B genotype.

THE ASSOCIATION BETWEEN HAEMOGLOBIN DECLINE AND SVR IS INDEPENDENT OF ITPA GENOTYPE DURING PEG-INTERFERON PLUS RIBAVIRIN THERAPY FOR HCV-1: ANALYSIS FROM THE CHARIOT STUDY

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Introduction: Two functional inosine triphosphatase (ITPA) genetic variants causing ITPase deficiency are strongly associated with protection from ribavirin (RBV)-induced anaemia. ITPA genotype is not associated with a lower SVR, despite the well-documented association between on-treatment anaemia and higher SVR after peg-interferon and RBV (PR) therapy. To examine this apparent inconsistency, we studied the relationships between ITPA genotype, on-treatment haemoglobin (Hb) reduction, and SVR in a large cohort of HCV-1 patients from the CHARIOT study.

Methods: In CHARIOT, patients were randomised to peg-interferon 180 or 360mcg/week from week 0–12, followed by peg-interferon 180mcg/week plus RBV until week 48. An association between anaemia and SVR has been described in this cohort. ITPA polymorphisms rs1127354 and rs7270101 were genotyped and ITPase activity defined as previously. Regression models were used to confirm the association between ITPA genotype deficiency and on-treatment anaemia. The LOWESS method was used to explore the relationship between SVR and on-treatment Hb change.

Results: ITPA genotype was determined in 546 patients: 65% had normal ITPase activity, and 35% reduced ITPase activity, balanced across treatment arms. ITPase deficiency was strongly associated with lower Hb reduction at week 4 (P = 10^-31), and during the treatment course. On-treatment anaemia (Hb ≤ 100 g/L) was associated with SVR (P = 0.036) however ITPase deficiency was not (P = 0.6912). The estimated local probabilities of SVR were plotted against the nadir Hb for patients with normal vs reduced ITPase activity (Figure).

The probability of SVR increased with lower nadir Hb levels. Trends were similar for patients with normal or reduced ITPase activity, with the curve shifted to the right with reduced ITPase activity. Peg-interferon dosing regimen was not associated with overall
treatment outcome, Hb decline >30 g/L during weeks 0–12, and did not confound the analyses by ITPase activity.

Figure: LOWESS plot: SVR probability by lowest Hb.

Conclusion: ITPase deficiency is strongly associated with protection from Hb decline during PR therapy. Hb decline is associated with SVR, but this is independent of ITPase activity. The data strongly suggests the link between Hb decline and virological response is not a direct effect of anaemia, but is likely to reflect individual RBV pharmacokinetics.

463 PHARMACOKINETIC INTERACTIONS BETWEEN THE HCV PROTEASE INHIBITOR BOCEPREVIR AND SIROLIMUS IN HEALTHY SUBJECTS

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Background and Aims: Boceprevir is a potent, orally administered, ketoamide inhibitor of the hepatitis C virus (HCV) NS3 protease. HCV-related end-stage liver disease and hepatocellular carcinoma are frequent causes of liver transplantation. Sirolimus is a macrolide immunosuppressant frequently used for the prophylaxis of transplant rejection. This study was conducted to evaluate the pharmacokinetic interaction of boceprevir and sirolimus in healthy volunteers.

Methods: In this open-label, 3-period, fixed-sequence trial, 12 subjects received single-dose sirolimus (2 mg) in period 1 and boceprevir (800 mg TID for 6 days) in period 2 with an intervening washout of 14 days. Period 3 immediately followed period 2 with no intervening washout: subjects received sirolimus (2 mg SD) plus boceprevir (800 mg TID) on day 1 and boceprevir (800 mg TID) thereafter for 9 days. Blood samples were collected for the pharmacokinetic assessment of sirolimus and boceprevir. Safety assessments included electrocardiograms, vital signs, clinical laboratory tests, physical examination, and adverse event monitoring.

Results: Twelve healthy volunteers were enrolled and 11 completed the trial. Coadministration of boceprevir and sirolimus was well tolerated. Boceprevir co-administration increased the geometric mean AU CO∞ and Cmax of sirolimus by 8.1-fold and 4.8-fold, respectively, with the corresponding 90% CIs of (7.08, 9.32) for AU CO∞ and (3.99, 5.88) for Cmax. The elimination half-life of sirolimus increased from 82.5 h to 98.3 h, and the apparent clearance decreased from 9.62 L/min to 1.18 L/min, respectively, with boceprevir co-administration. Sirolimus co-administration did not affect the pharmacokinetics of boceprevir (AU CO∞ and Cmax GMR [90%CI] of 1.0 [0.89, 1.01] and 0.9 [0.82, 1.07], respectively).

Conclusions: Concomitant administration of boceprevir and sirolimus resulted in increased steady-state exposures of sirolimus in healthy subjects. The magnitude of the potential interaction between sirolimus and boceprevir in organ transplant patients is not known, but could potentially be higher and more variable than that seen in healthy volunteers. Dose adjustment of sirolimus and/or prolongation of the dosing interval should be anticipated when administered with boceprevir, and should be guided by close monitoring of sirolimus blood concentrations, and frequent assessment of renal function and sirolimus-related side effects.

464 INFECTIVITY RATIO COULD EXPLAIN WHY SUBJECTS WITH FAVORABLE ILE-28B GENOTYPE HAD LOWER RATES OF SUSTAINED Virological RESPONSE

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Introduction: In patients with chronic hepatitis C genotype 1 (CHC-1), the expression of LDL-cholesterol receptor is regulated by sterol regulatory element-binding protein 2 (SREBP-2) as physiological pathway of entry of LDL-cholesterol. The degree of inhibition of Microsomal Triglyceride Transfer Protein enzyme (MTP) after administration of interferon could explain the different degree of secretion of very low-density lipoprotein (VLDL) associated with (virolipoparticles-LVP). This fact, together to the degree of activity of lipoprotein lipase (LPL) seems to be responsible for the level of hypertriglyceridemia. These virolipoparticles could compete with HDL-c, using Scavenger receptor (SR-B1) to introduce into hepatocyte as an alternative pathway.

Objective and Design: We wanted to know which factors could be related in patients with favorable ILE-28B genotype (CC) with absent of sustained virological response (SVR). We included 105 CHC-1, which were treated with dual antiviral therapy during 48 weeks.

Results: Rate SVR: 52.5% (74% in CC-group; 38% in CT/TT group). Patients with favorable genotype (CC) who did not achieve SVR had a higher baseline level of triglycerides than those subjects CC with presence of SVR (98 ± 25 versus 76 ± 21 mg/dl; OR 1.0 95%CI (1.0–1.1); p < 0.018); higher value of baseline VLDL (20.1 ± 5.1 vs 15.4 ± 3.5; OR 1.3 95%CI (1.0–1.7); p < 0.028); a higher value of “Infectivity Ratio” index = median value of triglycerides/median value of HDL during the 1st month-therapy (4.6 ± 3.2 versus 2.5 ± 1.1; OR 1.9 95%CI (1.1–3.4); p < 0.02).

Patients with (CT or TT) genotype achieved higher rates of RVR if they had a lower median level of LDL-cholesterol during the 1st month-therapy [74 ± 20 vs 91 ± 27 mg/dl; OR 1.0 95%CI (1.0–1.1); p < 0.04). Patients with favorable genotype (CC) who did not achieve SVR had a higher Infectivity Ratio (4.6 ± 3.2 versus 2.5 ± 1.1; OR 1.9 95%CI (1.1–3.4); p < 0.02).

Conclusions: High Infectivity Ratio would explain why patients with CC-ILE-28B genotype did not achieve SVR because of hypertriglyceridemia caused by a higher secretion of LVP and probably lower activity of LPL. Patients CT/TT-ILE-28B genotype who had a lower median level of LDL-cholesterol during the first month-therapy had a higher rate of RVR, fact that could be related to different activity of SREBP-2 (degree of expression LDLr).
465 POTENTIAL ECONOMIC IMPACT OF APPLYING ONUBA SCALE: A NOVEL PROGNOSTIC MODEL OF RESPONSE IN CHRONIC HEPATITIS C GENOTYPE 1

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Objective: We developed a powerful predictive model (PM) of response to dual therapy in chronic hepatitis C genotype 1 (CHC-1) called Onuba Scale (OS), based on scores obtained from using 4 baseline variables (BV) and viral and lipid kinetics, to establish who patients could be treated with dual or triple therapy. We wanted to know the potential economic impact that could generate in clinical practice.

Design: This double-blind randomized assay included 103 CHC-1 patients, who were randomized to receive a masked first induction-dose or a standard-dose plus ribavirin to compare the degree of sensitivity to interferon during 1st week-therapy, establishing who patients achieved “First Week Virological Response” and had a “Favorable Lipid Metabolism” (FLM), according with their fibro-virological and lipid requirement. Use of 4 BV plus 2 kinetical variables (FWVR and FLM) generated a final score, based on 3 different time points of therapy (baseline, 1st and 4th week-therapy). We calculated cost saving resulting from applying OS.

Results: PM made a correct prognosis in 94% subjects. Using scores obtained from Baseline Onuba Scale and Onuba-Week Scale, therapy could have been stopped in 21% patients at 1st week and 25% of them at 4th week-therapy. Total cost saving (antiviral therapy, Epoetin, Filgrastim, and medical visits) was 356,304€.

Conclusions: OS could become a strong, cost-efficient and very early PM of response for CHC-1 patients. Implementation of OS in clinical practice will probably generate significant financial savings, establishing who patients would not benefit from dual therapy, being better to treat with triple therapy.

466 INTERLEUKIN-28B rs12979860 C ALLELE IS PROTECTIVE AGAINST ADVANCED FIBROSIS IN CHRONIC HEPATITIS C GENOTYPE 1 INFECTION: ANALYSIS OF THE AUSTRALASIAN CHARIOT STUDY COHORT

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Background and Aims: Interleukin-28B (IL28B) genotype is the strongest pre-treatment predictor of sustained virologic response to therapy with pegylated interferon plus ribavirin in HCV genotype 1 (HCV-1) infection. However, the relationship of IL28B genotype to liver histology is yet to be determined.

Methods: Serum of 266 treatment-naïve Australian patients with HCV-1 and recent liver biopsy from the CHARIOT study was tested for the IL28B rs12979860 single nucleotide polymorphism. The relationship between IL28B genotype, demographics, baseline laboratory values, and advanced liver fibrosis (METAVIR F3/4) stage and high activity (A2/3) grade was analyzed by Chi-square test and multivariable logistic regression analysis.

Results: 44 (16.5%) patients had advanced fibrosis and 141 (53%) had high activity. Prevalence of advanced fibrosis was lower in those with IL28B CC genotype compared to those without (11% vs 21%), with an increasing number of T alleles associated with a higher frequency of advanced fibrosis as shown in Table 1. Predictors of advanced fibrosis on multivariate analysis are shown in Table 2. There was a trend towards increased prevalence of high activity grade in those with IL28B TT genotype when compared to non-TT genotype (69% vs 52%; P = 0.08). Factors associated with high activity grade on multivariate analysis were fibrosis stage (P < 0.0001), haemoglobin (P = 0.01) and GGT (P = 0.01), with no association between IL28B CC genotype and activity grade.

Table 1.

<table>
<thead>
<tr>
<th>IL28B genotype</th>
<th>F3/4 stage Prevalence</th>
<th>P value</th>
<th>A2/3 grade Prevalence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC 11% (13/118) 50% (60/119)</td>
<td>11% (13/118) 50% (60/119)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT 18% (20/111) 0.01</td>
<td>18% (20/111) 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT 33% (10/30) 0.19</td>
<td>33% (10/30) 0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>IL28B CC genotype</th>
<th>F3/4 vs F0–2 fibrosis stage Odds ratio 95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.36</td>
<td>0.14–0.93</td>
<td>0.03</td>
</tr>
<tr>
<td>High activity grade 5.68</td>
<td>1.86–17.32</td>
<td>0.002</td>
</tr>
<tr>
<td>Platelet count 0.98</td>
<td>0.97–0.99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AST 1.02</td>
<td>1.00–1.03</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Conclusions: In a large well-characterized cohort of treatment-naïve HCV-1 patients an increasing number of IL28B T alleles is associated with higher prevalence of advanced fibrosis, likely mediated via increased hepatic necroinflammation. In contrast CC genotype is protective against advanced fibrosis independent of activity grade. The mechanism of this effect requires further investigation.

467 COST IMPLICATIONS OF ONE-TIME HCV SCREENING OF THE 1945 TO 1965 BIRTH COHORT IN BRITISH COLUMBIA, CANADA

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Background and Aims: The Centers for Disease Control & Prevention (CDC) report that the 1945–1965 birth cohort accounts for about 75% of prevalent HCV infections in the US and many are unaware of their infection. This cohort is aging and increasingly at risk of liver disease. The CDC recommends one-time HCV screening of the birth cohort, in addition to current risk-based screening recommendations, to identify those at risk of HCV-related morbidity and mortality. We estimated the cost of screening and confirmation of HCV infection for the British Columbia (BC) 1945–1965 birth cohort.

Methods: We used BC data and published estimates to calculate the costs of screening, confirmation of chronic HCV infection
and genotype, and delivering results and counseling to those diagnosed.

Results: The BC 1945–1965 birth cohort is 1.36 M individuals (total population 4.4 M), of whom 357,426 (26%) have already been tested (based on existing risk-based screening recommendations) and 40,345 (11.3% of those tested; 3.0% of the cohort) are known to be HCV positive. This represents 64% of HCV positive individuals diagnosed to date. Based on an estimated overall HCV prevalence in the cohort of 1.5% to 3%, one-time screening of those still untested (n = 1.0 M) would identify an additional 15,018 to 30,035 individuals for a total of 55,363 to 70,380 HCV infections among the birth cohort. Screening, confirmation of chronic HCV infection, and genotyping, and delivery of positive results and counseling to the newly identified individuals is estimated to be a one-time cost of $15.6 M to $18.5 M.

Conclusions: One-time screening of the untested 1945–1965 BC birth cohort is expected to identify a substantial number of previously unidentified HCV infections. Many of these individuals would benefit from treatment to reduce HCV-related morbidity and mortality, but even if treatment were not offered to those with chronic HCV infection, appropriate counseling and associated behavior change (e.g., reduction of alcohol intake; linkage to care and assessment) would be expected to improve health outcomes for these individuals.

468 DATA MINING MODEL FOR IDENTIFICATION OF PATIENTS AT HIGH RISK FOR HEPATOCELLULAR CARCINOMA AFTER ERADICATION OF HEPATITIS C
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Background and Aims: Recent development of effective hepatitis C (HCV) treatment using direct acting antivirals (DAA) has dramatically improved viral eradication rates. However, hepatocellular carcinoma (HCC) may develop in some patients after viral eradication. This study aimed to develop a model to identify patients at high risk for HCC after eradication of HCV.

Methods: Chronic hepatitis C patients who had sustained virological response to by interferon therapy were recruited by Japanese Red Cross Liver Study Group, involving 18 hospitals and medical centers nationwide. Data at 24 weeks after the completion of therapy were collected from a total of 778 patients with a mean follow up of 6.5 years. Predictive model for HCC development was built by data mining (IBM-SPSS Modeler 14) based on 2/3 patients and was validated by remaining 1/3 patients.

Results: The cumulative incidence of HCC at 5 years was 7.1%. On the basis of factors such as age, aspartate aminotransferase (AST), albumin, platelet, and alpha-fetoprotein (AFP), the HCC risk prediction model identified subgroups with high-, intermediate-, and low-risk of HCC with a 5-year HCC development rate of 38.4%, 11.8%, and 0%, respectively. The reproducibility of the model was confirmed through validation (r² = 0.918). Older patients (>55 yrs) with either high AST (>30 IU/L), low platelet counts (<150 x 10⁹/L) or high AFP (>5.0 ng/ml) had high incidence of HCC development within 5 years (38.4%, 16.7%, and 16.7%, respectively), whereas the incidence was 0% in older patients with neither of these three factors or in younger patients (<55 yrs) with high serum albumin (>4.0 g/dl). Multivariable Cox regression analysis also confirmed that older age, low platelet counts, high AST, and high AFP were independent risk factors for HCC.

Conclusions: Predictive factors for development of HCC following eradication of HCV were older age, lower platelet counts, higher AST, and higher AFP levels. In the era of DAA with high probability of HCV eradication, the HCC risk prediction model using these simple and readily available factors allows physicians to identify patients requiring a careful HCC surveillance even after successful eradication of HCV by antiviral therapy.

469 GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY POTENTIAL SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH SPONTANEOUS HEPATITIS C VIRUS CLEARANCE AMONG CHRONIC HEPATITIS C PATIENTS
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Background and Aims: Host genetic susceptibility has been found to be associated with spontaneous and treatment-induced hepatitis C virus (HCV) RNA clearance. This study aimed to discover single nucleotide polymorphisms (SNPs) associated with spontaneous HCVRNA clearance among chronic hepatitis C patients without treatment experience through the genome-wide association study (GWAS).

Methods: A total of 751 anti-HCV-seropositive and HBsAg-seronegative participants included in the study. All of them were free of hepatocellular carcinoma cases during the follow-up year of 1991–2008. High quality human genomic DNA was extracted from each blood sample to perform genotyping. We applied the Axiom™ Genome-Wide CHB Array, a recently developed tool specifically on Chinese Han population that provides maximum power for GWAS and has capability for genomic researchers to identify trait-associated SNPs in the Han Chinese. The exact test was used to test disease–genotype association for each SNP based on different genetic models (allelic, dominant, and recessive). The multiple logistic regressions were used to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) of the potential SNPs associated with HCVRNA clearance.

Results: There were 267 (35.6%) ever occurred spontaneous clearance and the other 484 (64.4%) remained persistent of HCVRNA during follow-up. Females and individuals who had low serum levels of alanine aminotransferase were associated with higher probability of spontaneous HCVRNA clearance (p < 0.05). In total, 565,379 SNPs with call rate ≥95%, minor allele frequency >0.05, and without violation of the Hardy-Weinberg equilibrium (p > 0.01) were included in the analyses. We found 14 SNPs potentially associated with spontaneous HCVRNA clearance (p < 10⁻⁵). These SNPs were located on the chromosome 6, 8, 9, 10, and 12. After considering of age, gender, and serum levels of alanine aminotransferase, the adjusted odds ratios for the SNPs ranged from 1.4 to 6.4.

Conclusion: There were SNPs identified to be potentially associated with spontaneous HCVRNA clearance in chronic hepatitis C patients. However, these SNPs should be validated by an independent external population and functional studies would be needed.
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CHANGES IN INTERFERON-GAMMA INDUCIBLE PROTEIN-10 IN TREATMENT-NAIVE VERSUS TREATMENT-EXPERIENCED PATIENTS GIVEN AN ALL-ORAL ANTI-HCV REGIMEN


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Background and Objectives: Interferon (IFN)-free treatment regimens for hepatitis C virus (HCV) infection provide a unique opportunity to elucidate mechanistic and kinetic intracellular relationships between HCV and the innate immune response, potentially providing insight into the rate of decay of the viral replication complex itself. IP-10 is an IFN-inducible protein inversely correlated with response rates to interferon treatment. We assessed the effect of IFN-free DAA regimens for HCV on plasma IP-10 levels in 428 subjects from 3 Phase 2 studies.

Methods: We studied patients receiving DAA-therapy for chronic HCV genotype 1 in treatment-naive subjects (GS-US-248–0120), IFN-ineligible or -intolerant subjects (GS-US-248–132), and treatment experienced subjects (GS-US-248–0131). Plasma from 428 subjects (baseline, week 1, and week 2) was analyzed for IP-10 levels using the BMS 284INST Human IP-10 Instant ELISA assay.

Results: Increased baseline plasma IP-10 associated with a “T” allele in the IL28 gene (p < 0.0001), increased ALT (p < 0.0021), and increased BMI (p = 0.0041). Over the first two weeks of treatment, a bi-phasic pattern of plasma IP-10 (log10) decline was observed in all 3 studies (p < 0.0001). From baseline to week 1, average IP-10 decline was 34.8% overall. From week 1 to week 2, IP-10 declined on average 9% for treatment-naive subjects. However, treatment-experienced subjects had a substantially lower average decline in plasma IP-10 (1%). No significant associations between baseline plasma IP-10 levels or change in IP-10 levels and vRVR or SVR12 results were observed.

Conclusions: Potent oral DAA therapy is associated with a rapid reduction in plasma IP-10 levels that parallels the reduction in HCV RNA. These data suggest:
1. Higher baseline IP-10 levels may be associated with more hepatic cell turnover as manifest by higher ALT;
2. IP-10 does not predict relapse in patients.

However, IP-10 may serve as a surrogate marker of decay of intracellular viral replication complexes. Thus, the slower decline in IP-10 levels in patients who have previously failed anti-HCV therapy compared to the treatment naïve population suggests that treatment-experienced subjects may have delayed decay of intracellular HCV replication complexes after initiation of potent DAA treatment regimens.

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MOLECULAR EPIDEMIOLOGY AND SOCIAL NETWORK ANALYSIS TO TRACK HCV TRANSMISSION IN HIGH-RISK COMMUNITIES: INJECTING DRUG USERS IN PRISONS OF NEW SOUTH WALES, AUSTRALIA

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Background and Aims: The HCV epidemic is fuelled by transmission in high-risk communities, notably injecting drug users (IDU). Knowledge of the spatial-temporal and social factors characterizing IDU inmates is paramount to understanding and limiting the spread of HCV. This is an imperative for future treatment strategy for high-risk individuals. By integrating information of risk behaviours, geographic location, and viral sequences, we proposed a novel method that revealed the relationship between recent transmission and the social network of injecting inmates in prisons of New South Wales.

Methods: The Hepatitis C Incidence and Transmission Study cohort (HITS) is a prospective cohort of high-risk HCV-uninfected prisoners that are interviewed on IDU risk behaviours, and tested for HCV infection with Ab and RNA assays. Phylogenetical analysis (using Maximum Likelihood approach implemented in the software PhyML) was performed using viral sequences (N = 146) of the E1-HVR1 region of the HCV genome sequenced from 1–3 viraemic samples of 115 infected inmates within the HITS cohort. Clustering analysis was performed using the PhyloPart software, where putative clusters are tested via measures of genetic distances between any pair of sequences. The geographical location of each inmate (from 2005 to 2011) is available, including prison, ward and cell. Location and risk behaviours are used to construct the social network as validation of potential hot-spots identified via phylogenetic analysis.

Results: We have undertaken a comprehensive molecular epidemiological analysis of HCV sequences in incident cases in conjunction with analysis of geographical and socio-behavioural factors, to understand the spatial epidemiology of HCV in IDU prisoners from NSW. Clustering analysis revealed that in both Gt1a and Gt3a clusters of infected inmates shared closely related sequences of the E1-HVR1 segment of the HCV genome, indicating the existence of transmission networks. This was further corroborated by evidence that inmates within the identified clusters also shared same prison location at the estimated time of infection.

Conclusions: We proposed a novel method that integrates viral sequences and social network analysis to understand the network of HCV transmission among IDUs from their location, viral infection, and social network.
F.S. Macaluso1,

IN GENOTYPE 1 CHRONIC HEPATITIS C PATIENTS GENETIC DETERMINANTS, WITH SEVERITY OF LIVER FIBROSIS ASSOCIATION OF VITAMIN D SERUM LEVELS AND ITS COMMON ALLELE WITH pegIFN-

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with pegIFN-α

P

of SVR (P

pre-treatment hepatocellular MxA protein levels achieved an SVR (P

rs12979860 favourable allele as the strongest individual predictor of SVR using multivariate logistic regression analysis. Whilst the mechanisms by which genetic variation within IL28B gene influences ISG expression and ultimately treatment response remains unknown, these data suggest a role for both IL28B genotype and ISG expression in the successful eradication of HCV with pegIFN-α/RBV.

473 ASSOCIATION OF VITAMIN D SERUM LEVELS AND ITS COMMON ALLELE WITH SEVERITY OF LIVER FIBROSIS IN GENOTYPE 1 CHRONIC HEPATITIS C PATIENTS

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Background and Aims: Lower 25-Hydroxyvitamin D (25(OH)D) serum levels have been associated with the severity of liver fibrosis in genotype 1 chronic hepatitis C patients (G1CHC). In addition a recent genome-wide study identified genetic variants (rs12785878, near dehydrocholesterol reductase, DHCR7; rs10741657, near vitamin D binding protein, GC) affecting 25(OH)D serum levels in healthy populations. We aimed to assess a association between vitamin D serum levels and its genetic determinants, with the severity of liver fibrosis.

Methods: Two hundred sixty patients with biopsy-proven G1CHC were consecutively evaluated. The 25(OH)D serum levels were measured by high-pressure liquid chromatography. All patients were genotyped for DHCR7 rs12785878, CYP2R1 rs10741657, and GC rs7041 single nucleotide polymorphisms.

Results: DHCR7 GG genotype (p = 0.003), and the severity of fibrosis (p = 0.03) were independent factors associated with lower 25(OH)D serum levels in multiple linear regression analysis. Interestingly 53.8% (7/13) of patients with DHCR7 GG genotype had severe liver fibrosis, compared to 27.1% (67/247) of those with DHCR7 TT/TG genotype (p = 0.03). By multivariate logistic regression analysis, severe fibrosis was independently associated with older age (OR, 1.056; 95% CI, 1.023–1.089; p = 0.001), low cholesterol (OR, 0.984; 95% CI, 0.974–0.994, p = 0.002), high triglycerides (OR, 1.008; 95% CI, 1.002–1.015, p = 0.01), low 25(OH)D (OR, 0.958; 95% CI, 0.919–0.999, p = 0.04), DHCR7 GG genotype (OR, 4.222; 95% CI, 1.106–16.120; p = 0.03), moderate-severe steatosis (OR, 2.588; 95% CI, 1.355–4.943; p = 0.004), and moderate-severe necroinflammatory activity (grading) (OR, 2.437; 95% CI, 1.307–4.763; p = 0.001). No association was found between liver fibrosis and both CYP2R1 and GC genotypes.

Conclusion: In patients with G1CHC patients GG homozygosity for DHCR7 gene and lower 25(OH)D levels are independently associated with the severity of liver fibrosis.

474 ADVANCED FIBROSIS AND THE RISK OF LIVER DECOMPENSATION AMONG HIV/HCV-COINFECTED INDIVIDUALS: CONSEQUENCES FOR THE TIMING OF THERAPY AGAINST HCV

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Background: Directly acting antivirals against HCV (DAAs) in the pipeline are more potent and safer than telaprevir or boceprevir. Therefore, immediate therapy for patients at high risk of liver decompensation (DC) is the strategy in many countries. Panels of experts have proposed immediate therapy with DAA for individuals with F3, but most patients treated have been cirrhotic due to financial restrictions. However, the risk of DC among HIV/HCV-coinfected patients with F3 in the short-term could be high and not allowing delays. We aimed at assessing the risk of DC among HIV-infected individuals with chronic HCV infection and F3–F4.

Methods: 102 HIV/HCV-coinfected patients with chronic HCV infection, naïve or without SVR to HCV therapy, 58 (57%) of whom showed F3 and 44 (43%) F4, were included in this cohort study. The date of liver biopsy was baseline (BL). Fibrosis was staged by the Scheuer’s score. Survival analysis was carried out.

Results: Median (IQR) age was 39 (37–44) years, 88 (86%) patients were men. Median (IQR) follow-up was 5.6 (4–7.2) years. Median (IQR) CD4 cell count was 497 (331–666) cells/μL, and 72 (71%) individuals showed undetectable plasma HIV RNA at BL. 5 (8.6%) patients with F3 and 13 (30%) with F4 developed DC. The incidence of DC [95% confidence interval (95%CI)] was 0.12 (0.05–0.28) per 100 person-years for F3 vs. 0.45 (0.26–0.78) for F4. The probability of remaining free of DC for F3 vs. F4 was: at 1 year, 98% (88%–100%) vs. 86% (71%–93%); at 3 years, 94% (82%–98%) vs. 75% (59%–86%); at 5 years 84% (62%–94%) vs. 59% (38%–75%) (p = 0.007). The only factor independently associated with DC was BL platelet count <100x10³ vs. ≥100x10³ (hazard ratio [HR] 3.9; 95% CI, 1.4–11.2; p = 0.011). BL fibrosis F4 vs. F3 showed a HR 2.7 (95% CI, 0.93–7.95; p = 0.067) for DC.

Conclusions: As in patients with cirrhosis, immediate therapy against HCV is warranted for patients with F3 with HIV coinfection, as they are at risk of DC at 3 years, a period shorter than that required for newer DAA to become available. Platelet count may help to identify patients with priority among those who bear F3.

475 THE INFLUENCE OF PORTAL PRESSURE ON THE DISCORDANCE OF ABSOLUTE CD4+ COUNT AND CD4+ PERCENTAGE IN HIV/HCV-COINFECTED PATIENTS

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Introduction: Both absolute CD4+ count and CD4+ cell percentage expressed as a proportion of lymphocytes are used as predictors of disease progression in HIV infected, as well as HIV/HCV coinfected patients. The aim of our study was to investigate the association between portal pressure and discordance between absolute CD4+ count and CD4+ percentage.
Methods: Portal pressure was assessed through measurement of the hepatic venous pressure gradient (HVPG) in 97 HIV/HCV coinfected patients with compensated liver disease. In accordance with the definitions used by Hull and co-workers, CD4+ counts were considered concordant when the absolute CD4+ count matched the corresponding CD4+ percentage determined in HIV infected individuals (<100/<7%, 100–199/7–13%, 200–299/14–20%, 300–399/21–27%, 400–499/28–34%, >500/>35%). Higher CD4+ percentages than expected from the absolute CD4+ counts were referred to as high discordance, while lower CD4+ percentages than expected from the absolute CD4+ counts were referred to as low discordance.

Results: Patient characteristics: 76% male, mean age: 37.3±9.7 years, combined antiretroviral therapy: 72%, mean absolute CD4+ count: 519±261 cells/µl, mean CD4+ cell percentage: 28.6±10.4%, mean HVPG: 4.8±3.8 mmHg; cirrhosis: 19%. High and low CD4+ discordance was observed in 18% and 38% of patients, while 44% of patients had concordant CD4+ counts. There was a tendency toward a higher prevalence of high CD4+ discordance in patients with high portal pressure (±mmHg: 15% vs. 6–10 mmHg: 22%; vs. ≥11 mmHg: 29%, p=0.651). In contrast, low CD4+ discordance was observed more frequently in patients with low portal pressure (±mmHg: 42% vs. 6–10 mmHg: 39% vs. ≥11 mmHg: 0%, p=0.019) (Figure 1). Portal pressure was significantly correlated with the absolute CD4+ count/CD4+ percentage ratio (r = −0.201, p = 0.049) (Figure 2).

Conclusions: The observed trends did not attain statistical significance, which might be explained by the low number of cirrhotic patients who all had compensated liver disease. To the best of our knowledge, this is the first data to demonstrate that portal pressure influences the relationship between absolute CD4+ count and CD4+ percentage, which might be attributed to hypersplenism in patients with portal hypertension. In conclusion, clinicians should consider portal hypertension as a potential confounder when interpreting results on CD4+ counts.

476 LIVER STIFFNESS MEASUREMENT ASSESSED BY FIBROSCAN PREDICTS RESPONSE TO ANTI-VIRAL TREATMENT IN PATIENTS WITH GENOTYPE 2 AND 3 CHRONIC HEPATITIS C

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Background and Aims: Chronic hepatitis C virus infection (HCV) is a common cause of cirrhosis and end-stage liver disease. Pegylated interferon (PEG-IFN) and ribavirin (RBV) is currently the treatment of choice for genotype 2 and 3 (G2/3) HCV resulting in a sustained virological response (SVR) in 70–80%. Advanced fibrosis is known to be associated with failure of antiviral therapy. Increasingly, liver stiffness measurement (LSM) is being used to non-invasively assess fibrosis. However, it is not known whether LSM predicts response to antiviral therapy and whether there are predictive cut-offs. Our aim was to assess whether baseline LSM can predict SVR in HCV G2/3 patients treated with PEG-IFN+RBV.

Methods: Retrospective review of outcomes in naive patients with HCV G2/3 treated with PEG-IFN+RBV in our clinic between from Jan 2007 to Oct 2011. Post transplant and co-infected patients were excluded. Patients with a valid LSM within 1 year of starting treatment who completed ≥12wks and recorded outcome of treatment were included in the LSM analysis.

Results: 155 patients (mean age 40±11.56% male, 16% cirrhotic, 93% G2 and 42% high viral load) received PEG-IFN+RBV for HCV in the study period. 93% completed ≥12 wks treatment. 96% (62%) of patients had a valid LSM (median 6.7 kPa; 3.5 kPa to 39.1 kPa). 24% had a LSM >10.6 kPa consistent with advanced fibrosis. The overall SVR rate was 68%. 11% were lost to follow up and the outcome unknown. LSM was significantly associated with SVR (p=0.001). The AUROC for LSM in predicting treatment response was 0.752 (95%CI 0.60–0.90). The optimum cut-off to predict non-SVR was 10.6 kPa (71% sensitivity, 86% specificity). 90% with LSM ≤10.6 kPa achieved SVR versus 45% with LSM >10.6 kPa (p<0.001). All patients with low viral load (<600,000 IU/mL) and LSM <10.6 kPa who had >12 wks treatment achieved SVR (n=34).

Conclusions: Fibrosis assessed non-invasively with LSM can predict help predict response to antiviral therapy in patients with HCV G2/3. LSM (> or <10.6 kPa) could be factored into treatment algorithms to determine the optimum treatment course lengths.

477 PERSISTENCE OF HEPATITIS C VIRUS DURING AND AFTER CLINICALLY APPARENT SUCCESSFUL TREATMENT OF CHRONIC HEPATITIS C

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Introduction and Aim: Resolution of chronic hepatitis C (CHC) following therapy with PEG-IFN/ribavirin (RBV) is considered when serum HCV RNA becomes repeatedly undetectable and liver enzymes normalize. However, long-term persistence of low levels of HCV RNA in plasma, lymphoid cells and liver has been reported when highly sensitive assays and testing of serial plasma and PBMC.
samples were applied. The aim was to re-analyze sequential plasma and PBMC samples from patients who resolved CHC and became HCVRNA negative by clinical laboratory testing.

**Methods:** Plasma samples (n = 60) from 11 randomly selected patients who resolved CHC after a standard course of PEG/IFN/RBV therapy were collected before (n = 12), during (range 48–68 wks; n = 28) and up to 331 (range 12–88) wks post-treatment (n = 20). PBMCs (n = 26) from 4 patients before (n = 5), during (n = 13) and post-treatment (n = 8) were also analyzed. Total RNA was extracted from 250 or 750 μl plasma and intact or PHA-stimulated PBMCs. HCVRNA was detected by RT-PCR/nucleic acid hybridization (RT-PCR/NAH; sensitivity <5 copies/μg RNA or <2 IU/ml). Clone sequence analysis of the HCV 5′-UTR from sequential plasma and PBMCs was done in 2 patients.

**Results:** HCVRNA was detected in 9 of 20 (45.3%) plasma and 4 of 8 (50%) PBMC samples for up to 23.6 wks (range 12–59 wks) after completion of treatment. Among plasma samples identified during therapy as negative for HCVRNA by clinical assay, 64.3% were reactive by RT-PCR/NAH. Testing of RNA from 750μl plasma increased HCVRNA detection from 31.7% to 63.3% (38/60) compared to 250-μl samples. Testing naive versus PHA-stimulated PBMCs enhanced HCVRNA detection from 26.9% to 69.2% (18/26). Virus replicative strand was detected in 12/18 PBMC samples. Mutations identified in the 5′-UTR sequence persisted in plasma and/or PBMCs during and after PEG-IFN/RBV therapy. The frequency of HCVRNA detection tended to decline in both plasma and PBMCs with longer follow-up.

**Conclusions:** HCVRNA is detectable in plasma and PBMCs for up to 23.6 wks after treatment completion. Among plasma samples identified during therapy as negative for HCVRNA by clinical assay, 64.3% were reactive by RT-PCR/NAH. Testing of RNA from 750μl plasma increased HCVRNA detection from 31.7% to 63.3% (38/60) compared to 250-μl samples. Testing naive versus PHA-stimulated PBMCs enhanced HCVRNA detection from 26.9% to 69.2% (18/26). Virus replicative strand was detected in 12/18 PBMC samples. Mutations identified in the 5′-UTR sequence persisted in plasma and/or PBMCs during and after PEG-IFN/RBV therapy. The frequency of HCVRNA detection tended to decline in both plasma and PBMCs with longer follow-up.

**Background and Aims:** In therapy for chronic Hepatitis C Virus (HCV) infection, off label use of human recombinant erythropoietin is common to improve hemoglobin, maintain ribavarin dosing and attain sustained virologic response (SVR). The effect of erythropoietin use on mortality in HCV infected individuals receiving therapy for HCV is not known.

**Methods:** Retrospective analysis of 18,125 HCV infected Veterans who underwent treatment for chronic HCV infection from the Electronically Retrieved Cohort of Hepatitis C Infected Veterans (2001–2008). The association of erythropoietin use with mortality was estimated using Cox Proportional Hazard models with adjustment for potential confounders. Main outcome was all-cause mortality. Individuals with Human immunodeficiency virus infection, chronic kidney disease and decompensated liver disease were excluded from the analysis.

**Results:** 4,113 (22.69%) Veterans initiated on therapy for chronic HCV infection were treated with erythropoietin. Veterans treated with erythropoietin were likely to have a longer duration of therapy for HCV (OR 2.27, 95% CI 2.13–2.42) compared to those who were not treated with erythropoietin. The all-cause mortality rate in Veterans treated with erythropoietin [14.89 per 1000 person-years (95% CI 13.31–16.66 per1000 person years)] was not statistically different from Veterans not treated with erythropoietin [14.10 per 1000 person years (95% CI 13.23–15.03 per1000 person years)]. The adjusted hazards ratio for association of treatment with erythropoietin was 0.94 (95% CI 0.82–1.07). In multivariable cox regression analysis, risk of death was significantly associated with age (HR 1.05; 95% CI 1.04–1.06), diabetes (HR 1.42; 95% CI 1.21, 1.66), chronic obstructive pulmonary disorder (HR 1.60; 95% CI 1.33, 1.94), cardiovascular disease (HR 1.47, 95% CI 1.12, 1.93) and cancer history (HR 1.96; 95% CI 1.60, 2.41). Data on SVR was available on 212 Veterans who completed less than 24 weeks of therapy and 467 Veterans who completed 24–48 weeks of therapy. No significant difference in rate of SVR was noted with use of erythropoietin.

**Conclusion:** Erythropoietin use for HCV therapy related anemia is associated with longer duration of therapy but does not improve mortality or affect sustained virologic response.
Interferon Lamda-3 (IFN-λ-3), encoded by the human IL28B gene, has both antiviral and pro-inflammatory properties, though reports of its association with liver fibrosis are inconsistent. Homozygous recessive SNPs (rs12979860CC, rs8099917TT) in this gene are linked to spontaneous HCV clearance and better treatment response, potentially via non-synonymous functional variant rs8103142, which leads to a lysine-arginine substitution at position 70 (K70R).

**Aim:** Examining the relationship between specific IL28B genotypes and significant liver fibrosis as measured by the AST-to-platelet ratio index (APRI) ≥1.5 in HIV/HCV co-infected Canadians.

**Methods:** A case–control study was nested in the prospective Canadian Co-infection Cohort (n=1119). HCVRNA-positive participants free of fibrosis, end-stage liver disease and chronic Hepatitis B at baseline (n=679) were included. We matched on self-reported ethnicity and used incidence density sampling to select 2 controls per case. Cases (n=130) developed an APRI ≥1.5, while controls had APRI <1.5 when cases occurred. Conditional logistic regression was used, adjusting for sex, HCV genotype, alcohol use, age and baseline APRI.

**Results:** Overall 74% were male with median HCV duration=18 years. 130 participants developed fibrosis over 1290 person-years of risk (10/100 person-years, 95% CI=8.5–12/100 p-y). Univariate analyses suggested that each SNP may be linked to a higher risk of fibrosis. In multivariate analyses, rs8103142 had the strongest effect, although failed to reach statistical significance.

<table>
<thead>
<tr>
<th>Table: IL28B and liver fibrosis in co-infected Canadians</th>
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<tbody>
<tr>
<td>rs12979860 CC</td>
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<tr>
<td>Univariate</td>
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<tr>
<td>1.31 (0.84, 2.06)</td>
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<tr>
<td>Multivariate</td>
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<tr>
<td>1.18 (0.85, 1.66)</td>
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<tr>
<td>Female</td>
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<tr>
<td>1.14 (0.86, 1.57)</td>
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<tr>
<td>HCV genotype (1/4 vs. 2/3)</td>
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<tr>
<td>Alcohol use</td>
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<tr>
<td>1.46 (0.82, 2.68)</td>
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<tr>
<td>Baseline APRI</td>
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<tr>
<td>7.43 (2.75, 20.07)</td>
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<tr>
<td>Age</td>
</tr>
<tr>
<td>0.99 (0.93, 1.02)</td>
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**Conclusions:** Our results suggest that among the IL28B SNPs analyzed, rs8103142, which leads to structural changes in the IFN-λ-3 protein, is linked to a higher rate of liver fibrosis among HIV/HCV co-infected Canadians. Larger studies are needed to confirm this finding.

**481 THE EFFICIENCY OF ACOUSTIC RADIATION FORCE IMPULSE-IMAGING FOR THE STAGING OF LIVER FIBROSIS: A META-ANALYSIS**

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**Background and Aims:** Acoustic Radiation Force Impulse Imaging (ARFI) is an ultrasound-based elastography method that is integrated in a conventional ultrasound machine enabling the exact localization of measurement site. A systematic review and meta-analysis based on original and abstract publications were performed to evaluate the overall performance of ARFI imaging for the diagnosis of liver fibrosis.

**Methods:** Literature databases and conference abstracts were searched from 2007 up to February 2012. A random effects meta-analysis of the area under the ROC curve (AUROC) was performed as well as summary receiver operating curve techniques. Quality analyses were conducted to assess sources of heterogeneity.

**Results:** The systematic literature search revealed 37 studies with overall 3983 patients. The mean diagnostic accuracy of ARFI expressed as the AUROC was 0.84 (95%-CI: 0.80–0.87) for the diagnosis of significant fibrosis, 0.89 (95%-CI: 0.87–0.92) for the diagnosis of severe fibrosis and 0.92 (95%-CI: 0.89–0.94) for the diagnosis of liver cirrhosis. Subgroup analyses showed sources of heterogeneity between studies examining only HCV infected patients, studies with patients with mixed chronic liver diseases and studies without HCV infected patients (p <0.05 for the diagnosis of severe fibrosis and liver cirrhosis).

In addition, significant differences in AUROC for the diagnosis of severe fibrosis were observed between studies examining only HBV infected patients and studies with patients with mixed chronic liver diseases (p = 0.017). Body mass index had a significant influence on the diagnosis of significant fibrosis (p = 0.0062).

**Conclusions:** The present meta-analysis revealed a good diagnostic accuracy of ARFI-imaging for the staging of significant and severe fibrosis and an excellent diagnostic accuracy for the diagnosis of liver cirrhosis.
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IS LIVER FIBROSIS PROGRESSION FASTER IN HUMAN IMMUNODEFICIENCY VIRUS/HEPATITIS C VIRUS COINFECTED PATIENTS ON ANTIRETROVIRAL THAN IN HCV ONLY INFECTED PATIENTS?

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Background: Before antiretroviral therapy (HAART) era studies have demonstrated the rate of liver fibrosis progression in HCV-HIV coinfected patients is faster than in patients infected only by HCV, but few studies had compared fibrosis progression rate in HCV-HIV coinfected patients on HAART.

Aims: Compare the fibrosis progression rate in HCV-HIV coinfected patients on antiretroviral therapy with immune recovery with Hepatitis C monoinfected patients.

Patients and Methods: Only patients with known time of HCV infection were enrolled. HBsAg positive patients and hepatitis C treatment before liver biopsy were excluded. All coinfected patients were on HAART at least two years. All patients were submitted to liver biopsy and METAVIR was used. To compare liver fibrosis progression rate the coinfected patients were matched with HCV monoinfected patients by gender, alcohol consumption, age at HCV infection (± 2 years) and estimated time of HCV infection (± 2 years).

Results: 38 coinfected patients were enrolled and matched with 38 HCV only infected patients. 30 patients were male in each group. The estimated time of HCV infection and age on HCV infection were 17.1±5.1 years vs. 17.6±5.2 years (p=0.66) and 22.2±7.7 years vs. 21.6±8.3 years (p=0.75) in the monoinfected and coinfected patients, respectively. The CD4 count cell mean was 448±206 cells/mm³ and the HIV viral load median was 760 copies/mm³. The mean of the liver fibrosis stages (1.5±1.2 versus. 1.4±1.2; P=0.47), perportal inflammatory activity (1.9±1.4 versus. 1.8±1.2; P=0.81), liver fibrosis progression rate (0.081±fibrosis unit/year vs. 0.071±fibrosis unit/year; P=0.59) and proportion of liver cirrhosis was similar in coinfected and HCV monoinfected patients, respectively.

Conclusions: Coinfected patients on antiretroviral therapy and immunologic recovery has liver progression rate similar to HCV monoinfected patients.

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IL28B POLYMORPHISM IS ASSOCIATED WITH NECROINFLAMMATORY ACTIVITY AND ADVANCED FIBROSIS IN PATIENTS INFECTED WITH CHRONIC HEPATITIS DUE TO GENOTYPE NON-1 HCV

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Background and Aims: The natural course of hepatitis C virus (HCV) infection is partially determined by individual genetic background. IL28B polymorphisms on chromosome 19 have been recognized as the one of powerful predictors of spontaneous seroclearance and virologic response to the interferon based standard therapy of chronic hepatitis C. However, it is not clear whether these polymorphisms of IL28B are associated with disease progression of chronic hepatitis C. We investigated the impacts of IL28B genotype on histological findings in patients with chronic hepatitis C.

Methods: A total of 195 patients with histologically proven chronic hepatitis C were enrolled. One hundred-eleven patients and 84 patients were infected with genotype 1 and genotype non-1 HCV, respectively. IL28B related SNP, rs12979860 was analyzed by using the RMP and direct sequencing method. The correlation between IL28B related SNP and histologic features of chronic hepatitis (stage of fibrosis, grade of necroinflammatory activity, and steatosis) were analyzed.

Results: In multivariate analysis, rs12979860 favorable CC allele (OR 10.770, P=0.033) along with age (OR 1.079, P=0.002) were associated with advanced fibrosis in patients infected with genotype non-1 HCV. In addition, the rs12979860 CC allele tended to have relevance to more active portoportal inflammation and steatosis (P=0.081, P=0.098). On the contrary, no significant relationships were found between the histologic findings and IL28B related SNP in patients infected with genotype 1 HCV. However, age (P=0.033) and steatosis (P=0.077) were associated with advanced fibrosis in those with genotype 1 HCV.

Conclusion: In patients with infected with genotype 1 HCV, IL28B related SNP (rs12979860), a known predictor of treatment response and spontaneous viral clearance, was not associated with the activity and progression of chronic hepatitis. By contrast, rs12979860 appears to be involved in the progression of hepatic fibrosis in patients with genotype non-1 HCV infection, which may be secondary to necroinflammatory activity and steatosis. These findings suggest that the IL28B may modulate the immune reaction and the disease course according to genotype 1 or genotype non-1.

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IMPACT OF LIVER FIBROSIS IN DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN GENOTYPE 1 CHRONIC HEPATITIS C PATIENTS TREATED WITH ANTIVIRAL THERAPY: LONG TERM FOLLOW UP STUDY

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Background and Aims: The impact of sustained virological response (SVR) on hepatocellular carcinoma (HCC) in genotype 1 cirrhotic and chronic hepatitis C (CHC) patients treated with peginterferon (PEG-IFN) and ribavirin is not well established. The purpose of this study is to investigate the impact of antiviral treatment on the development of HCC in genotype 1 HCV infected patients treated with PEG-IFN plus Ribavirin.

Methods: Retrospective-prospective study including 172 HCV infected patients (115 CHC and 58 cirrhosis) treated with antiviral therapy.

Results: A total of 24 patients (14%) showed HCC during a median follow up of 60 months (range 6–240 months). Hepatocellular carcinoma was disclosed in 5 (3.3%) patients with SVR and in 19 (24%) patients without SVR. Five cirrhotic patients (26%) with SVR had HCC (5 over 19 patients), while no CHC patients (0%) with SVR developed HCC (0 over 74). Seventeen cirrhotic patients (44%) without SVR had HCC while only 1 CHC patient (2.5%) without SVR developed HCC. In multivariate analysis age≥60 years, presence of liver cirrhosis and no SVR were the most independent risk factors for HCC (Table). Multivariate analysis focused on cirrhotic patients showed that age≥60 years (OR 3.8, 95%CI 1–14.7 p<0.05) and male gender (0.27 95%CI 0.065–11 p<0.07) were independent risk factors for HCC.

Conclusions: Cirrhosis, age≥60 years and no SVR are predictors of HCC in genotype 1 chronic hepatitis C patients treated with antiviral therapy. In cirrhotic patients, an age≥60 and male gender are predictive risk factors for HCC. Despite SVR, cirrhotic patients still
present a risk of HCC although this is reduced respect to cirrhotic patients without SVR. A six months interval of ultrasonography surveillance for HCC represents a reasonable choice in cirrhotic patients, high incidence of HCC in cirrhotic patients could suggest for a short period of surveillance.

Table: Multivariate regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&gt;60)</td>
<td>4.1</td>
<td>(1.1–15.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>Gender</td>
<td>0.34</td>
<td>(0.08–1.3)</td>
<td>0.1</td>
</tr>
<tr>
<td>No SVR</td>
<td>3.58</td>
<td>(0.9–14.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>48.8</td>
<td>(6.1–120)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

485
HEPATITIS OUTREACH NETWORK: A PRACTICAL STRATEGY FOR HEPATITIS SCREENING WITH LINKAGE TO CARE IN FOREIGN BORN COMMUNITIES
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Background and Aims: Many foreign-born persons in the U.S. are at high risk of chronic hepatitis B (HBV) and C (HCV) infections, yet are not aware of their infection, and lack healthcare coverage or linkage to care.

Methods: A unique partnership, the Hepatitis Outreach Network, combines the expertise and resources of the Mount Sinai School of Medicine, the NYC Department of Health and Mental Hygiene, and community-based organizations, to provide education, screening and link to care in communities with high prevalence of chronic viral hepatitis. Comprehensive HBV and HCV screening identifies infected patients, who then receive further evaluation from either local or Mount Sinai physicians, combined with patient-navigators who organize follow-up visits.

Results: Of 1603 persons screened, 76 had HBV and 75 had HCV. Importantly, screening for HCV based on traditional risk factors would have missed 67% of those who tested positive. Of the 76 persons with HCV infection, 49 (64%) received a medical evaluation (26 with local providers and 23 at Mount Sinai). Of the 49 HCV-infected persons evaluated, treatment was recommended in 11 and begun in 8 (73%). Of the 76 persons with HBV infection, 43 (57%) received a medical evaluation (31 with local providers and 12 at Mount Sinai). Of the 43 HBV-infected persons evaluated, treatment was recommended and begun in 5 (100%).

Conclusion: Hepatitis Outreach Network has successfully established novel proof of concept for identifying HBV and HCV infections in foreign-born persons through use of several unique elements that effectively link them to care.

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INDUSTRIAL, BUT NOT FRUIT FRUCTOSE INTAKE IS INDEPENDENTLY ASSOCIATED WITH SEVERE LIVER FIBROSIS IN GENOTYPE 1 CHRONIC HEPATITIS C PATIENTS
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Background and Aims: Foods, and especially fructose have been showed to be linked to metabolic alterations, and to severity of liver fibrosis in patients with non-alcoholic fatty liver disease. In a cohort of patients with G1CHC, we tested the association of foods and in particular of fructose intake with histological features of the liver disease.

Methods: 147 consecutive biopsy-proven G1CHC patients were studied. Anthropometric and metabolic factors, including waist circumference (WC), WC hip ratio (WHR), dorso-cervical fat, and HOMA were assessed. Foods intake, including industrial and fruit fructose, was investigated by a three day detailed questioning and a computed database. All biopsies were scored by one pathologist for staging and grading (Scheuer), and graded for steatosis, which was considered moderate-severe if ≥20%.

Results: Mean total intake of industrial and fruit fructose was 18.0±8.7 g, 6.0±4.7 g, and 11.9±7.2 g, respectively. Intake of industrial, but not fruit fructose, was independently associated with younger age (p=0.02) and higher WHR (p=0.01). Patients with severe liver fibrosis (≥F3) had a significant higher intake of total and industrial (17.2±8.1 vs 20.8±10.2, p=0.04; and 5.5±4.2 vs 7.8±6.0, p=0.01 respectively), but not of fruit fructose (11.6±7.0 vs 12.9±8.0; p=0.34). Multivariate logistic regression analysis showed that older age (OR 1.053, 95%CI 1.009–1.098, p=0.01), severe necroinflammatory activity (OR 3.460, 95%CI 1.417–8.447, p=0.006), moderate-severe steatosis (OR 2.616, 95%CI 1.020–6.708, p=0.04), and intake of industrial fructose (OR 1.146, 95%CI 1.046–1.255, p=0.003) were independently linked to severe fibrosis. No association was found between fructose intake and both liver necroinflammatory activity and steatosis.

Conclusions: The intake of industrial, but not fruit fructose is a risk factor for metabolic alterations and severity of liver fibrosis in patients with G1CHC.
POSTERS

0.18 (0.05–0.31) P<0.001. Use of atazanavir or nevirapine did not change the results presumed by FT.

The spontaneous PTF4 from birth to baseline was dramatically higher in the 107 HIV-NT (0.41 (0.17–0.64)) vs 231 NoHIV-NT (0.01 (0.00–0.03); P<0.001). CD4 NADIR count [Cox-Risk-Ratio =0.995 (0.992–0.998)] P=0.001 was independently associated with PTF4 suggesting a role of HIV through immunosuppression.

Conclusion: In HIV-HCV coinfected patients, repeated biomarkers permitted to quantify the impact of treatment on the very high spontaneous fibrosis progression rate. SVR was associated with a slow regression of fibrosis and a worrisome remaining risk of liver cancer.

488 SERUM IP-10 LEVELS ARE ASSOCIATED WITH HIGHER DPPIV SERUM ACTIVITY AND INCREASED CIRCULATING CD4-CXCR3 POSITIVE CELLS IN PATIENTS WITH CHRONIC HEPATITIS C M. Rau1-2, J. Schmitt1-2, T. Kudlich1, K. Spanu1, H.-P. Tony1, H. Klinker1,2, B. Mühlhäupt1,2, A. Geier1-2, 1Division of Hepatology, Department of Internal Medicine II, University Hospital Würzburg, Würzburg, Germany; 2Department of Gastroenterology and Hepatology, 1Institute of Clinical Chemistry, University Hospital Zürich, Zürich, Switzerland; 4Division of Rheumatology/Clinical Immunology, Department of Internal Medicine II, 3Division of Infectious Diseases, Department of Internal Medicine II, University Hospital Würzburg, Würzburg, Germany

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Background: Interferon-gamma-inducible protein-10 (IP-10) serum levels are good predictors for antiviral therapy response. High serum concentrations correlate with nonresponse to peginterferon/ribavirin therapy and with viral kinetics in patients with chronic Hepatitis C infection. Elevated IP-10 serum levels were described as antagonist forms of IP-10 cleaved by DPPIV (Casrouge et al., JCI 2011). We recently showed in a cohort of 301 patients with chronic HCV infection an association between higher IP-10 serum levels, higher DPPIV serum activity and cholestasis.

Aims: To analyse abundance of CXCR3 positive peripheral blood cells together with DPPIV activity and IP-10 serum levels in patients with chronic Hepatitis C infection in the context of cholestasis (high bile acid serum levels).

Methods: 29 patients with chronic HCV genotype 1 infection were included in this study. In serum DPPIV activity was analyzed by enzymatic assay, IP-10 measured by ELISA and bile acids (BA) quantified using direct spectrophotometry. PBMCs were isolated and CD3-CXCR3, CD4-CXCR3 and CD8-CXCR3 cells characterized by FACS analysis.

Results: IP-10 serum levels were positively correlated to DPPIV activity. Patients with higher IP-10 serum levels had significantly higher BA serum levels (p=0.029) and showed higher frequency of CD4-CXCR3 positive peripheral blood cells. Interestingly, patients with cirrhosis had significantly higher IP-10 serum levels as well as serum BA concentrations in comparison to non-cirrhotic patients. Furthermore a trend towards higher frequency of CD4-CXCR3 positive peripheral blood cells was seen in cirrhotic vs. non-cirrhotic patients.

Conclusions: Higher frequency of circulating CD4-CXCR3 cells could be caused by higher antagonist IP-10 serum levels, which are associated with higher DPPIV activity as well as higher serum BA. Whether this reflects lower hepatic recruitment of CD4-CXCR3 cells remains to be investigated.

489 AN ALLELIC VARIANT IN THE IRF3 GENE IS ASSOCIATED WITH LIVER CIRRHOSIS IN HEPATITIS C VIRUS CARRIERS L.M. Real1, A. Caruz2, K. Neukam1, A. Rivero1, A. Rivero-Juarez3, D. Merino1, M. Márquez2, M.J. Gomez1, E. Pérez1, J. Macías1, J.A. Pineda1, 1Unidad de Enfermedades Infecciosas y Microbiología, Hospital Universitario Virgen de Valme, Sevilla, 2Unidad de Inmunogenética, Universidad de Jaén, Jaén, 3Unidad de Enfermedades Infecciosas, Hospital Universitario Reina Sofía, Córdoba, 4Unidad de Enfermedades Infecciosas, Hospital Juan Ramón Jiménez, Huelva

E-mail: lmreal67b@gmail.com

Introduction: Progression to cirrhosis in hepatitis C virus (HCV) carriers is a complex trait that is modulated by a combination of both clinical and host genetic factors. Host genetics factors could play an important role in the risk of developing this phenotype.

Objectives: To discover new genetic variants associated with the risk of developing cirrhosis using SNPs previously related to cholesterol metabolism and transport, fibrogenesis, immune response or viral treatment response.

Patients and Methods: Three hundred thirty-seven HCV carriers with available data on liver fibrosis status (FibroScan or liver biopsy) were selected from a genotype database containing 442 patients. Of them, 77 (22.85%) showed cirrhosis. Plink tools were used to perform quality control of genotype data and to carry out univariate association analyses. The variables associated with cirrhosis in the univariate analyses were entered in a multivariate logistic regression model.

Results: Only the SNP rs12104272, linked to IRF3 gene, was associated with cirrhosis in our sample after multiple testing corrections. A total of 330 (97.92%) patients out of 337 individuals were successfully genotyped for rs12104272. Of them, 76 (23%) were cirrhotic. In the overall population, the genotype frequencies were: 26 (7.87%) AA, 157 (47.57%) AG and 147 (44.54%) GG. A higher proportion of rs12104272 A allele carriers was observed in the non-cirrhotic group [60.63% (154 individuals)] than in the cirrhotic group [38.15% (29 individuals)]. Among patients with genotype AA/AG, the proportion of those who showed cirrhosis was 15.84% (29 patients) versus 31.97% (47 patients) with genotype GG (p=0.0005). In the multivariate analysis adjusted for age, sex, body mass index, HIV co-infection and HCV RNA load, rs12104272 genotype AA/AG was independently associated with cirrhosis (adjusted odds ratio=0.366, 95% confidence interval=0.180–0.746, p=0.006). The protective effect of rs12104272 AA/AG genotype was stronger in patients bearing the rs12979860 CC genotype of IL28B (adjusted odds ratio=0.069, 95% confidence interval = 0.014–0.349, p=0.001).

Conclusion: Due to its effect size, the rs12104272 SNP could have a clinical value, either alone or combined with the rs12979860 marker of IL28B, to select those individuals at low risk of cirrhosis development.

490 INFLUENCE OF PREDICTOR VARIABLES ON QUALITY OF LIFE OF PATIENTS WITH CHRONIC VIRAL HEPATITIS C N. Sarayants, Infectious Diseases, Armenicum Clinical Center, Yerevan, Armenia

E-mail: sknarina70@mail.ru

Background: The aim of the study is prediction of quality of life (QL) in patients with chronic hepatitis C.

Methods: QL was checked in 90 patients with chronic HCV-infection by SF-36 questionnaire and was compared with control group. The statistic analysis was done by SPSS 11.0 with multiple regression. The SF-36 8 domains considered as dependent variables: physical functioning – PF, role limitations due to physical health –
RP, bodily pain – BP, general health – GH, vitality – V, social functioning – SF, role limitations due to emotional problems – RE, mental health – MH. We evaluated influence of 10 independent variables (age, gender, body mass index (BMI), disease limitation, retreatment (standard or Peg-IFN in the past), presence of antibodies to HBV (HbcAb), alcohol abuse, drug abuse, level of ferritin (FERR), viral load (VL)) on QL. Predictor variables were coding as dichotomous or categorical. Thus, by age patients were subdivided on the following groups: <30, 30–40, 41–50, and >50 years old. According to BMI patients were divided: <25, 25–30 (overweight) and >30 (obesity). By presumably limitation of disease patients were split on categories <5 years, 5–10 years, >10 years. Gender, previous IFN-therapy, HBV-past infection, alcohol and drugs abuse, FERR and VL (high >400000 IU/ml) is considered as dichotomous.

### Table 1. Quality of Life score in HCV-infected patients (n=90)

<table>
<thead>
<tr>
<th>QL domains</th>
<th>PF</th>
<th>RP</th>
<th>BP</th>
<th>GH</th>
<th>V</th>
<th>RE</th>
<th>SF</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>83.2</td>
<td>63.3</td>
<td>69.4</td>
<td>58.0</td>
<td>56.3</td>
<td>69.1</td>
<td>54.1</td>
<td>55.3</td>
</tr>
<tr>
<td>SE</td>
<td>2.3</td>
<td>4.0</td>
<td>2.8</td>
<td>1.9</td>
<td>2.6</td>
<td>4.5</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Median</td>
<td>90</td>
<td>75</td>
<td>72</td>
<td>57</td>
<td>55</td>
<td>75</td>
<td>66.6</td>
<td>56</td>
</tr>
<tr>
<td>Mode</td>
<td>95</td>
<td>100</td>
<td>45</td>
<td>100</td>
<td>40</td>
<td>100</td>
<td>89.8</td>
<td>56</td>
</tr>
<tr>
<td>SD</td>
<td>21.7</td>
<td>38.25</td>
<td>26.5</td>
<td>18.0</td>
<td>19.9</td>
<td>24.8</td>
<td>42.9</td>
<td>18.85</td>
</tr>
<tr>
<td>Range</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>80</td>
<td>90</td>
<td>90</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Min</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>10</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>Max</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CI</td>
<td>78.7</td>
<td>55.4–87.6</td>
<td>63.8–74.9</td>
<td>54.3–61.8</td>
<td>52.2–64.5</td>
<td>45.2–51.4</td>
<td>60.4–74.2</td>
<td>63.8–59.2</td>
</tr>
</tbody>
</table>

### Table 2. Quality of Life score in patients with chronic hepatitis C and healthy control (m±SE)

<table>
<thead>
<tr>
<th>QL domains</th>
<th>Chronic hepatitis C</th>
<th>Healthy control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>83.2±2.3</td>
<td>97.0±0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RP</td>
<td>63.3±4.0</td>
<td>96.3±2.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BP</td>
<td>69.4±2.8</td>
<td>85.9±4.3</td>
<td>0.009</td>
</tr>
<tr>
<td>GH</td>
<td>58.0±1.9</td>
<td>79.5±3.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V</td>
<td>56.3±2.1</td>
<td>73.0±2.9</td>
<td>0.01</td>
</tr>
<tr>
<td>SF</td>
<td>69.1±2.6</td>
<td>82.5±5.4</td>
<td>0.03</td>
</tr>
<tr>
<td>RE</td>
<td>54.1±4.5</td>
<td>73.3±8.6</td>
<td>0.07</td>
</tr>
<tr>
<td>MH</td>
<td>55.3±2.0</td>
<td>73.2±4.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Results:

Table 2 show difference in QL scores on SF-36 scale HCV-infected and control group. Using all the predictors simultaneously 32% (adjusted R²=0.316) of the variance in MH and 33% (adjusted R²=0.325) in SF can be predicted from gender and pretreatment combined. The ANOVA table shows that the combination of the predictors significantly predict MH (p=0.017) and SF (p=0.015). The coefficients table shows significance of gender and pretreatment to the prediction of MH. The same independent variances plus BMI were significant in SF.

### Mental Health Model Summary

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. error of the estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.704</td>
<td>0.496</td>
<td>0.316</td>
<td>16.675</td>
</tr>
</tbody>
</table>

### Conclusions:

HCV-infected persons QL was significantly low in comparison with healthy control except role-emotional category. Gender and previous IFN-therapy have influence on mental health and social functioning.

### 491 MINIMAL NEUROPSYCHOMOTOR DISORDERS IN PATIENTS WITH CHRONIC HEPATITIS C

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### Introduction:

Hepatitis C virus causes long lasting liver injury in 50–80% of infected subjects. According to the published data persistent inflammation may negatively affect cognitive and motor functions.

### Aim:

To compare chronic hepatitis C (CHC) patients and healthy control neuropsychomotor functions with the aim to detect possible disorders.

### Patients and Methods:

68 patients with chronic hepatitis C (41/60% men and 27/40% women; mean age 41.5±13.6, education 13.7±2.7 years) and 15 subjects of control group (9/60% men, 6/40% women; mean age 40.0±12.8, education 14.8±2.2 years) were enrolled into the study. For diagnostic purpose standard biochemical tests and analysis of liver biopsy was done for all patients. All participants performed a standardized psychometric
test battery (PHES battery) including number connection tests A and B (NCT-A, NCT-B), the line-tracing (LTT), the serial-dotting (SDT) and the digit-symbol (DST) tests. Statistical data analysis was performed with SPSS 17.0 software. Differences in PHES tests results between control and CHC patients were evaluated with chi-square test. PHES tests results correlation with participant education, age and gender was assessed by calculation of Pearson correlation coefficient.

**Results:** In CHC patients PHES tests results were worse than in control: NCT-A - 36.6±4.3 sec. vs 24.4±6.1 sec., p<0.001; NCT-B - 89.6±11 vs 57.3±9.0 sec., p=0.002; LTT - 79.5±18.1 sec. vs 63.5±18.2 sec., p=0.026; SDT - 65.1±14.3 sec. vs 43.6±6.0 sec., p=0.009; DST - 59.3±11.8 sec. vs 46.1±11.2, p=0.002. Most tests time-span directly correlated with patient’s age: DST - r = -0.54, p=0.029; NCT-A - r = -0.41, p=0.006; NCT-B - r = -0.49, p=0.005; LTT - r = -0.54, p<0.001. Correlation between METAIVIR liver fibrosis score and NCT-B and LTT time-span was also found (r =-0.37, p=0.011 and r =-0.37, p=0.006, respectively). We did not find statistically significant correlation between PHES tests time-span and education, tests results did not depend on participant’s gender also.

**Conclusions:** According to PHES battery tests results CHC patients have impaired cognitive and motor functions in comparison with healthy control of the same education level as well as age and gender distribution. This impairment is more severe in older patients and in those with high liver fibrosis score.

**493 HOST GENETIC VARIANTS AROUND IL28A/IL28B ASSOCIATED WITH HCV-RELATED OUTCOMES BASED ON R.E.V.E.A.L.-HCV COHORT**

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E-mail: tzunwen@gmail.com

**Background and Aims:** The genome-wide association (GWAS) analysis promoted the identification of SNPs (single nucleotide polymorphism), e.g. rs8099971 and rs12979860, and haplotypes from IL28B region which was associated with drug-driven and spontaneous HCV clearance. Lied in the upstream of IL28B, IL28A, a paralogue of IL28B (>95% sequence identity), was included in this study to decipher novel SNP combinations, not a single SNP, and further investigated the possibility of cooperatively clearing HCV.

**Methods:** A prospective cohort comprised of participants who spontaneously cleared the HCV (n = 294) or had persistent infection (n = 595) was genotyped in this IL28 candidate gene study associated to the outcome of HCV infection. Then all genotypes were transformed to only three coding types: 2 for homozygote of major alleles, 1 for heterozygote, and 0 for homozygote of minor alleles. Based on Chi-squared test and odds ratio (OR), a pattern block of SNPs for HCV clearance or persistence were selected.

**Results:** Around IL28B region from 39729479 to 39745058, there were 12 SNPs (MAF=3.9–6.7%) significantly for HCV clearance, including four previously reported HCV-clearance associated SNPs: rs12980275, rs11881222, rs12979860, and rs8099971 showing the OR (95% CI) of 1.94 (1.16–3.25), 2.14 (1.25–3.67), 2.21 (1.29–3.78), and 2.07 (1.17–3.68) but no one for HCV persistent infection. Interestingly, 4, i.e. rs4803217, rs11881222, rs4803222, and rs8113007, of these 12 SNPs had different cooperation modes: in majority of patients, the four coordinate to clear HCV (2:01:117–3.45) by a pattern of heterozygotes; in minority of patients, they were beneficial for HCV persistence (0.46; 0.26–0.80) by a homozygotic pattern of major alleles. Furthermore, a subset of SNPs, i.e rs15499928, rs10853727, rs16697285, rs4803223, rs11304621, rs553114, and rs62120535, between IL28A and IL28B had insignificant associations for HCV clearance or persistence but were cooperatively significant for HCV clearance (2:51; 1:19–5:28) on 5.5% of patients.

**Conclusions:** We suspected that SNPs within a pattern block had equivalent contribution to HCV clearance. Furthermore, the tied SNPs from IL28A to IL28B implicated the possible cooperation of the two genes via genetic variants to clear HCV.

**494 METABOLIC SYNDROME AND INSULIN RESISTANCE ARE ASSOCIATED WITH MAXIMUM HEPATITIS C LIPOVIRAL PARTICLES IN GENOTYPE 1 INFECTION**

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E-mail: david.sheridan@ncl.ac.uk

**Background and Aims:** HCV has a direct role in the pathogenesis of insulin resistance. Patients with chronic HCV and insulin resistance are less likely to respond to antiviral therapy and are at risk for more rapid fibrosis progression. Insulin resistance is most strongly associated with HCV genotype (G)1. Infectious HCV particles have a low density due to association with triglyceride rich lipoproteins (TRLs) as lipoviral particles (LVP). We have reported that LVP levels are dynamic and can increase to a maximum level postprandially by transfer onto TRLs including chylomicrons, VLDL and lipid emulsions both in vivo and ex vivo [1]. We aimed to evaluate the relationships between the maximum amount of LVP in vivo and clinical and metabolic parameters.

**Methods:** 53 chronic HCV G1 patients provided fasting blood samples. The maximum LVP ratio (LVP-max) was measured by incubating fasting plasma with a lipid emulsion prior to separation using a size filter. HCV RNA was quantitated from the filtrate and retentate and LVP-max was calculated (retentate/retentate + filtrate).

**Results:** The median LVP-max was 0.28 but varied widely from 0.07 to 0.86. LVP-max was significantly correlated with HOMA-IR (r =0.362, p =0.008). LVP-max was significantly increased in patients with co-existing metabolic syndrome (median LVP-max 0.49, n =11) compared to those without metabolic syndrome (median 0.23), p=0.001. LVP-max did not correlate with plasma apoE concentration. There was no association of LVP-max with IL28B or PNPLA3 genotypes.

**Conclusions:** This study provides some mechanistic insights into the interaction of HCV with metabolic syndrome and insulin resistance, suggesting an association with increased amounts of post-prandial infectious HCV particles.

**Reference(s)**
MAJOR DEPRESSIVE DISORDER (MDD) IN PATIENTS WITH CHRONIC HEPATITIS C (CHC): PREVALENCE AND RISK FACTORS

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Background and Aims: Chronic hepatitis C (CHC) is a significant health problem on global scale. Like other chronic diseases, CHC is associated with an increased prevalence of psychiatric disorder, especially depression. However, the causes for the high frequency of depression in patients chronically infected with HCV are not completely understood. In this study, we examined a group of patients with hepatitis C awaiting interferon treatment to estimate the prevalence of psychiatric disorder and to identify factors associated with increased risk of major depressive disorder (MDD). We examined risk factors of mood disorder: sociodemographics, clinical comorbidities and viral characteristics. Additionally, we evaluated selected cytokines patterns [Interleukin-6 (IL-6) and IL-10].

Methods: 131 consecutive CHC patients were enrolled (61 males; mean age, 50.7±10.0 years). All patients completed several surveys including Mini-International Neuropsychiatry Interview (MINI-Plus 5.0), Hamilton Depression Rating Scale (HDRS) and Hospital Anxiety and Depression Scale (HADS). Clinical, laboratory and sociodemographic variables were evaluated in CHC patients with and without depression. Moreover, cytometric bead array assay was performed to detect serum levels of IL-6 and IL-10.

Results: The median time from diagnosis of HCV infection was 23.0±10.1 years. At the time of evaluation, 52 patients (39.7%) had clinical comorbidity [high blood pressure (HBP) or diabetes mellitus (DM)]. The most common psychiatric disorder was MDD (n=34, 26.0%), followed by anxiety disorders (n=10, 7.6%). In multivariate analysis, previous history of MDD (PR=1.89; IC95% 1.03–3.02) and diabetes (PR=1.93 IC95% 1.07–3.47; p=0.04) were associated with MDD. We found a significant positive correlation between IL-6 serum levels and severe cases of MDD (r=0.317; p=0.04) and HADS (r=0.456; p=0.003).

Conclusion: MDD have high prevalence in hepatitis C. However, the pathogenesis of HCV-related neuro-psychiatric symptoms remains poorly understood. The virus is able to cross the blood-brain barrier, therefore, one possible explanation is that the virus itself can directly cause depression. On the other hand, the role of host’s cytokines in psychiatric disorders in HCV-infected patients has been the focus of several investigations in recent years. PROEX, PRPb, CAPEb, CNPb, FAPEMIG.

RISK FACTORS FOR FIBROSIS PROGRESSION IN ADDICTED PATIENTS WITH CHRONIC HEPATITIS C ON METHADONE MAINTENANCE THERAPY

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Background: Intravenous drug abuse is the main road for HCV transmission. Methadone maintenance therapy (MMT) is effective pharmacologic treatment for chronic heroin addiction, which assists the majority of those taking it with achieving medical, psychological and psychosocial stability. However not all HCV positive patients on MMT are suitable for antiviral therapy.

Aim: The aim of this study was to assess risk factors for progression of liver fibrosis in patients with chronic hepatitis C (CHC) on MMT.

Patients and Methods: From 2004 to 2012 the study included 92 CHC patients on MMT, (72 males/20 females), mean age 24 (18–32 years) and 75 not addicted CHC patients (56 males/19 females), mean age 37 (20–41 years) (non-MMT group). All patients were HBV/HIV-negative and antiviral treatment naïve. Two consecutive liver biopsies were performed and the rate of fibrosis progression calculated as the difference between F scores at two biopsies divided by the time in years between them. Effect of twelve factors on fibrosis progression was assessed: age; gender; HCV genotype; HCV viremia; anti HBc Ab; occult HBV infection; ALT levels; histological steatosis; obesity; methadone dose; incidental drug use; alcohol consumption.

Results: The interval between biopsies in the groups ranged between 3 and 6 years. Fibrosis progression was observed in 28% of the patients in non-MMT group and in 54.3% in MMT group (p<0.0001). In both groups fibrosis progression was associated with male gender (p=0.030), positive anti HBc Ab (p=0.001), occult HBV infection (p<0.0001), elevated ALT (p=0.012), histological steatosis (p=0.022) and alcohol use (p<0.0001). In MMT group fibrosis progression was not associated with age (p=0.123), obesity (p=0.230), methadone dose (p=0.450) and associated with incidental drug use (p=0.002). In non-MMT group age (p=0.001) and obesity (p=0.032) were significant factors for fibrosis progression. HCV genotype (p=0.545) and HCV viremia (p=0.168) had no influence on fibrosis in both groups.

Conclusion: MMT, regardless of methadone dose, is associated with more rapid fibrosis progression in patients with CHC. Additional risk factors for fibrosis progression in MMT group are male gender, past HBV infection, biochemical activity, steatosis, alcohol consumption and incidental drug use.

CHANGES IN CIRCULATING HCV-RNA CONCENTRATIONS IN PATIENTS WITH CHRONIC HEPATITIS C IN THE ABSENCE OF ANTIVIRAL THERAPY

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Background: The concentration of circulating HCV-RNA at baseline and during antiviral therapy predicts response in chronic hepatitis C and allows implementation of early stopping rules. In HIV infection, plasma HIV-RNA levels are relatively stable over time. No similar information has been produced so far for HCV testing longitudinally from a large group of individuals followed during long periods off treatment.

Methods: Retrospective review of longitudinal plasma HCV-RNA determinations in untreated chronic hepatitis C patients. As comparison, plasma HIV-RNA measurements were examined in untreated HIV individuals.

Results: A total of 3169 HCV-RNA determinations over 66.2±27.4 months were available for 818 chronic hepatitis C patients (3.9±1.4 per subject). For 333 HIV individuals, 1998 HIV-RNA measurements were examined over 273±17.5 months (6 per subject).

Overall 44% consecutive specimens had >0.5 log variations in HCV-RNA IU/mL values compared to 23% for HIV-RNA (p<0.001). These figures were 15% and 4%, respectively, for variations >1 log (p<0.001). Mean variation in HCV-RNA and HIV-RNA concentrations in consecutive specimens was 0.47±0.29 log IU/mL and 0.33±0.15 log copies/mL, respectively (p<0.001).
A total prevalence of 14% HIV/HCV co-infection was found in the CC. Infection can occur and seems to be associated with IL28B genotype. Conclusions: Associated with spontaneous clearance of HCV. Demographical factors only a chronic hepatitis B infection was detected in 79%, indicating a chronic HCV infection. Among 5315 HIV patients in September 2010. Spontaneous clearance of HCV was defined as positive anti-HCV test and a negative HCV-RNA test in patients who had not been treated for HCV. Anti-HCV positive patients from the Stockholm area (n = 263) were tested for IL28B rs 12979860 SNP with a Taqman-based allele-specific PCR method. Human DNA was extracted from plasma out of EDTA blood. Results: A strong correlation with spontaneous HCV clearance for IL28B genotype CC was noted. Hence, 36% of patients with the CC versus 13% with the non-CC SNP cleared HCV, p = 0.0004, OR 5.02 (2.11–12.92). Furthermore, 3 patients with chronic HCV spontaneously cleared their HCV infection without therapy after receiving effective antiretroviral therapy. All three had IL28B genotype CC. The prevalence of anti-HCV in the total Swedish HIV positive cohort (n = 5315) was 14%. Among anti-HCV positive individuals HCV-RNA was detected in 79%, indicating a chronic HCV infection. Among demographical factors only a chronic hepatitis B infection was associated with spontaneous clearance of HCV. Conclusions: We found that the IL28B genotype CC in HIV/HCV co-infected patients was strongly correlated with spontaneous clearance of HCV. After immune reconstitution with ART in HIV/HCV co-infected patients spontaneous clearance of chronic HCV infection can occur and seems to be associated with IL28B genotype CC. A total prevalence of 14% HIV/HCV co-infection was found in the Swedish HIV infected population.

In multivariate analysis (odds ratio, 95% confidence interval, p), the likelihood of experiencing HCV-RNA variations >0.5 log was greater in patients with lower HCV-RNA (0.35 per log [0.26–0.47] 0.001). HIV coinfection (2.57 [95.64–4.23] and IL28B-CC (1.87 [1.28–2.74] 0.001).

Conclusion: Natural variation in circulating HCV-RNA concentrations may be clinically meaningful in a substantial proportion of chronic hepatitis C patients. It may influence the best time to prescribe antiviral therapy. Moreover, decisions based on early viral kinetics, such as early stopping rules, may require testing of baseline specimens the closest to treatment initiation.

497 SPONTANEOUS CLEARANCE OR CHRONIC EVOLUTION OF HCV IN HIV/HCV CO-INFECTED PATIENTS – INFLUENCE OF THE IL28B GENOTYPE
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Background and Aims: The IL28B genotype is correlated to spontaneous clearance of HCV, and sustained virologic response to peg-INF plus ribavirin treatment. Demographical factors have also been associated with spontaneous clearance of HCV in HIV/HCV co-infected persons. All Swedish known HIV/HCV co-infected patients have been included in a database (InfCare HIV). We analyzed the IL28B genotype and demographical factors in a subgroup of the total Swedish HIV/HCV co-infected cohort and the correlation to spontaneous HCV clearance.

Methods: Demographical and virological data for HIV/HCV co-infected patients were extracted from the total database including 5315 HIV patients in September 2010. Spontaneous clearance of HCV was defined as positive anti-HCV test and a negative HCV-RNA test in patients who had not been treated for HCV.

Anti-HCV positive patients from the Stockholm area (n = 263) were tested for IL28B rs 12979860 SNP with a Taqman-based allele-specific PCR method. Human DNA was extracted from plasma out of EDTA blood.

Results: A strong correlation with spontaneous HCV clearance for IL28B genotype CC was noted. Hence, 36% of patients with the CC versus 13% with the non-CC SNP cleared HCV, p = 0.0004, OR 5.02 (2.11–12.92). Furthermore, 3 patients with chronic HCV spontaneously cleared their HCV infection without therapy after receiving effective antiretroviral therapy. All three had IL28B genotype CC.

Conclusions: We found that the IL28B genotype CC in HIV/HCV co-infected patients was strongly correlated with spontaneous clearance of HCV. After immune reconstitution with ART in HIV/HCV co-infected patients spontaneous clearance of chronic HCV infection can occur and seems to be associated with IL28B genotype CC. A total prevalence of 14% HIV/HCV co-infection was found in the Swedish HIV infected population.

498 DRY BLOOD SPOT TESTING FOR HEPATITIS C IN PEOPLE WHO INJECTED DRUGS: REACHING THE POPULATIONS OTHER TESTS CANNOT REACH
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Background: Within the UK the main source of hepatitis C virus (HCV) infection is injecting drug use however diagnosing HCV in people who inject drugs (PWID) has often proved challenging.

Methods: This is a prospective cohort study of all individuals living in our region who had received a HCV dry blood spot test (DBST) between 2009 and 2011 Testing for HCV was offered to individuals who accessed needle exchange or drug treatment services.

Results: During the study 1123 DBSTs were carried out. 946 individuals had one test. 177 had a follow up test after 1 year. 295 (31.2%) individuals were HCV antibody positive on their first test. Overall 94.3% (902/956) individuals returned for the results of their test. 177 individuals were retested and there were 29 new cases.

249 individuals attended for further follow up and their PCR status was checked. 164 (65.5%) were PCR positive. All PCR positive individuals were offered further assessment for treatment and 138 patients attended for review. Assessment included factors affecting treatment outcomes including viral load, genotype and degree of fibrosis. 56.8% were genotype 3. 73.3% had a low viral load (under 800,000iu/ml). Only 9.6% had a Fibroscan score above 9 kPa which suggests moderate to severe fibrosis and/or cirrhosis. 35 have commenced treatment, 16 are in the assessment period for treatment. 54 are in continued follow up and working towards treatment, 30 individuals have declined further follow up and 3 individuals died from a drugs related death. Overall we have retained in services or treated 76% (105/138) of patients who accessed care. At the end of the study sustained viral response (SVR) was available for 7 patients and all patients who completed treatment had an SVR.

Conclusion: The study has shown DBST is a complementary technique for the diagnosis of HCV. We have shown that over 94% of PWID returned for their results. Many individuals have low viral loads and low fibrosis scores so that while this group of patients may be difficult to reach and may be challenging to maintain in therapy they are easier to cure.

499 VERY-EARLY VIRAL RESPONSE (WEEK-1) TO TRIPLE THERAPY WITH TELAPREVIR, PEG-INTERFERON AND RIBAVIRIN PREDICTS EXTENDED-RVR AND TREATMENT OUTCOMES IN PATIENTS WITH HCV GENOTYPE 1
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Background and Aims: The triple therapy with telaprevir, peg-interferon, and ribavirin increases the rate of sustained virologic response (SVR) in treatment-resistant patients infected with HCV genotype 1. However, this therapy has a high risk of adverse effects,
and early discontinuation of the treatment is preferable in patients with a low likelihood of SVR. We investigated whether very early response of HCV to triple therapy is predictive for extended-rapid virologic response (eRVR) which is strongly associated with SVR.

**Methods:** A total of 124 patients (median 59.2 years) with HCV genotype 1b and pretreatment HCVRNA >5.0 logIU/mL enrolled in this Japanese multi-center study. HCVRNA levels were measured before and 1, 4, and 12 weeks after the start of the therapy by real-time PCR. The likelihood of achieving eRVR were evaluated based on the HCVRNA at week-1 after starting therapy.

**Results:** eRVR was achieved in 57 of 64 patients (89.1%) in whom HCVRNA was measured at 4 and 12 weeks. When predictive values were compared between pretreatment HCVRNA levels, HCVRNA levels at week-1 after starting therapy, and reduction in HCVRNA levels between pretreatment and week-1, HCVRNA levels at week-1 after starting therapy has the strongest predictive value for eRVR (AUROC: HCVRNA levels at week-1, 0.930; pretreatment HCVRNA levels, 0.695; reduction in HCVRNA levels, 0.862). When HCVRNA levels at week-1 of 2.4logIU/mL was fixed as a cut-off, the sensitivity, specificity, PPV, NPV, and accuracy were 91%, 86%, 98%, 55%, and 91%, respectively. HCVRNA levels at week-1 of 2.4logIU/mL was a significant predictive factor of eRVR in univariate analysis along with the genetic polymorphisms near the IL28B gene and the gamma-GTP; in addition it was the only factor for eRVR in multivariate analysis (p=0.001); whereas the genetic polymorphisms near the IL28B gene was not a predictive factor by multivariate analysis (p=0.058).

**Conclusions:** HCVRNA levels (not simply reduction) at week-1 after starting triple therapy was a strong predictor of eRVR. Patients whose HCVRNA levels at week-1 after starting therapy was less than 2.4logIU/mL have a high likelihood of eRVR.

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**500 DIRECT AND INDIRECT COST BURDEN IS HIGHER IN HCV INFECTION COMPARED WITH A MATCHED NON-HCV INFECTED COHORT IN A PRIVATELY-INSURED POPULATION**

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**Background and Aims:** Hepatitis C virus (HCV) infection is associated with progressive liver disease, cirrhosis, and hepatocellular carcinoma and is the leading indication for liver transplantation. This study evaluates the direct healthcare and indirect work loss cost burden associated with chronic HCV.

**Methods:** Health insurance claims from 60 self-insured US companies and disability data for employees in 29 of these companies from 1/2001–9/2011 were analyzed. Adult patients with ≥2 diagnosis claims of chronic HCV (ICD-9-CM: 070.44, 070.54) were selected and matched 1:1 with non-HCV controls based on exact matching factors and propensity scores. Patients diagnosed with HIV were excluded from the study. Matched cohorts were compared for healthcare resource utilization using rate ratios (RRs), as well as direct healthcare costs and indirect work loss costs using per-patient per-year (PPPY) cost differences. Direct healthcare costs included costs attributable to pharmacy, hospitalizations, emergency room (ER) visits, and outpatient visits, whereas indirect costs included costs attributable to disability and medically-related absenteeism. HCV-related costs, defined as the subset of claims with a diagnosis of chronic HCV or HCV therapy, were also reported. Confidence intervals and p-values for cost differences were calculated using a nonparametric bootstrap.

**Results:** HCV and non-HCV patients (N=9,841 in each group) were well matched with respect to age (mean=52 years), gender (female=38.6%), Quan-charlson co-morbidity index (mean=0.54), and other demographics and clinical characteristics. Relative to the non-HCV cohort, the HCV cohort had significantly more hospitalizations (RR [95% CI]: 2.45 [2.37–2.54], P<0.001), ER visits (RR [95% CI]: 1.88 [1.83–1.92], P<0.001), and outpatient visits (RR [95% CI]: 1.67 [1.66–1.68], P<0.001), which translated into a ~$10,500 PPPY incremental direct healthcare cost associated with HCV ($16,721 vs. $6,063; cost difference [95% CI]: $10,503 [9,683–11,361], P<0.001). Among employees with disability coverage, HCV patients had significantly greater PPPY indirect costs compared to non-HCV patients ($3,310 vs. $1,723; cost difference [95% CI]: $1,523 [1,248–1,794], P<0.001). HCV-related costs (direct: $4,450; indirect: $415) accounted for 46% of direct and 27% of indirect cost differences.

**Conclusions:** This real-world matched-cohort study showed that HCV infection is associated with significantly greater levels of both direct and indirect costs.

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**501 PATIENT CHARACTERISTICS AND UTILIZATION OF PROTEASE INHIBITORS IN HEPATITIS C (HCV) PATIENTS IN A LARGE PAYER DATABASE**

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**Background and Aims:** Chronic Hepatitis C virus (HCV) infection represents a significant economic burden. This study investigated characteristics of HCV patients, and their co-morbid conditions, treatments and associated costs using a large healthcare payer database.

**Methods:** This retrospective study used data from Truven Health Analytics MarketScan databases from 01/01/2006–03/31/2012. Patients with ≥2 diagnosis claims of chronic HCV were selected. They were >18 years at index diagnosis date and had at least six months of continuous health plan and drug plan enrollment pre/post index date. HCV patients were grouped by disease severity as non-cirrhotic disease (NCD), compensated cirrhosis (CC), or end-stage liver disease (ESLD) based on ICD-9 diagnosis and current procedural terminology (CPT) codes. Treatment rates, healthcare utilization, and cost were assessed by disease severity, as were co-morbidities in the six-month pre/post index date.

**Results:** 57,084 HCV patients met inclusion criteria, of whom 43,356 (76.3%) were NCD, 6,830 (12.0%) were CC, and 6,693 (11.7%) were ESDL. Mean age was 50.6yrs (49.8yrs NCD vs. 52.6yrs CC and 54.2yrs ESDL). Over half of patients were male and over 70% had commercial insurance. Common co-morbidities included type II diabetes (13.3%), depression (11.7%), and substance abuse (14.5%). Type II diabetes and substance abuse increased with disease severity and were highest among the ESLD cohort following diagnosis. Depression also increased following diagnosis, but was fairly evenly distributed across disease severity cohorts. Anemia was highly prevalent following diagnosis (12.3%), especially in the ESLD cohort (28.3%). Only 10.9% of the population received treatment for HCV, the majority (83.1%) with dual therapy compared to 13.2% with telaprevir and 3.4% with boceprevir based triple therapy. In the 6 months following diagnosis, utilization of inpatient, hospital outpatient, emergency room, physician office, and pharmacy services increased significantly. Utilization and costs increased with disease severity and treatment rate was highest among CC patients.

**Conclusions:** A small percentage of HCV patients receive treatment. Type II diabetes, depression, and substance abuse are common among patients with HCV. Healthcare utilization and cost increased
after diagnosis and were highest among those with the most severe disease.

**502 ESTIMATING THE TIME POINT OF ACUTE HCV INFECTION; COMPARING SEQUENCE DERIVED ESTIMATES WITH HCV RNA MIDPOINTS**  
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**Background:** Duration of hepatitis C virus (HCV)-infection is usually unknown as during acute infection specific symptoms are often lacking. However, treatment outcome and pathogenesis are related to duration of the infection. In order to investigate whether sequence data can be used to estimate the time point of infection, we modeled HCV evolution with a Bayesian framework using molecular sequences from patients during and following acute infection. We compared the estimates for the time to the Most Recent Common Ancestor (tMRCA) with midpoint estimates from HCV RNA measurements.

**Methods:** Samples were selected from 29 HIV/HCV co-infected patients during and following sexually acquired acute HCV infection for each follow-up year. All 29 patients had an interval between the last negative and first positive HCVRNA test of ≤6 months and the midpoint was used as a clinical estimate for the actual time point of acute infection. The tMRCA was estimated with a Bayesian and Markov chain Monte Carlo statistical framework (BEAST v1.7.4) together with a relaxed uncorrelated lognormal molecular clock. Monophyly was imposed on sequences from each follow-up year derived from the same patients.

**Results:** On average, tMRCA was dated ~8 months before the HCVRNA midpoint (figure 1). The median interval between the lower and upper 95% higher posterior density (HPD) of the tMRCA was 1.6 years (IQR, 0.2–0.4) and the median interval between the last RNA negative and first RNA positive time point was 0.3 years (IQR, 0.2–0.4).

**Conclusion:** Our findings demonstrate a good concordance between the time point of acute HCV infection and tMRCA estimates, using sequences from the E2/HVR1 region. The common finding of tMRCA dates slightly prior to the RNA midpoint dates suggests transmission of multiple related viral variants from the same source.

**503 HCV NEUTRALIZING ANTIBODY RESPONSE DURING SPONTANEOUS AND TREATMENT-INDUCED VIRAL CLEARANCE OF ACUTE HCV INFECTION IN HIV-INFECTED MSM**  
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**Background:** High incidence of hepatitis C virus (HCV) reinfection in pegIFN-treated HIV-infected men who have sex with men (MSM) suggest that treatment-induced viral clearance does not lead to sterilizing immunity against HCV. We investigated the presence; breadth and persistence of neutralizing antibody (nAb) response together with general anti-HCV dynamics in HIV-infected MSM after both treatment-induced and spontaneous viral clearance of acute HCV.

**Methods:** Four HIV-infected MSM with acute HCV-1a infection were selected for this pilot-study. For two patients a 24-week pegIFN-based treatment-period was initiated at ~3 months following first RNA positive measurement in which the virus was cleared and a sustained viral response was achieved. The two untreated patients spontaneously cleared their infection after 2.5–10 months after first RNA positive measurement. Presence and breadth of nAbs in these patients was measured using a broad panel of HCV-pseudo-particles consisting of genotypes HCV-1a, -1b, -2a, -2b, -3a, -4a and 4d. Samples for neutralization were selected at 3, 6 and 12 months following the first RNA positive measurement. Viral load and anti-HCV titres were measured using the Roche Cobas Ampliprep and AxSYM v3.0 (Abbott) respectively.

**Results:** During follow-up all patients developed anti-HCV titres that declined over time. Cross-neutralizing activity was only observed in spontaneous clearers as seen in figure 1. Patient 38 developed high anti-HCV titres combined with broad neutralizing capacity against HCV-2a and HCV-3a that was still detectable at 2.5 months after viral clearance. Interestingly, patient 38 developed anti-HCV titres together with neutralizing activity against HCV-1a that was lost at 6 months, which coincided with clearance of the...
primary infection. Upon reinfection higher anti-HCV titres together with broader cross-neutralizing activity against multiple genotypes (HCV-1a, -2b and -3a) was observed. In contrast, patients with treatment-induced viral clearance did develop high anti-HCV titres, but no cross-neutralizing activity was observed.

**Conclusion:** Results from our pilot-study suggest that patients with spontaneous viral clearance might develop better long-term protection against HCV reinfection. In addition our data suggests that treatment-induced viral clearance in these patients indeed does not induce sterilizing immunity. Data from more patients with a larger patient-derived HCV pseudo-particle panel are pending.

**504**

**IMPROVEMENT OF INTERFERON-BASED THERAPY SUBSTANTIALLY REDUCED THE NUMBER NEEDED TO TREAT TO PREVENT HCC AMONG HCV GENOTYPE 1 INFECTED CIRRHOTICS**

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**Introduction:** Patients with hepatitis C virus (HCV) induced cirrhosis remain at risk for hepatocellular carcinoma (HCC) after sustained virological response (SVR). We aimed to assess the impact of improved antiviral therapy on the number needed to treat (NNT) to prevent HCC in consecutive interferon-treated chronic HCV-infected patients with cirrhosis.

**Methods:**

The occurrence of HCC was assessed in our cohort of patients with chronic HCV, who underwent protocol-based interferon treatment. SVR was defined as sustained undetectable serum HCV-RNA. Patients with cirrhosis remained at risk for HCC after interferon treatment. We calculated the NNT to prevent HCC in 1 patient in 5 years (NNT-5y) or 10 years (NNT-10y) and the 95% confidence interval, taking into account that treatment-induced viral clearance did develop high HCV titres, but no cross-neutralizing activity was observed.

**Results:**

Overall, 248 patients with HCV G1 infection and cirrhosis were followed for a median of 8.3 years (IQR 6.2–11.1). The mean age at baseline was 50.6 years (SD 9.2), 168 (68%) patients were male. In total, 59 (24%) patients attained SVR. During follow-up, 43 non-SVR and 3 SVR patients were diagnosed with HCC. The adjusted HCC-free survival probability in non-SVR patients was 94.9% after 5 years and 77.1% after 10 years. Time-dependent multivariate Cox analysis demonstrated that SVR was associated with reduced HCC occurrence (HR 0.20; 95% CI 0.06–0.69, p = 0.011). The NNT-5y to prevent HCC in 1 patient declined from >500 with SVR rates below 5% to a NNT-5y of 68 (95% CI 58–176) at an SVR rate of 35%, which might be expected from peginterferon and ribavirin therapy (Figure). The NNT is lower if one case with HCC is to be prevented over a longer period; the NNT-10y was 17 (95% CI 14–44) at an SVR rate of 35%. Availability of triple therapy during the enrollment period of our study, with an SVR rate of approximately 50%, would have decreased the NNT-5y to 48 (95% CI 41–123).

**Conclusion:** The NNT to prevent HCC in 1 cirrhotic patient with chronic HCV G1 infection has declined substantially with the increased SVR rate as a result of the improvement from interferon mono to peginterferon and ribavirin combination therapy.

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**DONOR IL28B TT-POLYMORPHISM IS ASSOCIATED WITH INCREASED INCIDENCE OF HEPATIC STEATOSIS IN LIVER TRANSPLANT PATIENTS WITH CHRONIC HEPATITIS C**

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**Background and Aims:** Polymorphisms in the IL28B gene are strongly associated with non-response to antiviral treatment for hepatitis C (HCV) infection as well as with the presence of metabolic complications of HCV infection such as hyperlipidemia and diabetes mellitus.

The aim of this study is to quantify the incidence of hepatic steatosis according to donor and recipient IL28B genotype in 211 liver transplant patients with chronic HCV, who underwent protocol-based liver biopsies during long-term follow-up after transplantation.

**Methods:** Consecutive HCV patients who underwent liver transplantation between January 1995 and July 2010 were studied. Genotyping of the polymorphism rs12979860 was performed on DNA collected from donors and recipients.

**Results:**

111 of 211 patients (53%) had histologic evidence of hepatic steatosis during follow-up. Kaplan-Meier analysis and life table analysis with interval censoring showed that patients with donor IL28B genotype TT had an increased incidence of graft steatosis over time (Log Rank p = 0.010 for CC versus TT) and Wilcoxon (Gehan) statistic p = 0.044 for CC versus TT. In contrast, recipient IL28B was not associated with steatosis.

Multivariate Cox regression analysis correcting for gender, HCV genotype, body mass index and presence of diabetes mellitus as a time-dependent covariate, showed that donor IL28B genotype TT was independently associated with occurrence of hepatic steatosis (hazard ratio 2.6; 95% CI 1.1–6.2; p = 0.033). Other factors associated with steatosis were gender, body mass index and HCV genotype 3.

**Figure 1.**
determination of RVR in 40% of all patients. Analyses of SVR rates are currently pending but will be presented at the meeting and will show whether different cut-offs are needed for different assays for response-guided triple-therapy.

507 SIMULTANEOUS DETECTION OF HEPATITIS C VIRUS ANTIGEN AND ANTIBODIES USING DRIED BLOOD SPOTS SAMPLES

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Background and Aims: Enzyme immunoassays (EIA) designed to simultaneously detect circulating hepatitis C virus (HCV) core antigen and anti-HCV antibodies (HCV Ag/Ab) can improve early detection of HCV infection when molecular diagnosis is not widely available. Blood sample collection is extremely difficult in remote areas, like Amazon Region, or in some risk groups, like drug users. The main objective of this study is to evaluate the applicability of DBS samples for simultaneous detection of HCV Ag/Ab using commercial EIAs.

Methods: Paired serum and DBS samples were obtained from 386 individuals referred to Viral Hepatitis Reference Centers in Brazil, of whom 40 were anti-HCV/HCV RNA reactive. Two commercial EIAs for HCV Ag/Ab were employed: EIA1 – MonolisaTM HCV Ag/Ab ULTRA (Bio-Rad) and EIA2 – Murex HCV Ag/Ab (Abbott) where manufacturer's instructions were followed for sera samples. For DBS samples, the volume of elution buffer, sample, diluent, and conjugate, the sample incubation period, and cut off (CO) values were evaluated. After optimization of these parameters, sensitivity, specificity, kappa value and limit of detection were determined. Stability of DBS samples for HCV Ag/Ab detection was also investigated in different conditions (22–26°C, 2–8°C and −20°C).

Results: In order to apply commercial EIA for HCV Ag/Ab detection among DBS samples, DBS sample volume should be four fold increased in both EIAs and CO values were recalculate using ROC analysis. Sensitivities were 97.5% for both EIAs and specificities were 99.7% for EIa1 and 95.9% for EIa2. The concordance of DBS and sera samples was excellent according to the Kappa index (k: 0.972 and 0.817, EIA 1 and EIA2, respectively). HCV Ag/Ab could be detected among DBS samples until 60 days in all conditions, but less variation among absorbance values were observed at −20°C. HCV limit of detection was 3.1 IU/mL among DBS using both EIAs.

Conclusion: DBS samples can be used for HCV Ag/Ab detection by commercial EIAs where excellent concordance compared to sera results was observed. DBS samples present high stability among different temperatures what can increase the feasibility of these samples for HCV diagnosis in some groups, like individuals living in remote areas.
508 IDENTIFICATION OF HBV AND HCV INFECTIONS IN THE PRIMARY CARE SETTING: PRE-DEFINED RISK SCENARIOS ARE A BETTER SCREENING STRATEGY THAN ELEVATED ALT VALUES

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Background: Elevated ALT values are a trigger to screen for hepatitis B (HBV) and hepatitis C (HCV) virus infection. However, both infections can present with normal ALT values. Treatment guidelines additionally define disease specific risk scenarios. We evaluated the relevance of these risk scenarios in a primary care setting.

Methods: HBsAg and anti-HCV (Architect, Abbott) screening was implemented in a routine check-up together with a questionnaire covering 15 risk scenarios defined by the German HCV-guidelines. HBsAg, anti-HCV, and ALT were analyzed at a central lab. We applied 2-sided Chi square statistics and discriminant analysis.

Results: 11845 patients were screened by 45 primary care physicians within 9 months. HBsAg and anti-HCV prevalence was 0.5% and 0.9%. Two cases were co-infected. Infections were previously known in 10/62 (16%) and 34/104 (33%) individuals, was 0.5% and 0.9%. Two cases were co-infected. Infections were previously known in 10/62 (16%) and 34/104 (33%) individuals.

Conclusion: A detailed analysis of risk scenarios may be a better approach to reduce the risk of hepatocellular carcinoma (HCC) in chronic hepatitis C patients treated with interferon/ribavirin (IFN/RBV)-based therapy. Lower IP10 levels during IFN/RBV-based therapy were associated with RVR and SVR during therapy. Our aim was to evaluate if IP10 levels before and during IFN/RBV-based treatment were related to response.

Patients and Methods: From 2002 to 2005, a cohort of difficult-to-treat chronic hepatitis C patients (n=85, naïve genotype 1 and 4 patients, and previous treatment failures of any HCV genotype) were treated for 6 weeks with high-dose interferon (6–18MU daily), followed by 24 or 48 weeks of peginterferon and ribavirin. IP10 levels were retrospectively measured (Quantikine human CXCL10/IP-10 immunoassay, R&D Systems) at baseline, day 1, week 1, 2, 4, and 6, at end of treatment and at end of follow-up (24 weeks after end of treatment) and SVR and non-SVR were established.

Results: Thirty-six of 85 patients (41%) achieved SVR, 26 (30%) had a null-response and 23 (29%) had a relapse, breakthrough or partial response. At baseline, median IP10 serum level was 385 pg/mL. An almost 10-fold average increase in IP10 levels was observed at day 1 upon this high dose of peginterferon (median 3614 pg/mL), although in a minority of patients no increase was observed at all (n=5). Thereafter, median IP10 levels diminished gradually with median IP10 levels returning to baseline levels between week 4 and 6 of treatment. Mean IP10 values were in general lower at all time points among SVR patients, although this failed to reach statistical significance. In addition, mean change in IP10 level at any time point compared to baseline was not statistical different between SVR and non-SVR patients.

Conclusions: In contrast to what has been reported previously, we did not show a statistical difference in baseline IP10 levels between patients achieving SVR or not. We observed a strong increase in IP10 levels one day after starting high dose interferon therapy. However, no difference in increase was present according to response to therapy.

510 DEVELOPMENT OF HEPATOCellular CARCINOMA IN CHRONIC HEPATITIS C PATIENTS WHO ACHIEVE SUSTAINED VIROLOGICAL RESPONSE TO INTERFERON THERAPY

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Background and Aims: Interferon (IFN) therapy is well known to reduce the risk of hepatocellular carcinoma (HCC) in chronic hepatitis C patients, especially in those who achieve sustained virological response (SVR). However, HCC development has been reported in SVR patients. Here, we describe the risk factors and characteristics of HCC in SVR patients and present a follow-up protocol for improved prognosis.

Methods: We retrospectively studied 524 consecutive SVR patients who had received IFN therapy. Their clinical characteristics were as follows: sex, 295 male and 229 female; age, 56 years (18–81 years); observation period, 4.8 years (1–20.1 years); and fibrosis stage, F0 (50), F1 (182), F2 (173), F3 (74), and F4 (45).

Results:
1. HCC was diagnosed in 30 patients (5.7%). Cumulative rates of HCC development were 3.3%, 10.2%, and 17.5% at 5, 10, and 15 years after the end of IFN therapy, respectively. Multivariate analysis identified advanced fibrosis stage (F0–1 vs. F2–4), advanced age (<50 vs. ≥50 years), habitual drinking (no vs. yes), and elevated alpha-fetoprotein levels (>8 vs. ≤8 ng/ml) as determinants of hepatocarcinogenesis, with hazard ratios of 8.65 (p < 0.001), 4.48 (p < 0.001), 3.35 (p < 0.001), and 2.06 (p < 0.05), respectively.
2. Eight of 30 HCC patients were diagnosed more than 10 years after the end of IFN therapy. 13 of 30 HCC patients were at a moderately fibrosis stage. Twenty-five of 30 patients received curative treatment. The 5-year survival rate was 85%. However,
the prognosis of some SVR HCC patients was poor, because of insufficient follow-up, resulting in a delayed diagnosis.

3. The age at the start of IFN therapy was significantly inversely correlated with the interval between the end of IFN therapy and carcinogenesis.

Conclusions: We recommend that SVR patients be observed at 6-month intervals, to facilitate HCC diagnosis at an early stage, for more than 10 years after the end of IFN therapy, even if these patients are not at an advanced fibrosis stage or cirrhosis.

511 THE IMPACT OF MODERATE AND EXCESSIVE ALCOHOL CONSUMPTION ON THE LONG-TERM OUTCOMES OF PATIENTS WITH CHRONIC HEPATITIS C (CH-C)

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Background and Aims: The impact of different amounts of alcohol consumption on the long-term outcomes of chronic hepatitis C (CH-C) patients remains controversial. Our aim was to assess this impact using population-based data.

Methods: Data were obtained from the Third National Health and Nutrition Examination Survey (NHANES III)—mortality linked files. Alcohol consumption was estimated as grams/day. Multivariate Cox proportional hazards model was utilized to assess the effects of CH-C and alcohol consumption on follow-up time to mortality from all causes, cardiovascular disease, and liver disease.

Results: A total of 8,985 participants were included. CH-C was diagnosed as positive HCV antibody and detectable HCV RNA by PCR. Of the entire cohort, 218 (2.4%) had CH-C. The median follow-up time was 162.95 months for CH-C patients and 178.27 months for controls. CH-C patients had increased risk for both overall mortality [aHR 2.44 (1.59–3.75)] and liver-related mortality of [aHR 74.25 (19.62–280.92)]. Furthermore, CH-C patients with excessive alcohol consumption had even higher risk for overall mortality [aHR 5.12 (1.97–13.28)] and liver-related mortality [aHR 183.74 (15.98–infinity)]. The risk of overall mortality associated with CH-C increased with moderate alcohol consumption of 1–19 gram/day: aHR=2.29 (1.36–3.88), excessive alcohol consumption of 20–29 gram/day: aHR=7.63 (1.48–39.31), and heavy alcohol consumption ≥30gram/day: aHR=3.50 (1.20–10.17).

Conclusions: CH-C is associated with increased risk of overall and liver-related mortality. These risks are substantially increased in CH-C patients consuming even moderate amounts of alcohol.

512 THE POTENTIAL IMPACT OF INTERFERON FREE ORAL REGIMENS ON CLINICAL AND COST OUTCOMES OF PATIENTS WITH CHRONIC HEPATITIS C, GENOTYPE 1: A DECISION ANALYTIC ASSESSMENT

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Background: Standard treatment for CH-C-G1 uses a combination of pegylated interferon-alpha (IFN), ribavirin (RBV) and direct acting antivirals (DAA) with 75–80% sustained virologic response (SVR). Still, side effects and complexity of these regimens remain important barriers.

Aim: To assess the impact of three different regimens for both clinical and cost outcomes.

Methods: A decision analytic Markov model that simulated patients up until death was used to compare three strategies for treating CH-C-G1: Triple regimen (IFN, RBV, DAA) with biopsy (TT), oral interferon-free regimen with biopsy (OT), and oral interferon-free regimen treating all patients without biopsy (OTA). Strategies with biopsy initiated treatment after reaching fibrosis stages F2–F4. Stages F0–F1 underwent biopsy every-5–years until age 70. Triple therapy utilized a response-guided approach for 24 or 48 weeks. Oral-IFN-Free regimen was given for 12 weeks. Reference case was a 50 year CH-C with no previous treatment. Model parameters were taken from the published literature. Treatment outcomes for Oral-IFN-Free regimen were assumed to include 6% dropout, 4% relapse, and 90% SVR based on data presented at scientific meetings. Baseline cost of oral therapy was calibrated to equal the cost of triple therapy. Effectiveness was measured in quality-adjusted life years (QALYS). Cost and QALYS were discounted at 3% per year.

Conclusions: OT was evaluated using the incremental cost-effectiveness ratio (ICER). Results: See Table 1. OTA was associated with lowest risk of developing advanced liver disease. Life expectancy was highest for OTA, followed by OT and TT. In baseline analysis, TT had the highest cost and worst outcomes. OTA was more expensive than OT, but more effective. OTA was the most cost-effective strategy, with an ICER of $14,205/QALY. The ICER remained below $15,000/QALY for any value of utility on oral therapy, and remained under $50,000/QALY even if the cost of oral therapy was increased.

Conclusions: OTA reduced the number of CH-C patients reaching advanced liver disease and increased life expectancy. Additionally, OTA was the most cost effective approach for treating CH-C-G1 patients. Nevertheless, the efficacy and safety of these regimens must be confirmed in controlled trials.

Table 1

<table>
<thead>
<tr>
<th>Associated Outcomes</th>
<th>TT</th>
<th>OT</th>
<th>OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
<td>27.0%</td>
<td>11.0%</td>
<td>6.8%</td>
</tr>
<tr>
<td>Decompensated</td>
<td>12.4%</td>
<td>6.1%</td>
<td>4.9%</td>
</tr>
<tr>
<td>HCC</td>
<td>11.5%</td>
<td>7.5%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Advanced liver disease (HCC and decompensated)</td>
<td>22.8%</td>
<td>13.1%</td>
<td>11.0%</td>
</tr>
<tr>
<td>Transplant</td>
<td>5.1%</td>
<td>3.2%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Life expectancy from baseline (yrs)</td>
<td>28,448</td>
<td>29,796</td>
<td>29,977</td>
</tr>
<tr>
<td>ICER ($/QALY)</td>
<td>$14,205</td>
<td>Dominated</td>
<td>—</td>
</tr>
</tbody>
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11. ALCOHOLIC LIVER DISEASE AND DRUG INDUCED LIVER DISEASE

513 CCL20 AS POTENTIAL MEDIATOR OF INFLAMMATION AND FIBROSIS IN PATIENTS WITH ALCOHOLIC HEPATITIS: EVIDENCES OF A TRANSLATIONAL STUDY

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Background and Aims: Alcoholic hepatitis (AH) is a form of acute-on-chronic liver injury and carries a high mortality rate. AH is commonly associated with hepatic failure, portal hypertension and endotoxemia and is characterized by hepatocellular damage,
fibrosis and inflammation. In previous studies, we identified CCL20 as the most up-regulated cytokine in patients with AH. The aim of this study was to investigate the role of CCL20 in AH.

**Methods:** CCL20 hepatic expression was assessed by quantitative PCR in normal livers, patients with AH, non-alcoholic steatohepatitis, chronic hepatitis C, compensated-alcoholic cirrhosis, and animal models of chronic, acute and acute-on-chronic liver injury. CCL20 and LPS serum levels were determined by ELISA and Limulus Amebocyte Lysate assays, respectively. Flow cytometry was used to select hepatic cell populations. CCL20 effects were analyzed in hepatic stellate cells (HSCs). Cell migration was evaluated by Boyden chamber assay and cell signaling activation was assessed by Western blotting.

**Results:** CCL20 hepatic gene expression and serum levels were up-regulated in patients with AH compared to normal livers and patients with other liver diseases (p<0.001). CCL20 hepatic expression and serum levels positively correlated with the degree of liver fibrosis (p<0.05) and LPS serum levels (p<0.01), respectively. CCL20 was up-regulated in animal models of chronic and acute liver injury induced by CC14 and LPS (p<0.05 for both). Importantly, hepatic levels of CCL20 were much more increased in model of acute-on-chronic liver injury (p<0.05), which mimics AH. By flow cytometry and quantitative PCR macrophages were identified as the main hepatic source of CCL20 in this model, followed by hepatocytes, lymphocytes, HSCs and neutrophils. Finally, CCL20 induced pro-inflammatory (MCP-1 and ICAM-1) and pro-fibrogenic (TGF-β and collagen a1) gene expression and promoted ERK-dependent migration in HSCs.

**Conclusions:** CCL20 is markedly overexpressed in patients with AH and correlates with the degree of fibrosis. Human and animal data suggest that LPS leads to CCL20 up-regulation. CCL20 is up-regulated in models of acute-on-chronic liver injury, mainly in macrophages, and exerts pro-inflammatory and pro-fibrogenic effects on HSCs. These results suggest that CCL20 could be an important mediator of inflammation and fibrosis in AH.

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**DECOMPENSATED ALCOHOLIC LIVER DISEASE (ALD): HIGH LONG-TERM MORTALITY DESPITE INITIAL SURVIVAL**

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**Background and Aims:** Early management and outcome of decompensated ALD has been extensively studied, there are few published data on long-term outcome. We have (McFarlane, Gut 2006; 55:A2 and 55:A36) reported on early and 4-year mortality in 249 patients (163 men, age (mean (range)) 50 (27–77) yr) admitted consecutively to our unit between 1/4/1998 and 31/12/2005 with first presentation of decompensated ALD (Child grade B or C). We aimed to assess long-term mortality and its associations in this cohort.

**Methods:** We reviewed available hospital records, death certificates and contacted surviving patients and general practitioners to assess who had died, the causes of death and the patients’ overall alcohol drinking behaviour subsequent to the index hospital episode (classified as: abstinent, continued drinking but reduced, and did not reduce).

**Results:** 37 patients died during the index hospital episode, all because of liver disease. The other 212 patients (including one transplanted during the index episode) were followed up for 4.3 (0.03–13.0) years. Only one other patient was transplanted. 154 patients have subsequently died. Cause of death is known in 134 (87%) and was due to liver disease in 95 (71%) of these. Only 4 patients died of hepatocellular carcinoma. Overall 5- and 10-year total mortality rates were 52±(SEM)4% and 75±3% respectively; corresponding rates from causes known to be liver related were 41±5% and 51±4%. Patients who were abstinent (n=52) had lower total and known liver-related mortality (61±9% and 20±6% after 10 yr) compared to those who continued but reduced (n=105; 73±5% p=0.122 and 53±6% p=0.013) and to those who did not reduce (n=53; 91±4% p<0.001 and 71±7% p<0.001). In Cox regression analysis, both total and known liver-related mortality were independent of age, gender and severity of liver dysfunction at index presentation (Child, MELD, Glasgow and Maddrey scores) but were strongly associated with subsequent drinking behaviour (both p<0.001) and inversely associated with serum albumin at discharge following index hospital episode (p=0.001 and 0.019).

**Conclusions:** Patients with decompensated ALD who survive their first hospital episode have high long-term mortality, mainly due to liver disease, which is reduced but not prevented by abstinence.

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**MECHANISTIC BIOMARKERS PROVIDE EARLY AND SENSITIVE DETECTION OF PARACETAMOL-INDUCED ACUTE LIVER INJURY AT FIRST PRESENTATION TO HOSPITAL**

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**Background and Aims:** Paracetamol overdose is a common reason for admission to hospital and the most frequent cause of acute liver failure in the western world. Early identification of liver injury would facilitate patient risk stratification. We investigated the potential of novel biomarkers – which demonstrate either enhanced liver expression or have been linked to the mechanism of toxicity – to identify patients with paracetamol-induced acute liver injury at first presentation to hospital when current liver injury markers are still normal.

**Methods:** In plasma samples taken from patients at first presentation to hospital following paracetamol overdose, we measured the following biomarkers: microRNA-122 (miR-122; high liver specificity), High Mobility Group Box-1 (HMG1; marker of necrosis), full length and caspase-cleaved Keratin-18 (K18; markers of necrosis and apoptosis, respectively) and glutamate dehydrogenase (GLDH; marker of mitochondrial dysfunction). Receiver operator characteristic (ROC) curve analysis was used to compare the sensitivity of each marker to report liver injury versus standard liver function test parameters.

**Results:** In all patients (n=129); the biomarkers (miR-122, HMG1, necrosis K18, apoptosis K18 and GLDH) at first presentation all correlated with peak hospital stay ALT/INR (all p<0.0001). In patients with normal ALT/INR at presentation, miR-122, HMG1 and necrosis K18 identified the development of liver injury (n=15) or not (n=84) with a high degree of accuracy (miR-122, HMG1 and necrosis K-18: ROC curve AUC values (sensitivity at 90% specificity); 0.93 (0.83), 0.97 (0.91) and 0.94 (0.90), respectively. All p<0.0001).

**Conclusion:** Elevations in plasma miR-122, HMG1, and necrosis keratin-18 identify subsequent development of acute liver injury in patients on admission to hospital, soon after paracetamol overdose, and in patients with ALTs in the normal range. The clinical development of such a biomarker panel could improve the speed of clinical decision-making, both in the treatment of acute liver injury and in the design and execution of clinical trials for new treatment strategies that aim to refine the management of this common hepatotoxin.
516 EFAVIRENZ INDUCES ENDOPLASMATIC STRESS IN HUMAN HEPATIC CELLS BY A MECHANISM DIFFERENT THAN THAT ELICITED BY THE PHARMACOLOGICAL INDUCER THAPSIGARGIN

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Background and Aims: The endoplasmic reticulum (ER) is a multifunctional organelle involved in synthesis, processing and folding of proteins, lipid biosynthesis, and regulation of calcium and redox homeostasis. Altered ER-function due to accumulation of misfolded/unfolded proteins, oxidative stress or depletion of ER calcium, lead to a condition called “ER-stress”, particularly important in cells with a high burden of protein synthesis as hepatocytes. Efavirenz, a non-nucleoside analog reverse transcriptase inhibitor, is a cornerstone in the combined therapy of HIV1 infection. Despite being considered generally safe, it has been related to development of adverse events such as hepatic toxicity. Here we report the presence of specific ER-stress in human hepatic cells.

Methods: Hep3B cells and primary human hepatocytes were exposed to short-term treatment (24h) with clinically relevant concentrations of Efavirenz. General cell biology methods were employed (RNA interference, RT-PCR, Western blot and fluorescence microscopy).

Results: ER-stress markers indicative of several ER-stress pathways were up-regulated concentration-dependently by Efavirenz, including the expression of CHOP and GRP78, at protein and mRNA level, and the phosphorylated form of eIF-2α. Furthermore, we also detected an increase in the transcription of ATF4 and XBP-1, and presence of the spliced form s-XBP-1. Efavirenz also induced morphological changes in ER with enhanced ER-content and dilatation of its cisternae, and led to an increase in the cytosolic calcium level. All these changes were similar to those produced by the standard pharmacological ER-stressor, Thapsigargin. On the contrary, while Thapsigargin induced an increment in the mitochondrial calcium level, Efavirenz provoked a decrease. A differential effect was also observed with transient silencing of CHOP. The stimulating effect of Thapsigargin on the generation of reactive oxygen species and the increment in the ER-content was more pronounced in CHOP-depleted cells, but this exacerbated action was not observed when cells were treated with Efavirenz.

Conclusions: Clinical concentrations of Efavirenz induce ER-stress in hepatic cells in a compound-specific manner. Given the lifelong and widespread use of Efavirenz in the multidrug therapy of HIV, this novel mechanism of cellular response to drug-induced stress could help to understand the frequent hepatic toxicities that accompany the treatment of HIV1 infection.

517 EFFECTIVENESS AND SAFETY OF BACLOFEN TREATMENT IN ALCOHOL-DEPENDENT PATIENTS WITH OR WITHOUT LIVER CIRRHOSIS: RESULTS OF AN OPEN STUDY IN TRUE LIFE

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Introduction: Baclofen (BAC) is a gaba B-receptor agonist. Several studies have suggested its efficacy in reducing alcohol consumption at low or high dose. Since April 2012, the use of BAC for alcohol-dependent patients is authorized by the french drug agency (ANSM) and approved by EASL guidelines in cirrhotic patients. The aim of the study was to assess effectiveness and safety of BAC at 12 months in alcohol-dependent patient with or without liver cirrhosis. We present 3 months preliminary results.

Patients and Methods: Between June 2010 and October 2012, 54 consecutive patients from 2 liver units began BAC treatment and were included in this prospective open label study. Patients received written information consent before treatment. BAC was orally administered at a dose of 15mg/day and weekly increased until obtaining alcohol indifference.

Results: Fifty-four patients (82% male, 53 years old) were treated, 59% were cirrhotic (Child Pugh score A, B and C: 59%, 19%, 22% respectively). After 3 months, at a mean BAC dosage of 38 mg/day (10–160), mean daily alcohol consumption reduced from 126 to 31 g/day (p < 0.001) and 18 patients (33%) achieved abstinence. A significant decrease of mean ASAT activity from 2.3N to 1.3N (p < 0.001), mean γGT activity from 6N to 1.8N (p = 0.005) and mean erythrocyte globular volume from 101 to 95 μL (p < 0.001) was noted. In cirrhotic patients, total bilirubin serum concentration significantly decreased from 40 to 25 μmol/L (p = 0.007) and prothrombin time increased from 68 to 70% (p = 0.04). Two patients (4%) stopped treatment because of non serious side effects. No serious event nor liver function deterioration occurred in cirrhotic patients.

Conclusion: Preliminary results of this study suggest that baclofen is effective to reduce alcohol consumption, even at low dose, in patients with or without cirrhosis. It led to significant improvement in biological markers of alcohol abuse and liver function tests. Six months data will be available in April 2013. In our study, treatment was well tolerated even in severe cirrhosis. These results should be validated by a large multicentric study.

C. Barrault and H. Lison participated equally to this work.

518 THE SPANISH–LATIN AMERICAN DILI NETWORK: PRELIMINARY RESULTS FROM A COLLABORATIVE STRATEGIC INITIATIVE

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Background and Aims: Idiosyncratic hepatotoxicity induced by drugs or herbal remedies (DILI) is an important health problem. DILI is expected to differ across geographical areas due to differential drug polices, prescription habits, drug consumption and genetic factors. In 2011 the Spanish DILI Registry contacted leading Latin American hepatologists in order to establish a Latin American DILI Registry. The objectives of this initiative were to stimulate detection and collection of well phenotyped cases to provide information on the Latin American DILI profile and corresponding risk factors.
Methods: Reference hepatologists were identified in Argentina, Uruguay, Chile, Brazil, Mexico, Peru, Venezuela and Bolivia, who in turn were commissioned to establish national specialist networks contributing to the project. Data would be obtained using the methodology in place at the Spanish DILI Registry. Identified cases would be remitted to the coordinating centre in Málaga for causality assessment and information storage.

Results: Seventy-three DILI cases have been analyzed up to November 2012, having a mean age of 52 years (range 15–86) and female predominance (60%). The therapeutic groups most frequently implicated were NSAIDs (22%) including nimesulide (5 cases) and diclofenac (4 cases); antilinfecives (19%) including nitrofurantoin (3 cases), herbal remedies (12%) including Morinda citrifolia, Peumus boldus and Monascus purpureus; hormonal therapy (12%) including cyproterone acetate (4 cases); and central nervous system drugs (11%). Hepatocellular injury (50%) was the most common type of liver damage. Jaundice was seen in 71% of cases, 53% required hospitalization and 38% fulfilled Hy’s Law criteria (66% of hormonal therapy cases, 44% of herbal cases). Positive autoantibody titers were present in 29% of cases, mainly antinuclear. Six cases were autoimmune hepatitis DILI (8%) and five cases had experienced a second DILI episode (7%).

Conclusions: This initial analysis demonstrates similar phenotypic characteristics as observed in registers outside Latin America with respect to type of injury and severity. However, female cases seem to predominate in Latin America. With regards to causative agents, elevated representation of NSAIDs, hormonal treatments and herbal remedies were evidenced.

Funding: Agencia Española del Medicamento. Proyecto Excelencia SAS PI0CTS-6470. ISAEc. CIBERehd is funded by ISCIII.

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CELLULAR TH1 AND TH2 IMMUNE RESPONSES TO ALCOHOL DEHYDROGENASE WITHIN THE LIVER OF PATIENTS WITH ALCOHOL-RELATED CIRRHOSIS DESPITE ABSTINENCE

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Alcohol-related cirrhosis (ARC) patients demonstrate proliferative peripheral blood mononuclear cell (PBMC) immune responses against the enzyme alcohol dehydrogenase (ADH) which are associated with active alcohol consumption and characterized by Th helper 1 (Th1) cytokine secretion.

Aim: To define whether ADH-specific cellular immune responses are present in the liver of patients with ARC despite long-term abstinence from alcohol.

Methods: Hepatic mononuclear cells (HMCs) were collected from second passage perfusate of explanted liver from seven ARC patients undergoing transplantation (Group A). PBMCs were collected from one of these seven patients and from 13 ARC patients after long-term alcohol abstinence [median 3.3 yrs (9 mo – 20 yrs); median CP Score=6] (Group B). 25 overlapping peptides, spanning the 375 amino acid human ADH β1 subunit, were constructed using fmoc solid phase chemistry. Proliferative responses to ADH peptides (1×105 cells/well cultured with 10 nM peptides for 7 days) were assessed by [3H]-thymidine incorporation. Stimulation index (SI) ≥2.5 was considered positive. Levels of IFNγ (Th1), IL-17 (Th17) and IL-4 (Th2) from culture supernatant collected on day 6 were measured by ELISA from 3/7 Group A and 9/14 Group B patients.

Results: HMCs from 3/7 (42%) Group A patients recognized 1 to 2 peptides (median 1) with SI up to 4.26 (median 3.4); HMCs and PBMCs from the same patient recognized similar peptides (peptides 4 and 13 by HMCs; peptides 3 and 13 by PBMCs). PBMCs from 8/14 (57%) Group B patients recognized median 2 peptides (range 1 to 5). In Group A, IFNγ (mean±SEM: 209.6±58.1 pg/ml) was detected in 36/75 (48%), IL-17 (8.6±2.9 pg/ml) in 7/75 (9%) and IL-4 (485.8±40.9 pg/ml) in 48/75 (64%) wells. In Group B, IFNγ (312.6±52.8 pg/ml, p=0.09) was detected in 70/225 (31%), IL-17 (49.2±10.1 pg/ml, p<0.0001) in 43/225 (19%) and IL-4 (191.6±13.1 pg/ml, p<0.0001) in 62/225 (28%) wells.

Conclusion: Abstinent ARC patients demonstrated weak proliferative responses to ADH peptide stimulation despite high frequency inflammatory T cell responses being present. In HMC, Th1 and Th2 responses were more vigorous than in PBMCs, and were stronger than Th17. HMC ADH-specific immune responses in ARC may favour fibrosis progression.

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TRANSPLANTATION FOR ALD: LESSONS FROM THE EXPLANT

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Background and Aims: Alcoholic liver disease (ALD) remains one of the commonest indications for liver transplantation in Europe. The histological features of ALD vary, depending on extent and stage of injury, No features are reliably pathognomonic of ALD. We describe the histological spectrum of explants of well characterised cohort of patients undergoing transplantation for ALD.

Methods: Consecutive explants (n=84) of patients transplanted for ALD in our institution between 2002 and 2011 were selected for retrospective histological assessment. Explants were scored blinded by two pathologists using a predetermined pro-forma. Histological assessment including the presence and degree of cirrhosis, steatosis, inflammation, inclusions, siderosis and neoplastic changes were scored semi-quantitatively.

Results: Median age was 54 and the majority (70%) were male. All patients had a long history of alcohol excess but reported abstinence for at least 6 months by transplantation. The aetiology was ALD (n=80) and mixed ALD/HCV (n=4). The majority (n=83) had a mixed or macronodular cirrhosis with evidence of re-modelling in a significant number; one had pre-septal cirrhosis. Alpha-1 antitrypsin inclusion bodies were seen in 9 (10.7%); only 4 of these had serum A1AT levels below normal. Parenchymal siderosis was present in 39 (46.4%); in 19 (22.6%) this was grade 3–4. Amongst these, only single mutations of the HFE gene were identified. Induced cell change was seen in 67 (79.8%) and 47 (56%) had the “abstinent cell” phenotype. While 46 (54.8%) had Mallory-denk bodies (MDB), 22 (26.1% of total) patients had both “abstinent cells” and MDB. Ballooning (n=45, 53.6%) and steatosis (n=31, 36.9%) were also seen. HCC was present in 14 (16.7%), with dysplastic nodules in 15 (17.9%), small-cell change in 20 (23.8%) and large-cell change 50 (59.5%). Phlebosclerosis and parenchymal extinction were universal findings.

Conclusion: We describe a wide spectrum of histological features in a large cohort transplanted for end-stage ALD. We demonstrate that despite abstinence, over half have residual MDB and ballooning. Conversely, over half had the recently described “abstinent cell” phenotype. Therefore, the presence of MDB should not be used as evidence of continued alcohol consumption; the presence of induced or abstinent cells correlates more strongly with reported abstinence.
Background and Aims: Disease progression can be reduced by nucleos(t)ide analogues (Nucs) in chronic hepatitis B patients with cirrhosis or advanced fibrosis. The effect of viral suppression with Nuc-naïve and Nuc-experienced patients or between patients with continuous and interrupted treatment.

Methods: A consecutive cohort of HBeAg-negative hepatitis B cirrhotic patients treated with ETV 0.5 mg/day was included. Patients with a history of HCC or who developed HCC within the first 6 months of therapy were excluded. The follow-up was censored when development of HCC was confirmed. The incidence of HCC was compared between Nuc-naïve and Nuc-experienced, and between patients with continuous therapy and those who had stopped ETV therapy after demonstration of undetectable HBV DNA at 3 occasions at least 6 months apart. Age, gender, genotype, HBV DNA, Nuc-experienced and interrupted treatment were analyzed in Cox hazard proportional regression analysis for the development of HCC.

Results: Among this cohort, 382 patients with mean age of 54.5 ± 9.2 years were followed up for mean period of 36.3 ± 19.3 months. HCC developed in 13 of 239 Nuc-naïve and 11 of 143 Nuc-experienced patients with estimated annual incidence of 2.03% and 2.18%, respectively, lower than 3.5–8.7%/year in earlier natural history studies from this country. The cumulative incidences of HCC in these two groups were comparable (15.8% vs. 11.1%, p = 0.984). In multivariate analysis, age (HR 1.107, 95% CI 1.052–1.166, p < 0.001) was the only independent factor for HCC development. Of the 242 patients being followed up for more than 24 months, 104 were treated with ETV continuously and 138 had interrupted ETV therapy. There was no significant difference in the cumulative incidence of HCC development (10.5% and 8.4%, p = 0.41) between these two subgroups. In multivariate analysis, age (HR 1.227, 95% CI 1.160–1.303, p < 0.001) and HBV DNA (HR 3.156, 95% CI 1.334–7.466, p = 0.009) were the independent factors for HCC development.

Conclusions: In HBeAg-negative hepatitis B cirrhotic patients treated with ETV, the incidence of HCC was 2.1%/year, and was similar between Nuc-naïve and Nuc-experienced patients or between patients with continuous and interrupted treatment.

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Conclusions: In HBeAg-negative hepatitis B cirrhotic patients treated with ETV, the incidence of HCC was 2.1%/year, and was similar between Nuc-naïve and Nuc-experienced patients or between patients with continuous and interrupted treatment.
Results: Patients with AH had a greater proportion of CD14+CD16+ monocytes compared with HVs (0.31 v 0.11; p = 0.002) and fewer CD14+CD16- cells (0.01 v 0.03; p = 0.002). In HVs, CD14+CD16- monocytes expressed more interleukin (IL)-10 mRNA (3.1 fold difference; p = 0.02) and less TNFα (0.6 fold; p = 0.002) than CD14+CD16+ monocytes. CD14+CD16+ monocytes expressed particularly high levels of TNFα, and CD14+CD16- monocytes were less effective than CD14+CD16- cells at driving memory T-cell proliferation (14.6% v 28.4%; p = 0.001).

Conclusions: Consistent with previous reports in CLD, patients with AH have a greater proportion of CD14+CD16+ monocytes. Detailed phenotypic analysis of these cells in HVs questions whether CD14+CD16- cells are pro-inflammatory as they express more of the anti-inflammatory cytokine IL-10, and are less able to drive memory T-cell proliferation, than CD14+CD16+ cells. Further studies are being conducted to assess the role of these 3 monocyte subsets in AH.

524 IN VITRO STEROID SENSITIVITY ACCURATELY PREDICTS 6 MONTH MORTALITY IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS

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Background and Aims: Severe alcoholic hepatitis (SAH) has a high mortality especially in those who fail to respond to steroid treatment. Early identification of steroid resistant patients may allow rapid implementation of other therapies, which may improve patient outcome. An early change in bilirubin has prognostic value but requires 7 days of steroid treatment first. Our group have previously reported that a simple 48h in vitro method for assessing steroid sensitivity (dexamethasone inhibition of lymphocyte proliferation; DILPA) correlates with 6 month mortality in patients with SAH (di Mambro et al, Hepatology 2011). We aimed to determine the accuracy of the DILPA in predicting outcome and to compare it to existing models of prognosis in SAH.

Methods: Peripheral blood was drawn from consecutive patients with a clinical diagnosis of severe alcoholic hepatitis (Maddrey discriminant function [MDF]-32). Peripheral blood mononuclear cells were cultured for 48h in media supplemented with 10% FCS and the mitogen PHA in the presence or absence of dexamethasone. Tritiated thymidine was added for the final 6h of culture before proliferation was measured on a beta counter. Maximum suppression of lymphocyte proliferation by dexamethasone was calculated (Imax). An Imax of <60% indicates in vitro steroid resistance as previously determined.

Results: 43 patients were recruited (21% female, median age 45). Patients who survived 6 months had a significantly higher Imax than those who didn’t (79.2% v 41.7%; p = 0.0003). There were no significant differences between baseline MDF or early change in bilirubin between patients that survived versus died at 6 months. An Imax of >60% had a 88% sensitivity and 78% specificity in predicting outcome. A Lille score >0.45 or a Glasgow score ≥9 had poorer sensitivity and specificity for predicting 6 month outcome (Lille: 53%, 57%; Glasgow: 67%, 61%). Neither score was significantly different between patients who survived versus died at 6 months.

Conclusions: The DILPA provides a simple, fast and accurate in vitro method of predicting clinical outcome in patients with SAH. This could be applied in clinical practice to identify patients who will not respond to steroid therapy within 2 days of starting treatment.

525 BMI BUT NOT AETIOLOGY OR STAGE OF LIVER DISEASE AFFECTS THE DIAGNOSTIC SENSITIVITY OF CARBOHYDRATE DEFICIENT TRANSFERRIN

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Background and Aims: A reliable biomarker is required in hepatology clinics for detection and follow-up of heavy alcohol consumption. Carbohydrate-deficient transferrin (CDT) increases with sustained heavy alcohol consumption and is the most specific biomarker of ethanol consumption [1]. Recent introduction of a standardised method for measuring CDT has improved its clinical application. This study was designed to determine whether alcohol-independent factors influence CDT levels in patients with chronic liver disease (CLD).

Methods: The relationship between serum %CDT and self-reported history of alcohol consumption was examined in 254 patients referred for evaluation of liver disease. CDT analysis was performed on serum collected at time of liver biopsy. The volume distribution of ethanol was estimated from lean body weight using the Janmahasatian equation [2].

Results: CDT levels were not affected by aetiology or severity of CLD. 13 of 254 subjects had a %CDT >1.7, predictive of heavy alcohol intake, 6 of whom did not acknowledge heavy drinking. 12 of these 13 subjects were suspected heavy drinkers on review of their medical records and clinical results. Conversely, not all acknowledged heavy drinkers had %CDT >1.7. Heavy drinkers with a BMI in the overweight or obese range had significantly lower %CDT than lean heavy drinkers (Figure 1). This persisted even when lean body weight was used as an approximation of the ethanol volume of distribution.

Figure 1. BMI affects %CDT. Alcohol consumption vs %CDT in lean and overweight drinkers. (Horizontal line represents the male heavy drinking threshold (>420g/week), vertical line represents 1.7% CDT, # is female.) (Alcohol groups: 1 = females <0.0-140g/week, males >0.0-210g/week; 2 = females >140–<350g/week, males >210–<420g/week; 3 = females ≥350g/week, males ≥420g/week).

Conclusion: An elevated BMI reduces the diagnostic utility of CDT at higher alcohol intake in subjects with CLD using the standardized method. In a hepatology outpatient setting, this assay is likely to be useful to confirm suspicion of heavy drinking in subjects who are not overweight, but cannot reliably identify moderate drinkers or heavy drinkers who are overweight.

Reference(s)
Background and Aims: It is well known that some inflammatory markers are elevated in alcoholic liver disease (ALD) but they are not routinely used to predict survival or select for transplantation. Dysregulated antibody secretion and increased circulating levels of IgG, IgM and particularly IgA are associated with ALD. Recent studies report raised combined k+ serum free light chains (cFLC) in patients with chronic inflammatory disease leading us to investigate their use compared with MELD scores as prognostic markers in ALD.

Methods: cFLC (Comblyte™, The Binding Site Group Ltd, The Binding Site Group, Ltd., Birmingham, UK) was measured in stored sera from 340 ALD patients (157/340 (46%) with alcoholic cirrhosis (AC); 70/340 (21%) required transplant) median follow up 9.9 months (range 0–46 months). Results were compared to patients MELD scores at sample collection (median 12; range 6–37) and cFLC concentrations from healthy controls (median 20 mg/L; range 9–50 mg/L).

Results: In all patients, median cFLC levels were 49.9 mg/L (range, 11.0–337.4). Patients progressing to transplant had higher cFLC levels and MELD scores (cFLC: 61.9 mg/L, 21.3–164.2 mg/L vs 47 mg/L, 11–337.4 mg/L; p = 0.001; MELD: 16, 6–25 vs 11, 6–37; p < 0.001). Raised concentrations of cFLC (>50 mg/L) were associated with shorter time to transplant (T TT, Hazard Ratio=1.8; 95%CI=1.1–3.0, p=0.014, transplants in 25% patients >50 mg/L=9 months, <50 mg/L=23 months, p=0.012) as were elevated MELD values (>11) (TTT Hazard Ratio=2.9; 95%CI=1.7–4.8, p<0.001). A simple model including raised cFLC (>50 mg/L) and MELD (>11) as categorical risk factors identified patients with 0 (n = 102), 1 (n = 132) or 2 (n = 106) risk factors with transplant in 25% patients after 30 vs 13 vs 7 months, respectively p<0.0001. In addition, the model stratified AC patients more effectively than MELD alone. Patients with 0 risk factors had a longer TTT than those with a high MELD score (>11) with transplants in 25% patients after 30 vs 23 months (3/38, 8% vs 10/71, 14%) respectively.

Conclusion: The addition of cFLC measurements improved the prediction of TTT of the MELD score in ALD. Prospective studies will determine the role of cFLC and MELD in predicting outcome in patients with ALD but our data suggest a model incorporating cFLC will improve predictive capacity in liver.

Background: The branched short-chain fatty acid valproic acid (VPA) is widely used as an anticonvulsant, primarily in the treatment of epilepsy, bipolar disorder and migraine prophylaxis. The mechanisms of action remain unclear, although it has been shown to alter a wide variety of signaling pathways and a small number of direct targets (e.g. GSK3β). Moreover, VPA targets mitochondria and induces mitochondrial permeability transition. There is evidence that glutathione (GSH) homeostasis may be altered as a consequence of reactive metabolites and/or reactive oxygen species produced during VPA treatment and may play an important role in VPA-induced hepatotoxicity. Therefore, VPA use in patients is associated with mitochondrial dysfunction, weight gain and hepatic steatosis. Thus, the aim of our study was to investigate the role of VPA in cholesterol homeostasis and intracellular trafficking, in particular, if VPA induces mitochondrial cholesterol accumulation and mitochondrial GSH (mGSH) depletion, and if this effect sensitizes to acetaminophen (APAP) toxicity.

Methods: Mitochondrial cholesterol trafficking and GSH levels were analyzed in F2-CHO cells transfected with Cyp11a1 treated with VPA (100–1000 μM), measuring mitochondrial cholesterol loading by confocal imaging and pregnenolone levels by ELISA. Expression of StAR, MLN64, ER stress markers and lipogenic transcription factors were examined by qPCR and WB. Liver damage, cholesterol trafficking, GSH homeostasis and mitochondrial function was examined in mice following VPA treatment (400 mg/Kg). VPA-treated mice were administered APAP (300 mg/kg) and liver injury examined by ALT and H&E.

Results: VPA selectively depleted mGSH levels (40–50%) in F2-CHO cell line and increased pregnenolone levels, indicating enhanced mitochondrial cholesterol levels. In parallel to these observations, VPA induced the expression of MLN64, StAR and mitochondrial cholesterol accumulation was observed by confocal imaging after VPA treatment. Hepatic extracts from VPA treated wild type mice exhibited microvesicular steatosis, liver injury, mitochondrial cholesterol accumulation and mGSH depletion. VPA treatment significantly induced ER stress markers and the expression of lipogenic transcription factors. Importantly, VPA treatment in fed mice sensitized to APAP treatment (4000U/dL in VPA-treated mice vs 300U/dL in control mice).

Conclusions: VPA-induced mitochondrial cholesterol trafficking leading to subsequent mitochondrial GSH depletion, which in turn sensitized to APAP mediated liver injury.
positive serum C reactive protein (CRP) had high hepcidin-to-ferritin ratio compared to those with negative CRP ($0.45 \pm 0.05$ vs. $0.31 \pm 0.15$, $P < 0.01$). Serum IL-6 of ALD was also decreased after abstaining (from $3.15 \pm 2.23$ to $2.20 \pm 1.16$ pg/ml during 3 month).

**Conclusions:** Hepcidin secretion responds to iron condition and this response is enhanced by systemic inflammation in ALD. Because the enhanced hepcidin secretion is usually causative to iron deficiency, iron overload seen in ALD may be caused by bluntness of duodenal enterocytes to hepcidin by ethanol.

**529 ETHANOL STIMULATES PRODUCTION OF EXTRACELLULAR MATRIX PROTEINS IN HEPATIC STELLATE CELLS**

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**Background:** Consumption of alcohol is a leading cause of liver fibrosis; however the mechanisms underlying ethanol-induced liver fibrosis are still unknown. Transdifferentiation of hepatic stellate cells (HSC) generates hepatic myofibroblasts, which are the key cells responsible for onset and progression of liver fibrosis. We investigated epigenetic regulation that causes expression changes of extracellular matrix (ECM) proteins in ethanol-treated cultures of primary rat HSC.

**Methods:** Rat hepatic stellate cells were treated at day 1, 2, 3, 5, 7 and 10 of culture for 24h and 48h with 86mM ethanol (dose equivalent to heavy drinking in humans). Expression levels of collagen I, III, IV, elastin and fibronectin were assessed and measured by qRT-PCR. Chromatin immunoprecipitation assay (ChIP) was used to examine epigenetic events at the ECM proteins.

**Results:** Expression of ECM proteins is upregulated during HSC transdifferentiation, however ethanol treatment causes further increase in mRNA expression for collagen I, III, IV and elastin. The biggest changes were observed during early stages of HSC transdifferentiation (day 1–3) at 48h of ethanol treatment. Small or no difference was seen in expression of fibronectin. ChIP assay showed that ethanol-induced gene expression is associated with altered methylation patterns of histone H3.

**Conclusions:** There were significant differences in ECM proteins expression between ethanol-treated and control HSCs. Changes in expression of ECM proteins in ethanol-stimulated HSC appear to be regulated by modifications in epigenetic events. Evidence for epigenetic changes in ethanol-induced liver fibrosis might be helpful in diagnosis and may provide a future target for treatment of liver fibrosis.

**530 CURCUMIN NANOPARTICLES: A POTENTIAL ORAL FORMULATION IN PREVENTING ALCOHOL INDUCED LIVER DAMAGE IN RAT MODEL**

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**Background and Aims:** Alcoholic liver disease results from dose and time dependent exposure to alcohol. Alcohol mediated liver cirrhosis is growing at an alarming rate in the western world. Massive oxidative stress and depressed antioxidant status induced by ethanol metabolism play a major role in alcohol manifestation and liver damage. Curcumin, a very well known dietary antioxidant is known to have beneficial effect in circumventing alcohol induced liver damage. However its poor oral bioavailability is a major drawback limiting its potency and impelling the use of large doses for having beneficial effects. To overcome this limitation our aim was to investigate the efficacy of consuming oral curcumin encapsulated nanoparticles at a much lower dose as compared to free curcumin in preventing alcohol induced liver damage.

**Methods:** Alcoholic liver damage was induced in rats by consumption of ethanol. Biodegradable curcumin nanoparticles were given orally daily prior to alcohol consumption. ROS generation, membrane lipid peroxidation, microviscosity, antioxidant enzymes, NF-κB translocation, inflammatory proteins like NOS-2 and Cox-2 and mitochondrial cytochrome C release in the cytosol were measured in the liver tissue. Histopathology of the liver sections was done.

**Results:** Alcohol consumption led to a massive generation of ROS. Alcohol induced liver damage was confirmed by histopathological analysis of liver sections and factors related to cell degeneration. NF-κB translocation in the nucleus, increase in Nos-2 and Cox-2 expression and release of cytochrome C in the cytosol were seen in alcohol induced control group of rats. Nanoparticulated curcumin treatment however prevented increased ROS, restored membrane integrity, prevented downregulation of membrane microviscosity and antioxidant enzymes with an inhibition of NF-κB activation and reduced the expression of inflammatory proteins. Histopathological analysis confirmed the pathological improvement of the liver. Free curcumin at the same dose was ineffective.

**Conclusion:** Delivering a herb origin antioxidant curcumin in biodegradable nanoparticles containing a very low dose of the compound proved to be useful in overcoming the limitation of free curcumin and may be recommended as a very potent formulation in preventing alcohol induced liver damage.

**Acknowledgements:** Council of Scientific & Industrial Research.
Alcoholic hepatitis (AH), in its severe form, is a lethal disease in the short-term. Although infections are frequent complications of AH, the incidence of invasive aspergillosis (IA), and its impact on short-term survival remain unknown.

**Methods:** We retrospectively analyzed 82 patients prospectively followed for biopsy-proven severe AH (modified Discriminant Function (mDF) >3) from June 2006 to December 2011 with a follow-up of 3 months after biopsy. AH were treated in 58 patients with corticosteroids, 4 with corticosteroids and pentoxifylline (PTX), 1 with PTX alone and 20 did not receive specific treatment. Demographic, bacteriological and therapeutic data were collected. The diagnosis of IA was based on the revised criteria of EORTC/Mycoses Study Group and the AspICU except for host factors.

**Results:** Forty cases of IA classified as proven (n=5), probable (n=8) or possible (n=1) were diagnosed (17%) after a median delay of 34 [0–79] days after AH diagnosis. The sites of infection were the lungs (n=10) and the central nervous system (n=2) and was disseminated in 2. Aspergillus fumigatus was isolated in 10 cases (71% of IA): 5 in bronchoalveolar lavage (BAL), 4 in bronchial secretions and 1 in a brain biopsy. Diagnosis of other IA was based on radiological signs and galactomannan detection. Patients with IA were younger, had higher total bilirubine, creatinine and Prothrombin Time at day 28 (p<0.01) and were more frequently admitted in ICU. 12 patients with IA received corticosteroids but 2 did not receive any treatment for AH. The occurrence of IA was similar in non-responders to corticosteroids vs responders as defined by the Lille score. The 3-month mortality was higher in patients with IA than without IA (93 vs. 50%, p<0.01). Multivariate logistic regression analysis showed that age ≥53 y, a Lille score ≥0.45 and the presence of IA were associated with a higher risk of mortality at 3 months.

**Conclusions:** IA is a frequent complication of corticosteroid-treated severe AH, carrying a high risk of mortality. Systematic screening for IA should be recommended in these patients while further studies are needed to identify high risk population requiring antifungal prophylactic treatments.

**Introduction and Aim:** Severe alcoholic hepatitis (SAH) implies 50% mortality at 2 months. Studies show that therapy with steroids has reduced mortality to 35% at 6 months. However, mortality is still high. A recent study has demonstrated that in vitro steroid-treatment resistance is high and correlate with adverse clinical outcome. It is need to explore new therapeutic alternatives to improve survival rate in patients who fail to respond to steroid therapy.

Oxidative stress and depletion of mitochondrial glutathione are important implied factors in liver injury. Metadoxine (MTD) has shown to inhibit hepatic lipid accumulation. In tissues, ion pair molecules can be separated forming N-oxide molecules that work as rotating traps capable of capturing reactive oxygen species. The aim of this study was to evaluate the impact on mortality rate at six months of MTD added to steroid therapy in patients with SAH. Also, risk factors implicated in increased mortality in the next six months were analyzed.

**Patients and Methods:** Randomized clinical trial, open label, conducted in Mexico’s General Hospital (Registry Key: DIC/10/107/03/043). We randomized 70 patients with SAH criteria, 35 received prednisone (PDN) 40 mg/day and 35 received PDN 40 mg/day plus MTD 500 mg three times daily. The duration of treatment in both groups was 30 days.

**Results:** In the group supplemented with MTD significantly improved the following parameters: At the end of treatment, survival was better (74.3% vs. 45.7% P=0.02); there was less development or progression of complications such as encephalopathy (28.6% vs. 60.0% P=0.008) and hepatorenal syndrome (31.4% vs. 54.3% P=0.05). Survival was also higher at 6 months follow-up (48.6% vs. 20% P=0.003). Cox regression shown that relapse in alcohol consumption is strongly associated with 6-month mortality (HR 8.4; 95%CI 2.8 to 25.4).

**Conclusions:** MTD added to steroid therapy improves survival at six months in patients with SAH. Relapse in alcohol consumption is the main independent risk factor associated with mortality within the first 6 months of follow-up.

**Acknowledgment:** This work was supported by ‘Estimulo Angeles Espinosa Yglesias 2010’.
mice in whom ALF was induced. Phenotypically, these animals are normal and have normal circulating glutamine levels.

Methods: GS-KO/M mice were obtained by a selective elimination of GS expression in striated muscle of MCK-Cretg/−/c-MetloxP (GS-KO/M) mice. Four groups of animals were studied: FVB Flox expressing normal GS levels (WT) and GS-KO/M received acetaminophen (IP 250 mg/kg) to induce liver failure (ALF), or saline (IP) (n=12 in each group). Plasma was measured for: ammonia and standard biochemical markers (AST, ALT, bilirubin, urea, lactate, glucose, creatinine, COBAS-Roche). Brain water and ammonia were measured. TNF-α was determined by ELISA in liver tissue homogenates.

Results: Plasma ammonia was found elevated in WT ALF versus. WT (122±37.3 vs. 58.9±9.9 μmol/l; p = 0.26) and in GS-KO/M ALF (299.5±7.5 μmol/l; p < 0.05 vs. GS-KO/M). Severity of liver injury measured using ALT, AST and lactate were similar between groups but hepatic TNF-α was higher in the GS-KO ALF versus WT ALF (6.4±2.25 vs. 1.9±0.5 ng/mg protein; p < 0.05). Brain water was increased in GS-KO ALF vs. WT ALF (79.8±0.5 vs. 78.7±0.4%; p < 0.05), which was associated with greater mortality in the GS KO animals (p < 0.05).

Conclusions: The results of this study provides direct evidence for the importance of muscle GS in regulating ammonia levels and hepatic inflammation which contributed to increased brain swelling and mortality. The mechanism of increased hepatic inflammation in the GS-KO animals is unclear but likely to be due to the alterations in gut integrity and known immunomodulatory function of glutamine. As glutamine supplementation cannot be undertaken due to its ammoniagenic affects, direct augmentation of GS expression may provide novel approach to therapy.

Acknowledgment: Professors Lamers & Hakvoort.

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HGF/c-Met IS PROTECTIVE IN THE MODEL OF ACETAMINOPHEN INDUCED TOXIC LIVER INJURY

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Background: Overdosage of the free-available analgetic drug paracetamol (Acetaminophen) leads to fatal liver damage. To date knowledge about underlying mechanisms and specific therapeutic interventions is still limited. In this study we aim to define the role of the HGF receptor c-Met in the model of Acetaminophen-induced toxic liver injury.

Methods: Conditional c-Met knockout mice (c-MetloxP) and control mice (c-MetloxP+/+) were subjected to Acetaminophen (300 mg/kg ip.) treatment. A second experimental group was treated with N-Acetylcystein – a commonly used antidot – after Acetaminophen injection. Liver tissue and blood samples were analysed 6 and 48 hours after Acetaminophen treatment.

Results: Consistent with significantly enhanced liver transaminases (>10-fold stronger) in c-MetloxP mice histopathological stainings revealed large haemorrhagic necrotic areas as compared to c-MetloxP+/+ mice. This is reflected in an enhanced inflammatory phenotype and corroborated through increased numbers of apoptotic and more importantly of liver infiltrating cells (Ly6G) in the c-MetloxP group. Oxidative stress is known to play an important role in Acetaminophen-induced toxic liver injury. Analysis of antioxidants genes like Haemoxigenase-1, CCL-2, Methallothionine1/2 and Nrf revealed significantly increased expression levels in c-MetloxP mice indicating a compensatory reaction to stronger liver damage. In contrast c-MetloxP mice showed a dramatically decreased activation of the Cytochrome P450 system (Cyp2e1 and Cyp1a2) – which is known to play a crucial role in drug metabolism – in these mice. We additionally showed a stronger expression of the gap junction subunit Connexin-32 in c-MetloxP mice, suggesting an enhanced distribution of tissue damaging toxic acetaminophen-dependent degradation products. Investigation of the c-Met dependent phosphorylation of AKT and ERK displayed a reduced activation of these protein kinases in c-MetloxP mice. The therapeutic intervention with N-Acetylcystein leads to significantly decreased liver damage, in both c-MetloxP and c-MetloxP+/+ mice and is also reflected in a lower expression of oxidative stress related genes.

Discussion: These results show a clear protective function of c-Met in the pathogenesis of Acetaminophen-induced toxic liver injury. This is mediated through a dysregulation of cytochrome p450 genes and the oxidative stress response. A modulation of the HGF/c-Met pathway is thus a promising novel approach for the treatment of drug-induced liver injury.

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SHORT-TERM SURVIVAL IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS TREATED WITH PENTOXIFYLLINE vs. CORTICOSTEROID: A NON-INFERIORITY TRIAL – A PRELIMINARY REPORT

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Background: Both corticosteroid and pentoxifylline reduce short-term mortality in severe alcoholic hepatitis. However, few studies have directly compared the efficacy of pentoxifylline and corticosteroid in patients with severe alcoholic hepatitis.

Methods: In this ongoing prospective, multicenter study (target sample size: 126 patients), we randomly assigned 93 patients with severe alcoholic hepatitis (Maddrey discriminant factor ≥32) to receive either the pentoxifylline (400 mg 3 times daily, in 47 subjects) or the prednisolone (40 mg daily, in 46 subjects). The primary end point was non-inferiority in survival at 1-month time point for the pentoxifylline as compared with prednisolone. Secondary outcomes included survival at 3-month, hepatitis complications, and adverse events.

Results: There were no significant baseline differences between the pentoxifylline and prednisolone groups in demographics or disease severity parameters including age, gender, and Maddrey discriminant factor. The 1-month survival rate of patients receiving pentoxifylline was 74.5% as compared with 87.0% in those of taking prednisolone, for a treatment difference of 12.5% (95% confidence interval, −3.8% to 28.1%). The confidence interval included the predefined margin of non-inferiority (15%) and zero, indicating that the difference in survival was non-significant but the result regarding non-inferiority was inconclusive. The 3-month survival rates were similar (72.3% in pentoxifylline group vs. 76.1% in prednisolone group; 95% confidence interval in difference, −13.9% to 21.0%). Hepatitis complications including hepatic failure and/or hepatorenal syndrome and side effects such as infection and gastrointestinal bleeding were similar in the two groups.

Conclusions: Although the trial is intended to assess the non-inferiority of pentoxifylline as compared with corticosteroid, the
findings demonstrate that the efficacy of the pentoxifylline is not statistically equivalent to the efficacy of the corticosteroid, supporting corticosteroid as a preferred treatment option in patients with severe alcoholic hepatitis.

537 PREDICTION OF LIVER-RELATED MORTALITY AND RISK STRATIFICATION IN PATIENTS HOSPITALIZED FOR ALCOHOLIC HEPATITIS

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Background and Aims: The accurate prognostic stratification of alcoholic hepatitis (AH) is essential for individualized therapeutic decisions. The aim of this study was to develop a new scoring system to predict liver-related mortality in Asian AH patients.

Methods: We conducted a population-based, retrospective cohort study using the AH registry data of the Seoul Metropolitan Government from 1999 to 2011. A new predictive scoring model was constructed using the Cox regression method. The new prognostic scoring system was validated by the bootstrap sampling method and compared with other scoring systems by analyzing the area under the curve. The predictability for mortality on 30 and 90 days was presented as a nomogram.

Results: Of the 1410 AH patients, 709 were included in the final study cohort. The difference in serum bilirubin from day 0 to day 7, the serum blood urea nitrogen on day 7, and the prothrombin time, serum albumin, creatinine, sodium, and potassium levels at admission independently predicted liver-related mortality. The model for alcoholic hepatitis to grade the severity in an Asian patient cohort (MAGIC) was generated from these factors. For risk stratification, three different risk groups were identified with cutoff points of 199 and 262, based on the different survival probabilities. In addition, MAGIC showed a better predictive performance for liver-related mortality than any other scoring system.

Conclusions: MAGIC is the first prognostic scoring system for predicting liver-related mortality in the Asian population with AH.

538 A CLINICAL–BIOCHEMICAL–HISTOLOGICAL (CBH) MODEL IMPROVES LONG-TERM MORTALITY PREDICTION IN MALE AND FEMALE PATIENTS WITH ALCOHOLIC LIVER DISEASE

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Patients with non-severe alcoholic liver disease (ALD) are at lower risk of short-term mortality but die at later time points with worse prognosis for women than men. The aims of our study were to develop a non-invasive prognostic model for prediction of long-term mortality for male and female patients with ALD and to investigate if histological parameters of ALD can improve the diagnostic accuracy of the non-invasive model. A cohort of 189 consecutive patients with a history of alcohol consumption of >40 g/d for women (n = 69) and >60 g/day for men (n = 120) enrolled between 1985–2008 was studied. Other causes of liver disease were excluded. All patients underwent liver biopsy for staging of ALD. Cox regression models were used for univariate and multivariate analyses. Variables independently associated with mortality were used to build new prognostic models. The parameters included in the non-invasive clinical-biochemical (CB) model were sex, alkaline phosphatase, bilirubin, creatinine, INR and thrombocyte count. The clinical-biochemical-histological (CBH) model included fibrosis stage and pericellular fibrosis in addition to the parameters of the CB model. Diagnostic accuracies of the CB, CBH and Child Pugh score (CPS) were evaluated by ROC analysis for prediction of mortality at one and five years for the entire study cohort and the male and female subgroups. In all patient groups the performance of the CBH model was superior to CPS at both time points. Each of the prognostic models showed better performance in men as compared to women with improved accuracy for the CBH over the CB model or CPS (Table 1).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>AUROC (95% CI)</th>
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<tbody>
<tr>
<td></td>
<td>1 Years</td>
<td>5 Years</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>CBH</td>
<td>0.92 (0.86–0.98)</td>
<td>0.75 (0.59–0.92)</td>
<td>0.85 (0.78–0.92)</td>
</tr>
<tr>
<td>CB</td>
<td>0.86 (0.75–0.97)</td>
<td>0.69 (0.48–0.85)</td>
<td>0.80 (0.71–0.89)</td>
</tr>
<tr>
<td>CPS</td>
<td>0.82 (0.70–0.95)</td>
<td>0.69 (0.50–0.87)</td>
<td>0.76 (0.66–0.87)</td>
</tr>
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The CBH model is a new prognostic tool for prediction of intermediate- and long-term mortality particularly in men with ALD. Long-term prognostic models should be evaluated in larger patient cohorts with emphasis on gender difference.

539 DRUG-INDUCED LIVER INJURY: THE IMPACT OF NON STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

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Background and Aim: Drug-induced liver injury (DILI) is increasingly being recognized as a cause of clinically significant liver disease. We aimed to evaluate the rate of hospital-admitted severe cases of DILI, and to identify the drugs most commonly responsible of severe clinical course.

Methods: Diagnosis of DILI was made when at least three of International Consensus Criteria were present. Between 1996 and 2011, all consecutive patients diagnosed as DILI were analyzed. Data were collected from in- and outpatient visit charts. Liver damage was defined as hepatocellular, cholestatic or mixed, according to clinical and laboratory data. Liver stiffness measurement was carried out by Fibroscan©. All patients had regular follow-up visits every three months for at least one year, and were contacted to update their clinical outcomes.

Results: Out of 10,270 patients, 157 (73 males, 46.5%), mean age 54.2 years (range 11–88) fulfilled diagnosis of DILI; 29 patients (18.5%) had pre-existing compensated chronic liver disease (CLD). Hepatocellular pattern was more commonly observed (53.2%) followed by cholestatic (26.7%) and mixed pattern (20.1%). The most frequent drugs were non-steroidal anti-inflammatory drugs (NSAIDs, 33.3%), followed by antibiotics (19.7%), anti-diabetic drugs (13.4%), anti-platelet agents (9.6%) statins (9.3%) immunosuppressant (6.7%), psychotropic drugs and herbal products. In 38 cases, two or more drugs were involved. Among NSAIDs, nimesulide was the most frequently involved (43%). NSAIDs were more frequently associated with hepatocellular pattern and signs of hepatic encephalopathy. Patients with DILI from NSAIDs were younger (48.1 vs. 55.1 years, p = 0.02) and the duration of drug intake was shorter (p < 0.001) as well as the average latency (p = 0.02). ALT values (p = 0.02) and eosinophil count (p = 0.02) were
significantly higher in NSAIDs-induced liver damage compared to other drugs (p < 0.007). NSAIDs were involved in six cases of acute liver failure and one died while on waiting list for OLT. The overall clinical pattern of DILI in CLD was similar to other non CLD cases and none had hepatic decompensation.

**Conclusions:** NSAIDs was the most common drug class as cause of DILI among patients younger than 50 years. Acute liver disease induced by NSAIDs represents an important cause of hospitalization.

540 **HEAVY ALCOHOL INTAKE INCREASES HEPATOCELLULAR CARCINOMA IN HEPATITIS B VIRUS-RELATED CIRRHOSIS**

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**Background and Aims:** Taiwan has high prevalence of hepatitis B viral (HBV) infection and hepatocellular carcinoma (HCC) with increasing consumption of alcohol. We investigate the impact of heavy alcohol consumption and HBV infection on HCC in cirrhotic patients.

**Methods:** 966 cirrhotic patients including 132 patients with HBV infection and alcoholism, 632 patients with HBV infection, and 202 patients with alcoholism between 2000 and 2009 were enrolled and followed until 2011. The primary end-point is newly developed HCC.

**Results:** The mean age of patients with HBV infection and alcoholism was much younger than those with HBV infection alone or with alcoholism alone (43.9 vs. 47.8 vs. 49.3 years, P = 0.03). Within the three patient groups respectively—cirrhotic patients with HBV infection and alcoholism, HBV infection, and alcoholism—thirty-eight (28.8%), 100 (15.8%), and 21 (10.4%) showed newly developed HCC. The 10-year cumulative incidence (52.8% vs. 39.8% vs. 25.6%, P < 0.001) and annual incidence (9.9%, 4.1%, and 2.1%) of HCC were significantly higher in cirrhotic patients with HBV infection and alcoholism than those with HBV infection or those with alcoholism (Figure 1).

The cumulative incidence of HCC was higher in Child–Pugh class A or B patients with HBV infection and alcoholism than those with HBV infection or those with alcoholism. In multivariate linear regression models, baseline serum HBVDNA levels (OR = 1.81, P = 0.008), HBV genotypes (OR = 2.61, P = 0.047), and serum α-fetoprotein (OR = 1.08, P = 0.016) were risk predictors for HCC. The cumulative incidence of HCC was higher in patients with higher baseline serum HBV DNA. The cumulative incidence of HCC was higher in those patients with HBV genotype C than those with HBV genotype B (Figure 1).

**Conclusions:** Heavy alcohol consumption significantly increased the risk of HCC in HBV-related cirrhotic patients. Alcoholic cirrhotic patients with concomitant HBV infection were found to have significantly higher incidence of HCC than those without HBV infection. Elevated baseline serum HBV DNA was a strong risk predictor of HCC in cirrhotic patients with HBV infection and heavy alcoholism.

541 **SUSCEPTIBILITY TO INFECTION IN PATIENTS WITH ACUTE ALCOHOLIC HEPATITIS: A NOVEL ROLE FOR PD-1 AND GALECTIN-9?**


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**Background and Aims:** Sepsis is the major cause of mortality in patients with Acute Alcoholic Hepatitis (AAH). Susceptibility to infection has been shown to be consequent to the failure of a robust and co-ordinated innate and adaptive immune response. However, there is a paucity of studies analysing both arms of the host immune response in AAH patients. Furthermore, there is no information regarding the role of immuno-inhibitory signatures, such as the Programmed-death (PD-1/PD-1-ligand) and TIM-3/Galectin-9 pathway, on the functions of innate and adaptive immune cells during excessive alcohol abuse. The aim of this study was to characterise the role, relationship and contribution of the innate and adaptive immunity to the susceptibility to infection in well-defined patients with AAH.

**Patients and Methods:** Peripheral blood and plasma was collected from treatment naive patients with severe AAH (n = 12, DF>32), alcohol-related cirrhosis (n = 12, Child–Pugh B/C) and healthy controls (HC). The frequency, phenotype and expression of immuno-inhibitory signatures (including PD-1, PD-L1, TIM-3, Galectin-9) and toll-like receptors (TLR) on neutrophils and CD4+CD8+T-cells was analysed using FACS. Basal and bacterially-challenged neutrophil function was assessed by phagocytosis and oxidative burst assays. T-cell responses were assessed by measuring the frequency of T-cells producing IFNγ/IL-10 in response to bacterial challenge by Eslip Assays. Plasma cytokine levels were analysed by CBA and ELISA.

**Results:** Patients with AAH had impaired neutrophil phagocytosis (p < 0.001), which correlated with reduction of neutrophil activation markers (CD16, p < 0.001, CD11b, p = 0.01), TLR2/4 (p = 0.02/0.03/0.01 respectively), and PD-1 (p = 0.004). Neutrophil oxidative burst was also compromised in AAH (p < 0.001), whilst spontaneous oxidative burst was elevated (p < 0.001). AAH patients had significantly elevated T-cell expression of PD-1/PD-L1 (p = 0.009/0.03), Galectin-9/TIM-3 (p = 0.01/0.02) and displayed compromised IFNγ/IL-10 T-cell responses to bacterial challenge.
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Only plasma from AAH patients showed elevated levels of pro-inflammatory IL-6/IL-8 (p = 0.03/0.02) and Galectin-9 (p = 0.04), a known negative regulator of the T-cell immune response.

**Conclusions:** Patients with AAH have impaired anti-bacterial innate and adaptive immunity, which is characterised by the hyperexpression of immune-inhibitory receptors on neutrophils and T-cells. We propose that PD-1/PD-L1 and TIM-3/Galectin-9 signatures not only represent markers of infection susceptibility in patients with AAH but therapeutic targets whereby blockade of these pathways could allow reconstitution of effective host immunity.

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**NEW ALGORITHM TO ASSESS FIBROSIS STAGE IN ALCOHOL LIVER DISEASE BY TRANSIENT ELASTOGRAPHY**

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**Aim:** Liver stiffness (LS) is the novel gold standard to assess fibrosis stage in patients with alcoholic liver disease (ALD). However, we could recently demonstrate that alcoholic steatohepatitis needs to be considered (transaminase levels) prior to fibrosis assessment since it increases LS independently of fibrosis stage. We here validate our novel algorithm in a larger two-center study population of heavy drinkers undergoing alcohol detoxification.

**Methods:** 417 patients admitted for alcohol detoxification were included both from France (n = 137) and Germany (n = 280) undergoing sequential LS measurement by transient elastography and lab tests at day of admission and release. 251 patients had valid LS measurements (IQR <30%, success rate>60%) for both time points.

**Results:** Overall, LS decreased in 10% with a mean decrease of LS by 1.9 kPa (7.2%) over the mean observation interval of 6.4 days. The decrease of LS translated into a lower fibrosis stage in 24.7%

**Conclusion:** This larger two-center trial confirms previous findings that coexisting steatohepatitis of GOT>100 U/L markedly increases LS in patients with ALD independent of fibrosis stage. Ca. 20% of patients with elevated GOT levels eventually have a lower LS-based fibrosis stage. Therefore, normalization of GOT levels is required to correctly interpret liver stiffness in patients with ALD.

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**INTERLEUKIN 17F CONCENTRATION IS ASSOCIATED WITH DEGREE OF LIVER DAMAGE IN ALCOHOLIC LIVER DISEASE**

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**Background and Aims:** The Th17 lymphocytes, subset of T helper CD4+ lymphocytes, play a role in pathogenesis of inflammatory and autoimmune diseases. Recent reports have proven the influence of Th17 lymphocytes on inflammatory and autoimmune reactions in alcoholic liver disease (ALD). Activated Th17 T-cells, infiltrating liver tissue, recruit neutrophils thus enhancing inflammatory response in ALD. Our study aims to assess serum interleukin 17F (IL-17F) and interleukin 17A (IL-17A) concentration in ALD with regards to the advancement of liver damage.

**Methods:** Serum concentrations of IL-17F and IL-17A were assessed by ELISA method in 88 patients with ALD. The results were analysed with regard to the degree of hepatic damage classified according to MELD (Model of End-Stage Liver Disease), Child–Pugh score and GAHS (Glasgow Alcoholic Hepatitis Score). Control group included 30 healthy volunteers.

**Results:** Serum concentration of IL-17F was significantly elevated in ALD compared to control group (median: 15.46 pg/mL vs. 8.17 pg/mL, p = 0.006). There were significant positive correlations between serum IL-17F concentration and serum bilirubin (Rs=0.23, p = 0.03), INR (Rs=0.29, p = 0.005) and degree of liver damage in MELD (Rs=0.23, p = 0.03), but not in Child–Pugh and GAHS classification. Serum IL-17A concentrations were comparable in ALD and control groups. In ALD group serum IL-17A positively correlated with serum IL-17F (R²=0.26, p = 0.01) and INR (R²=0.22, p = 0.03).

**Conclusions:** Serum IL-17F, but not IL-17A, is increased in ALD. IL-17F correlates with the progression of liver damage in ALD demonstrated through MELD classification.
Conclusion: This pilot study permitted to validate an ‘ALT-free strategy’ for planning the statistical analysis of the forthcoming validation phase of new DILI-biomarkers.

**545** HEAVY ALCOHOL USE IN THE ELDERLY IS ASSOCIATED WITH HEPATIC STEATOSIS BUT ONLY RESULTS IN SIGNIFICANT FIBROSIS IN A MINORITY OF SUBJECTS

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**Background and Aim:** We investigated the prevalence of liver steatosis and fibrosis among heavy alcohol drinkers in a large elderly cohort.

**Methods:** This cross-sectional study was based on participants of the population-based Rotterdam Study. Participants were interviewed, had a fasting blood collection, liver ultrasonography (US), and liver stiffness measurement (LSM) using Fibroscan. Excessive alcohol intake was defined as >14 drinks/week. Diagnosis and grading of fatty liver was determined according to the protocol by Hamaguchi et al., and classified as ‘no’ (0–1), ‘mild’ (2–3), or ‘moderate to severe’ fatty liver (4–6). Significant fibrosis was defined as reliable LSM>8 kPa, and cirrhosis as reliable LSM>13 kPa.

**Results:** Data on alcohol use and US were available for 3160 participants; 255 subjects were classified as healthy drinkers (HD) and 2905 as non-heavy drinkers (NHD). HD were younger than NHD (72±4.4 vs. 76±6.0; p<0.001). Males accounted for 72% and 40% of HD and NHD, respectively (p<0.001). 130 (51%) of 255 HD demonstrated hepatic steatosis, which was graded as ‘mild’ and ‘moderate to severe’ in 16 (6.3%) and 114 (45%) participants, respectively. Among NHD, 6% demonstrated mild and 30% moderate to severe fatty liver (p<0.001; compared to healthy drinkers).

Independent risk factors of hepatic steatosis were excessive alcohol intake (OR 1.8; p<0.001), lower age (OR 0.98; p=0.001), higher BMI (OR 1.3; p<0.001), higher fasting triglyceride level (OR 2.2; p<0.001), higher fasting glucose level (OR 1.3; p<0.001), and presence of hypertension (OR 1.5; p=0.006). Among HD, alcohol intake remained an independent predictor of hepatic steatosis (OR 1.07; p=0.01) after adjustment for age, BMI, presence of hypertension, serum triglyceride, glucose and HDL-C levels. Reliable LSM was available in 158 of 255 HD. More HD had undergone LSM than NHD (62% vs. 40%; p<0.001). LSM did not differ between HD and NHD (5.6±1.9 vs. 5.5±2.4; p=0.64). Of HD, 13 (8.2%) were classified as having significant fibrosis and none as having cirrhosis, which was similar to NHD (9% and 12%, respectively).

**Conclusion:** In the elderly population, heavy alcohol intake is associated with hepatic steatosis, yet it only results in significant liver disease in a minority of subjects.

**546** SEVERE ALCOHOLIC HEPATITIS (AH) HAS THE SAME PROGNOSIS IN PATIENTS WITH OR WITHOUT GASTROINTESTINAL BLEEDING (GIB)

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**Background:** All trials published in severe AH have included patients with “pure” AH, i.e. without concomitant GIB. However, diagnosis of severe AH is often suspected in alcoholic cirrhotic patients admitted for GIB. The aims of this study were: (1) to compare the prognosis of AH in patients with or without GIB; (2) to assess the performance of Lille model patients with AH and GIB.

**Methods:** All alcoholic cirrhotic patients admitted in our unit between January 2005 and March 2011 meeting the following criteria were retrospectively included: (1) jaundice <3 months; (2) DF ≥32 at admission; (3) total bilirubin >50 μmol/l; (4) active drinking; (5) AH on LB. Exclusion criteria: (1) advanced hepatocellular carcinoma; (2) other etiologies of cirrhosis; (3) severe co morbidities; (4) DF <32 after stabilization. When severe AH was diagnosed, patients were treated with prednisolone. Lille model was retrospectively assessed.

**Results:** 125 pts met the inclusion criteria; 5 died before corticosteroids therapy. 2 patients were not treated with corticosteroids. 188 patients were included (74 [62.7%] with GIB and 44 [37.3%] without GIB, mean age 54.8±9.3 yr, MELD score 24.7±5.6, Child–Pugh score 11.4±1.7, DF 64.4±29.3). Demographic data were not different in patients with or without GIB. Despite comparable MELD, Child–Pugh score and DF, INR (2.6±0.9 vs 2.1±0.6, p<0.10–3) and bilirubin (145±126 μmol/l vs 211±133, p=0.008) were statistically different in patients with or without GIB. Prednisolone was started 5 (3–6) days after admission in case of GIB. Actuarial probabilities of survival were similar in patients with/without GIB at day-28 (91.8±0.03 vs 87.2±0.05%, p=0.53), and month 3 with a trend towards better survival in patients with GIB (80.7±0.05 vs 65.9±0.07%, p=0.01). AUC of Lille model was 0.76±0.07 in patients without GIB and 0.75±0.06 in patients with GIB. In multivariate analysis, independent factors associated with 3-month survival were Lille model and development of infection (p<10–3).

**Conclusion:** Although not significant, there was a trend towards better 3-month survival in patients with severe AH and GIB, as compared to patients without GIB. Lille model had a good prognostic value in patients with GIB.
POSTERS

Results: OB was significantly higher in AAH in resting neutrophils compared to HC (p < 0.05) and following stimulation with fMLP (p < 0.05). Compared to HC, baseline and stimulated TLR2 expression was reduced in the AAH group (both p < 0.01). TLR4 expression tended to be lower at baseline compared to HC but following stimulation was significantly reduced (p = 0.02). Neutrophils from AAH patients failed to upregulate TLR2 and TLR4 expression appropriately in response to stimulation with lipopolysaccharide.

Figure demonstrating reduced baseline and stimulated (0.2 ng/mL lipopolysaccharide) neutrophil TLR 2 and TLR 4 expression in patients with acute alcoholic hepatitis (AAH) compared to healthy controls.

Plasma GCSF/GM-CSF was below the limit of detection in AAH but levels of IL-6 and IL-8 were elevated compared to HC. Plasma IL-8 (a potent neutrophil chemo-attractant) was higher in non-survivors (n = 3/15; p = 0.02).

Conclusion: Circulating neutrophils in AAH demonstrate reduced TLR 2/4 expression and fail to appropriately up-regulate TLRs in response to bacterial challenge. This suggests that neutrophils become endotoxin tolerant contributing to the increased susceptibility to infection. Paradoxically, neutrophils show increased OB at baseline and following stimulation which may underlie their role in hepatic inflammation and the organ dysfunction seen in AAH.

548 LONG-TERM OUTCOMES OF PATIENTS WITH ALCOHOL-RELATED CIRRHOSIS ADMITTED TO A GENERAL INTENSIVE CARE UNIT AT A TERTIARY HOSPITAL IN THE UNITED KINGDOM

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Background and Aims: Patients with cirrhosis admitted to intensive care unit (ICU) have been shown to have a poor prognosis. A recent study from a non-transplant ICU setting in the UK reported much better ICU (38%) and hospital (47%) mortality compared to transplant ICUs, attributed to less severe disease in their patient cohort. However, whether this is sustained long term is unclear. We evaluated the early to1 year outcomes of patients with cirrhosis admitted to a general ICU.

Methods: Retrospective analysis of the ICU admissions database of all patients admitted to the Royal Liverpool Hospital from July 2003 to September 2011. For readmissions, only the first episode was included. Comparisons between survivors and non survivors and between patients with alcoholic and non-alcoholic cirrhosis were performed using the Mann-Whitney test for continuous variables and either the Chi-squared test or Fisher’s exact test for categorical variables.

Results: There were 4178 admissions, 135 (3.2%) with cirrhosis (87% from alcohol and 13% from other causes). 57% of the cirrhotic patients were male, with a median (IQR) age of 51 (42–59) years, Model for End Stage Liver Disease (MELD) score of 21.1 (14.9–28.4), Acute Physiology and Chronic Health Evaluation II (APACHE II) score of 18 (14.8–24.0), and ICU length of stay of 3.9 days (1.6–10.4). Unit, hospital, 6-month and 1 year mortality was 54.8%, 64.4%, 66.7% and 70.4% respectively. Compared to hospital survivors, non-survivors had higher admission MELD scores (25.3 [19.3–31.2] vs. 15.1 [11.4–21.5]; p < 0.001) and APACHE II scores (20 [16–25] vs. 15 [11–20]; p < 0.001), and more had received renal replacement therapy (31.4% vs. 10.4%; p = 0.013). More survivors presented with variceal bleeding compared to non-survivors (31.3% vs. 20.7%; p = 0.2). Although unit mortality was similar between patients with alcoholic cirrhosis and non-alcoholic cirrhosis (54.7% vs. 55.5%), at 1 year, 19% of survivors with alcoholic cirrhosis had died compared to none with non-alcoholic cirrhosis.

Conclusion: Outcomes in ICU admissions with cirrhosis in our cohort are comparable to previously published figures. The main determinants of early mortality are disease severity, but not aetiology. A significant proportion of survivors with alcoholic cirrhosis die after hospital discharge.

549 INHIBITION OF p53 MITIGATES ETHANOL-INDUCED TIGAR UPREGULATION, REDUCTIVE STRESS AND SUBSEQUENTLY IMPROVES HEPATIC INSULIN SIGNALING IN THE LIVERS OF ETHANOL-FED LONG–EVANS RATS

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Background and Aims: Tp53-Induced Glycolysis and Apoptosis Regulator (TIGAR) is a downstream target of p53 that is upregulated in the livers of chronically ethanol-fed Long–Evans rats. TIGAR is a bisphosphatase that depletes hepatic fructose-2,6-bisphosphate and subsequently increases the cellular NAD(P)H/NAD(P)+ ratio. We hypothesized that ethanol-induced activation of p53, followed by a TIGAR-mediated increase in reducing equivalents enhances ethanol-induced reductive stress, which subsequently abrogates the activation of mTORC2, an enzyme complex responsible for the phosphorylation of AKT. Our aim was to assess the effect of the p53 inhibitor – pifithrin-α p-nitro (PFT) – on TIGAR upregulation, oxidative/reductive stress, hepatic insulin resistance and subsequent liver injury in chronically ethanol-fed LE rats.

Methods: Male LE rats were fed ad libitum with control diet or an ethanol-containing liquid diet for 10 weeks. The rats were i.p. injected with 0.8 mg/kg PFT or DMSO vehicle three times per week. Serum, liver and colon samples were collected for histological, biochemical assays and Western-blotting. Additionally, mitochondria were used to assess oxygen consumption, as a measure of oxidative stress-mediated mitochondrial damage.

Results: PFT treatment abrogated serum ALT elevation suggesting that PFT diminished liver injury. Furthermore, in situ oligoligation assay detected fewer cells harboring apoptotic DNA in the livers of ethanol-fed, PFT-injected rats compared to their control counterparts. PFT treatment significantly decreased ethanol-induced oxidative stress, as evidenced by diminished protein carbonylation and improved mitochondrial oxygen consumption. Inhibition of the transcriptional activity of p53 did not alter the total cellular abundance or non-transcriptional functions of p53; however, it significantly decreased the transcriptional activity of p53 and prevented the ethanol-induced upregulation of TIGAR. Subsequently, the level of reductive stress was significantly lower in the ethanol-fed, PFT-injected rats, as demonstrated by the decreased reduced/oxidized thioredoxin-2 ratio. Concordantly, PFT
improved the mTORC2-mediated phosphorylation of AKT at Ser\textsuperscript{473} in the ethanol-fed, PFT injected animals.

**Conclusions:** Inhibition of p53 improved liver injury, apoptosis, oxidative and reductive stress as well as insulin/PI3K/AKT signaling in the livers of ethanol-fed LE rats. This observation underlines the importance of p53 in the pathogenesis of ALD. This study also identifies TIGAR that may mediate ethanol-induced hepatic insulin resistance.

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**TIGAR PROMOTES INSULIN RESISTANCE BY INCREASING REDUCTIVE STRESS IN ETHANOL-TREATED HUMAN HepaRG CELLS**

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**Background and Aims:** Tp53-Induced Glycolysis and Apoptosis Regulator (TIGAR) is a downstream target of p53 that has been shown to be upregulated in the livers of ethanol-fed Long–Evans rats. TIGAR is a bisphosphatase that depletes hepatic fructose-2,6-bisphosphate (F26BP) and subsequently increases the cellular NAD(P)/H/NAD(P)\textsuperscript{+} ratio. The abundance of TIGAR has been found to correlate with the severity of hepatic insulin resistance in rats. The aims of this study were to demonstrate the causative role of TIGAR in ethanol-induced hepatic insulin resistance and elucidate the mechanisms by which TIGAR upregulation may lead to suppressed phosphorylation of AKT in response to insulin stimulation. We hypothesized that TIGAR may further enhance ethanol-induced reductive stress, resulting in ribosomal protein aggregation and thus insufficient ribosomal binding/activation of mTORC2, which is an enzyme complex responsible for the phosphorylation of AKT at Ser\textsuperscript{473}.

**Methods:** Full-length human TIGAR cDNA was overexpressed in HepaRG liver cells and the levels of F26BP, NAD(P)/H/NAD(P)\textsuperscript{+} were measured. The insulin/PI3K/AKT pathway was stimulated with 10 minutes of 100 nM insulin treatment in the TIGAR-overexpressing or empty vector-transfected cells, in the presence or absence of 50 mM ethanol. In order to demonstrate that TIGAR-mediated suppression of hepatic insulin signaling involves reductive stress, we used well-known inhibitors of reductive stress, such as β-lapachone or 2-deoxyglucose. The interaction between ribosomal proteins and mTORC2 was analyzed by immunoprecipitation using anti-Rpl26 antibody.

**Results:** TIGAR overexpression significantly lowered F26BP levels and markedly increased the NAD(P)/H/NAD(P)\textsuperscript{+} ratio in HepaRG cells. TIGAR-mediated reductive stress was significantly decreased by β-lapachone and 2-deoxyglucose. TIGAR overexpression further facilitated the ethanol-mediated suppression of AKT phosphorylation as Ser\textsuperscript{473} after insulin stimulation. β-lapachone treatment successfully restored insulin sensitivity in the TIGAR-overexpressing, ethanol-treated HepaRG cells by facilitating the mTORC2-Rpl26 interaction and increasing the activity of mTORC2.

**Conclusions:** TIGAR has a causative role in hepatic insulin resistance, which involves augmented reductive stress by promoting increased generation of NAD(P)/H. This biochemical event further enhances ethanol-induced reductive stress, ribosomal protein aggregation and thus decreases mTORC2 activity to phosphorylate AKT in response to insulin stimulation. Our study identifies TIGAR as a potential target to improve insulin signaling in alcoholic liver disease.

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**A PROTEOMIC ROAD MAP OF THE HepaRG CELL LINE: METABOLIC PATHWAYS PRESENT AND THEIR RESPONSE TO ACETAMINOPHEN TREATMENT**

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**Background and Aims:** The NOTOX project (notox-sb.eu) aims at generating large-scale-omics data and in silico prediction of drug induced hepatotoxicity using human cellular in vitro systems. The selected cell line, HepaRG, was already shown to exhibit many features of a possible surrogate for primary human hepatocytes for the use during in vitro toxicity studies. However, a more comprehensive overview of the pathways active in these cells based on proteomics data and their response to drug treatment is still lacking. Here, we present a road map of metabolic pathways present in HepaRG and examine the acute toxic effects of acetaminophen (ACAP) treatment on the proteome.

**Methods:** HepaRG cells were cultivated for 20h and treated with either 5mM or 15mM acetaminophen. Cellular proteins were extracted and separated by SDS-PAGE. After systematic excision of protein bands and in-gel digestion, proteins were identified using liquid chromatography coupled to mass spectrometry (LC-MS) and subsequent protein database search using the Mascot algorithm. In order to quantify global proteome changes, a label-free relative quantitation based on spectral counting was performed. Pathway analysis and functional annotation (Gene Ontology) was performed using Ingenuity pathway analysis\textsuperscript{12} and a home developed interface (msda.unistra.fr).

**Results:** Approximately 1500 proteins were identified across all different conditions. Many proteins of pathways predominately active in hepatocytes were identified, e.g. glycogen metabolism, ammonia detoxification, lipid metabolism as well as drug detoxification. 348 proteins showed significant changes upon ACAP treatment compared to the control. These proteins indicate ongoing impairment of mitochondrial function, activation of retinoic X receptor (RXR) and glutathione depletion in a dose-dependent manner.

**Conclusion:** The HepaRG cell line expresses many proteins indicative of a hepatocyte-like phenotype. The response to ACAP treatment resembles the situation described for primary hepatocytes. In addition to its unlimited availability and higher reproducibility in terms of gene/protein expression, this makes HepaRG a suitable model for in vitro toxicity studies. In-depth systems biological characterisation of the HepaRG cell line within the NOTOX consortium will then further assist the understanding of drug induced hepatotoxicity.

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**CIRCULATING NEUTROPHIL GRANULE SUBSET RELEASE IN RESPONSE TO BACTERIAL STIMULUS IS AUGMENTED IN PATIENTS WITH ALCOHOL-RELATED LIVER DISEASE AND MAY CONTRIBUTE TO ORGAN BYSTANDER DAMAGE**

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**Background and Aims:** Alcohol-related liver disease (ALD) represents a growing burden of disease in the UK amidst burgeoning drinking behaviours. Susceptibility to infection remains a concern in acute alcoholic hepatitis (AAH) and ALD precipitating
multiple organ failure with high mortality. Neutrophils are abundant innate immune cells and function via controlled release of their primary, secondary and secretory granule subsets with specific targeted functions. The aim of this study was to characterise neutrophil granule phenotype and function, comparing patients with ALD/AAH against healthy controls (HC) to interrogate whether granule dysfunction contributes to the susceptibility to infection.

Methods: A case–control study was undertaken comparing peripheral blood neutrophils from patients with advanced [Child Pugh B/C] ALD (n = 15) and AAH (n = 5) against HC (n = 7).

Results: Circulating neutrophils from ALD patients had increased expression of the surface endothelial adhesion marker CD11b at baseline (p = 0.04) with augmented intracellular and extracellular responses to stimulation with fMLP. Primary granules were also upregulated at baseline (p = 0.04) and demonstrated exaggerated responses to stimulation (p = 0.03). ImageStream flow cytometry following incubation with FITC-labelled E. Coli confirmed robust granule co-localisation of CD11b, CD16, CD63, myeloperoxidase and CD66b with phagocyted E. Coli similar to that of HC. Plasma lactoferrin (released from secondary granules) and interleukin-8 (potent neutrophil-attracting chemokine) were elevated across the spectrum of ALD, most marked in AAH (p = 0.04 and p = 0.001, respectively).

Conclusions: Circulating neutrophils in patients with ALD/AAH are pre-primed, with increased endothelial adhesion markers and augmented intracellular granule mobilisation and extracellular release. Impaired granule mobilisation does not appear to contribute to the functional immunoparesis observed in patients with ALD/AAH but may contribute to bystander damage (myeloperoxidase) inducing systemic inflammation and organ dysfunction.

553 LEPTIN ADMINISTRATION REGULATES HEPATIC CHOLESTEROL SYNTHESIS IN A MOUSE MODEL OF ALCOHOLIC FATTY LIVER DISEASE

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Alcohol induced fatty liver disease is the most common and earliest response to the progression of fibrosis and/or cirrhosis. The mechanism by which ethanol causes fatty liver disease is complex and not fully understood, however, enhanced hepatic lipogenesis has been proposed as an important biochemical mechanism. The purpose of this study was to evaluate the effect of exogenous leptin administration on ethanol induced hepatic cholesterol synthesis in mice.

Methods: CD-1 mice (n = 10/group) were studied for 45 days. Four groups were studied.

1. control, 2. leptin+control (230 mg/kg intraperitoneal every alternate day from day 15), 3. alcohol (6.32 g/kg daily by gastric lavage, for 45 days) and 4. alcohol plus leptin (as prior dosing).

Results: Compared to control, ethanol supplementation significantly (p<0.05) increased levels of plasma total and ester cholesterol and the activities of the enzymes HMG CoA reductase and cholesterol ester synthase (CES) which were normalized by addition of leptin (p<0.05). Increased SREBP2 protein expression found in ethanol fed mice was also normalised by leptin treatment. The activities of hepatic lipoprotein lipase (LPL), plasma lecithin cholesterol acyl transferase (LCAT) and tissue cholesterol ester hydrolyase (CEH) were significantly (p<0.05) lowered following ethanol supplementation compared to control mice. These features were significantly increased by addition of leptin. Furthermore, significantly increased excretion of total bile acids and neutral sterols were observed on leptin administration to ethanol fed mice. Liver histology showed that mice given ethanol had macro and micro vesicular steatosis. However, ethanol + leptin treated liver showed sinusoidal dilatation and no fatty change. Leptin injection in control mice showed mild sinusoidal dilatation and normal hepatocytes.

Conclusion: Thus, administration of exogenous leptin to ethanol-supplemented mice markedly decreased the synthesis and increased the catabolism of cholesterol in the liver.

554 EFFECTS OF URSODEOXYCHOLIC ACID AND L-ORNITHINE-L-ASPARTATE ON HEPATOCYTES CHANGES IN ALD PATIENTS

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Methods: In 48 ALD patients [age, 44.2±6.8 yr] with alcoholic hepatitis and 12 healthy volunteers [age, 44.3±5.5 yr] circulating type IV collagen and basic fibroblast growth factor (bFGF) levels, electromicroscopic, morphometrical, cytogenetic parameters of the hepatocytes and hepatocytes nuclei were determined. 26 ALD patients treated with 10 mg/kg/day of UDCA (I group), 22 ALD patients treated with 10 mg/kg/day of UDCA+L-ornithine-L-aspartate 9 g/day for six months (II group).

Results: After therapy the condensed chromatin content was decreased in hepatocytes in II group (p<0.05). The quantity of the pathologically changed nuclei was decreased more than 2.5 times and 1.8 times (p<0.05) in II and I groups vs. patients before treatment. In II group the area of the hepatocytes profile (AHP) was increased up to (165.15±11.88) μm2 vs. (119.65±10.53) μm2 (p<0.05); in I group – (139.65±11.53) μm2 vs. (120.07±10.26) μm2 (p<0.05) before treatment and decreased vs. (182.17±2.54) μm2 (p<0.05) in control. After therapy 5 (22.73%) patients in II group and 13 (46.4%) in I group have AHP from 50.0 to 150.0 μm2 vs. 18 (81.8%) and 21 (80.76%) patients before treatment; in healthy volunteers AHP was from 100.0 to 250.0 μm2 (85.42%). The area of the nuclear profile of patients in II group was increased – up to (25.28±1.84) μm2 vs. (16.96±1.02) μm2 (p<0.05), in I group – (21.70±1.25) μm2 vs. (17.35±1.19) μm2 (p<0.05) before treatment and decreased vs. (35.32±0.60) μm2 in control. The circulating type IV collagen levels decreased more than 1.8 times and 1.4 times (p<0.05), bFGF levels decreased more than 2.2 times and 1.6 times (p<0.05) in II and I groups vs. patients before treatment (P<0.02).

Conclusions: The UDCA+L-ornithine-L-aspartate combination in long-term use has antifibrotic effects with beneficial cytoprotective properties and improves electromicroscopic, morphometrical and cytogenetic markers of the hepatocytes and hepatocytes nuclei in ALD patients.
ALCOHOL WITHDRAWAL ALLEVIATES SUBCUTANEOUS ADIPOSE TISSUE INFLAMMATION IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

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Background and Aims: Patients with alcoholic hepatitis display increased level of inflammation in the subcutaneous adipose tissue (SAT) which positively correlates with liver histological lesions. In this study, we assessed the cytokines and chemokines involved in this process and examined the effect of alcohol withdrawal on cytokine/adipokine expression, macrophage infiltration and polarization in the SAT of alcoholic patients.

Patients and Methods: Forty-seven patients with different stages of alcoholic liver disease (ALD) underwent liver biopsy at inclusion for the investigation of cytokines and liver histology. SAT and blood samples were collected at inclusion and after one week of alcohol withdrawal. Cytokine, chemokine and adipokine expression levels, macrophage infiltration and polarization were assayed in liver at inclusion and in SAT at inclusion and after one week of alcohol withdrawal. Adipokine serum levels were also investigated.

Results: Production of chemokines involved in the recruitment of immune cells (osteopontin, IL8) was an early event in ALD and began in both the liver and SAT as soon as the steatosis stage. In patients with mild and severe ALD, one week of alcohol abstinence decreased production of proinflammatory cytokines/chemokines (IL18, CCL2, osteopontin, semaphorin 7A) and oriented adipose tissue macrophages (ATM) towards an anti-inflammatory M2 phenotype. In patient with mild ALD, one week of abstinence was sufficient to obtain a decrease in ATM infiltration. Adiponectin production and serum concentration, which were negatively correlated with steatosis and fibrosis scores, decreased after one week of abstinence. There was also a decreased expression level of adiponectin receptor 1 and 2 in SAT.

Conclusions: Adipose tissue inflammation is an early event in ALD and correlates with liver histological lesions. Adipokine dysregulation may participate to the harmful effect of adipose tissue in ALD. One week of alcohol withdrawal orients ATM towards a M2 phenotype and alleviates SAT inflammation, suggesting the direct role of alcohol in SAT inflammation.
01a. LIVER TRANSPLANTATION/SURGERY: EXPERIMENTAL

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THE EFFECT OF ISCHAEMIA–REPERFUSION INJURY ON THE MORPHOLOGY OF BILIARY EPITHELIAL CELL MICROVILLI AND BILE REGULATION IN VIVO

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Introduction: Both warm ischaemia and altered bile are implicated in the development of non-anastomotic biliary strictures following liver transplantation. However a causal link between warm ischaemia and altered bile composition remains to be proven. Our aim was to examine the effects of ischaemia-reperfusion injury (IRI) on bile homeostasis and on BEC microvilli in vivo.

Methods: Sprague Dawley rats were divided into two groups (n = 30 per group): IRI group subjected to 90 minutes of left hepatic ischaemia followed by reperfusion and a sham-operated group. Bile flow was measured and liver tissue was harvested at various time points (3hrs up to 28 days post IRI) in addition to serum and bile collection for biochemical analysis. Liver tissue was examined using transmission electron microscopy to assess the morphology of BEC microvilli.

Results: The IRI group demonstrated significantly raised serum bile acids and alkaline phosphatase which peaked on day 1 and 3 respectively. Bile flow was significantly lower in the IRI group immediately following reperfusion and normalised by day 3 compared to the sham group. Biliary bile acid concentration was significantly higher in the IRI group on day one. A delayed rise in bile acids and alkaline phosphatase which peaked on day 1 and 3 respectively. Biliary bile acid concentration was significantly higher in the IRI group on day one. A delayed rise in bile acids and alkaline phosphatase which peaked on day 1 and 3 respectively.

Conclusion: This study demonstrates that IRI results in changes to the composition of bile which may play an important role in the development of biliary strictures. It also results in persistent changes to BEC microvilli suggesting an adaptive response to a prolonged pathology.

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IL-4 AND IL-13 OVER-EXPRESSION IN SEVERE RECURRENT HEPATITIS C AFTER LIVER TRANSPANTATION

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Hepatitis C frequently recurs after liver transplantation, resulting in accelerated progression toward fibrosis. The mechanisms underlying accelerated liver fibrosis after HCV recurrence are poorly understood but immunological factors are probably involved. Interleukin (IL)-4 and IL-13 have been shown to induce fibrogenesis using in vitro models.

To determine the role of IL-4 and IL-13, in the accelerated progression of fibrosis in recurrent hepatitis C after liver transplantation, we have retrospectively evaluated the in situ expression of IL-4 and IL-13 in transplanted patients with or without hepatitis C recurrence by using immunohistochemistry followed by semi-quantitative analysis of positive cells.

Fifteen patients have been analysed, five with severe recurrent hepatitis C (Metavir: ≥F2) have been compared with 5 patients with minimal recurrence (≤F1) and with 5 stable HCV negative transplanted patients.

IL-4 and IL-13 in situ expression was low in transplanted patients without HCV recurrence (2.3±0.7, 1.2±0.58 respectively), was significantly increased in mild HCV recurrence and further increased in severe recurrence, (for IL-4 23.4±2.29 versus 7±1.12, p<0.0001; for IL-13 23.9±5.18 versus 5±1.21, p=0.001) IL-4 and IL-13 protein were overexpressed in graft recipients with severe recurrent hepatitis C.

In conclusion, IL-4 and IL-13 expression is upregulated in severe recurrent hepatitis C, and may play a central role in the progression of hepatic lesions, particularly in fibrosis after liver transplantation. An immunointervention planned to inhibit IL-4 and IL-13 pathway could be helpful in the treatment of recurrent hepatitis C after liver transplantation.

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HCV KINETICS DURING AND AFTER THE ANHEPATIC PHASE: IS THE LIVER THE PRIMARY SITE FOR HCV CLEARANCE?

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Background and Aims: The role of the liver in clearance of circulating HCV is not known. Previous HCV kinetic studies during liver transplantation have suggested that HCV is cleared at approximately the same rate during the anhepatic phase (ANH) and during liver transplantation.

The role of the liver in clearance of circulating HCV is not known. Previous HCV kinetic studies during liver transplantation have suggested that HCV is cleared at approximately the same rate during the anhepatic phase (ANH) and during liver transplantation. However, these analyses were based mainly on HCV measurements that were limited to the beginning and end of ANH and 4h post RP. Here, we analyzed HCV kinetics based on very frequent sampling during the ANH and RP.

Methods: A detailed investigation was performed in five patients (P1–5). All received methylprednisolone at RP. Blood samples were taken every 5–15 minutes during the AHP and 4h post RP. We calculated the viral slope during the ANH and during the first 4h post RP, which represents an eclipse-phase in which the new liver does not release virions. Viral slope was considered flat/plateau if the slope was not significantly (p<0.05) different from 0.
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Results and Conclusions: During ANH (range 1.25–1.90h) virus levels were flat in three patients (P2, P3 and P4) while in two patients (P1 and P5) the serum HCV half-lives, t1/2, were 4.4h and 2.2h, respectively. A viral plateau during ANH may indicate a static viral equilibrium i.e., no production and clearance. Interestingly, P1 and P5 received the largest volume of albumin (500 and 750 ml, respectively), and blood (P1=450 ml) during ANH. During RP, P1 experienced an extremely-slow viral decline compared to the others (t1/2=26.7h vs. 2.1h (P2), 2.1h (P3), 2.9h (P4) and 1.8h (P5)). In P2, P3 and P4, the transition from viral plateau to a significant viral decline, suggests a major role of the liver in virus clearance. Finally, if one computes t1/2 in all patients, from the current study, with only two HCV measurements (i.e., at the beginning and end of ANH), t1/2=1.3±0.4h. Therefore, the results indicate that previous studies overestimated the HCV-RNA t1/2 during ANH.

Acknowledgements: NIH grants: R56/R01-AI078881 and P20-RRO18754, Germany Academic Exchange Service (DAAD) and U.S. DOE contract DE-AC52–06NA25396.

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SEMI-AUTOMATIC ANALYSIS OF THE BRANCHING TOPOLOGY AND GEOMETRICAL CHARACTERISTICS OF HEPATIC VASCULAR TREES

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Introduction: Computational models for hepatic flow, based on a precise knowledge of the hepatic vasculature, could be useful to better understand liver flow under normal and pathological conditions, and could be applied for liver surgery and transplantation. Previously, we studied the branching patterns and geometrical characteristics of liver circulation in individual human and rat livers based on a labour-intensive manual inventory method. As an alternative, we designed a semi-automatic method to analyse the vascular anatomy of hepatic vascular trees.

Methods and Results: After vascular corrosion casting and high resolution micro-CT scanning of the human hepatic vasculature (liver graft that failed rescue allocation for transplantation), all vascular trees (arterial, portal, hepatic venous) of the 3D dataset were segmented. Subsequently, an automatic vessel tracking algorithm was initialized for every tree by identifying its inlet vessel(s) to calculate the vascular tree centerlines. After typically 3 or 4 automated runs to track all vessels and post-processing to correct for small artifacts, a graph structure is provided that represents the tree skeletons (Fig. 1a) and carries information on the connectivity, length and diameter of all blood vessel segments. A vessel ordering algorithm (based on the (diameter-defined) Strahler system) allowed quantifying the branching topology and the corresponding geometrical characteristics of the vessel elements (series of vessel segments having an equal generation number). The result of this semi-automated vessel analysis are illustrated in Fig. 1b for the liver studied, showing the vessel generations of the macrocirculatory hepatic arterial tree, having terminal vessels of generation 1 going up to generation 6 at the inlets.

Conclusion: Semi-automatic vessel tracking and ordering enables the quantitative anatomical analysis of complex branching structures such as hepatic vascular trees. This technique can be used to make an inventory of vascular tree architectures and to develop computational perfusion models to better understand liver flow and hemodynamics under normal and pathological conditions (e.g. cirrhosis), which may be helpful in the context of surgical planning and transplantation.

Acknowledgment: This research was supported by the Agency for Innovation by Science and Technology in Flanders (IWT), Belgium.

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OAS AND IL-28b GENETIC POLYMORPHISMS IN RELATION TO HCV RECURRENCE POST LIVING RELATED LIVER TRANSPLANTATION

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Background and Aims: The influence of HCV infection on allograft histology is highly variable. Several factors have been suggested to accelerate HCV re-infection of the allograft. Genetic variants of oligoadenylate synthetase (OAS) have been reported to influence response to antiviral treatment and to virus-related disease progression. Interleukin-28b (IL-28b) plays a role in immune defense against viruses. Its genotype C/C is associated with viral clearance during acute HCV infection. The sharp decline in the C/C genotype from healthy subjects till its absence in end stage liver disease suggests a central role of this genotype against HCV disease progression. We aimed in this study to confirm the role of OAS and IL-28b in prediction of HCV recurrence in post transplant recipients.

Methods: This study was conducted on fifty adult patients with HCV related liver cirrhosis who underwent living donor liver transplantation at El-Sahel Teaching Hospital, Cairo, Egypt. Patients with etiologies for cirrhosis other than HCV were excluded. Patients were subjected to detailed assessment and laboratory work up. Testing for genetic variants of OAS and IL-28b were performed. Results were interpreted in relation to disease free survival (DFS).
Factors Predictive of Tolerance after Liver Transplantation

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Liver transplant recipients may develop immunological tolerance, but there are no established ways to identify tolerant patients before they withdraw immunosuppressive therapy. We tried to find factors predictive of tolerance in a series of patients who underwent a gradual reduction of immunosuppression.

Patients and Methods: Twenty-four adult liver transplant recipients with side effects of immunosuppression underwent a gradual reduction of their immunosuppressive therapy until withdrawal or alteration of liver function tests. Tolerance was defined as complete immunosuppression withdrawal while maintaining normal liver function tests. The baseline clinical and immunological characteristics of tolerant and non-tolerant patients were compared. The immunological characteristics studied were lymphocyte count and lymphocyte subpopulations (T, B, NK, CD4+, CD8+, Treg), and stimulation index (SI) (the ratio between lymphocyte proliferation after stimulation with phytohaemagglutinin and proliferation without stimulus).

Results: Among the 24 evaluable patients, 15 (62.5%) were tolerant. The median time of follow-up after complete immunosuppression withdrawal was 12 months (IQR 6.5–20.5). Tolerant patients had a longer interval between transplantation and immunosuppression withdrawal (median 156 versus 71 months; p=0.003) and a lower SI (median 7.49 versus 41.73; p=0.01) than non-tolerant patients. When the series was divided in groups: time from transplantation >10 years (n=12) or <10 years (n=12) and SI >20 (n=12) or SI <20 (n=12), three groups were defined. Group A (>10 years and SI <20; n=7): tolerance in 100%. Group B (>10 years and SI >20 or <10 years and SI <20; n=10): tolerance in 60%. Group C (<10 years and SI >20; n=7): tolerance in 28%.

Conclusions: A high proportion of liver transplant recipients are tolerant in the long term. The combination of two simple variables (time from transplantation and stimulation index) may be useful to predict immune tolerance.

Hepatic Inflow Modulation after Extended Liver Resection via Shunt Surgery

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Background: One of the most challenging issues after extended liver resection is decrease of the hepatic artery flow and increase of the portal vein flow and pressure in the remnant liver with the risk of small for size syndrome (SFSS). The aim of this study was to evaluate the role of shunt surgery in hepatic inflow modulation after extended liver resection.

Methods: 24 pigs were divided into three groups: Group A: 75% liver resection without shunt (n=8); Group B: 75% liver resection with side to side portocaval shunt (PCS S–S); Group C: 75% liver resection with side to portocaval shunt (PCS E–S). The flow of the hepatic artery (HAF) and portal vein (PVF) in relation to the 100 gram remnant liver as well as the pressure of the portal vein (PVP) were measured and compared between the groups.

Results: In Group A, extended liver resection (75%) decreased the HAF (20%) and increased the PVF (119%) and PVP (306%). In Group B, the PCS S–S following extended liver resection (75%) could increase the HAF (12%) and decrease the PVF (78%) and PVP (48%) in comparison to Group A. In Group C, the PCS E–S following extended liver resection (75%) could increase the HAF (44%) and decrease the PVP (38%) in comparison to Group A.

Conclusion: Portocaval shunt can increase the dropped HAF and reduce the increased PVF and PVP, after extended liver resection. In increasing the HAF after extended liver resection, the PCS E–S seems to be more effective than PCS S–S. In case of the risk of SFSS, the hepatic inflow modulation after extended liver resection through portocaval shunt might prevent the consequent postoperative complications.

A Novel Recombinant Form of the Human Manganese Superoxide Dismutase Protects Rat Liver Grafts Procured for Transplantation

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Introduction: Ischemia–reperfusion during liver transplantation causes hepatic injury and early graft dysfunction. The mechanisms involved include vascular dysfunction, inflammation and oxidative stress. Recently a new recombinant human manganese superoxide dismutase (rMnSOD) has been generated. This protein form freely enters the cells and is constitutively active. rMnSOD represents a new pharmacological strategy against oxidative stress. The present study aimed at evaluating the protective effects of rMnSOD on the hepatic and endothelial function and viability of liver grafts obtained from healthy rat donors undergoing cold storage and warm reperfusion injuries.

Methods: 1. Effects of rMnSOD on oxidative stress levels (DHE staining) and NO bioavailability (DAF staining) were tested in freshly isolated liver sinusoidal endothelial cells (SEC) preserved in cold storage conditions.

2. Rats were intravenously treated with rMnSOD, or its vehicle, 30 minutes before liver graft harvesting and preservation in Celsior solution for 16h. Afterwards, grafts were warm reperused for 1h and hepatic injury (ALT, AST, LDH), endothelial function (vasorelaxation response to acetylcholine), antioxidiant capacity (SOD activity), oxidative stress (O2–, ONOO−), inflammation (ICAM-1), and nitric oxide bioavailability (cGMP and NOx) were evaluated.
3. Antioxidant capability of rMnSOD as a supplement of a commercially available preservation solution was evaluated in hepatic biopsies cold stored for transplantation.

**Results:**
1. Cold storage induced a marked increase in O$_2^-$ levels and a decrease in NO bioavailability in SEC, those detrimental effects were abolished in cells preserved with rMnSOD.
2. In rats, administration of rMnSOD ameliorated hepatic injury and endothelial dysfunction derived from cold storage and warm reperfusion injuries. The beneficial effects of rMnSOD were associated with a reduction in hepatic O$_2^-$, ONOO$^-$ and inflammation together with an improved antioxidant activity and nitric oxide bioavailability.
3. rMnSOD added to a conventional preservation solution maintains its marked antioxidant activity avoiding oxidative stress formation in hepatic tissue preserved for transplantation.

**Conclusions:** This study demonstrates that rMnSOD markedly improves liver viability and endothelial function after cold ischemia and warm reperfusion. rMnSOD represents a new therapeutic strategy to protect liver grafts undergoing transplantation.

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**TLR4 INDUCTION BY TUDCA IN EXPERIMENTAL MODELS OF SYNGENEIC AND ALLOGENEIC STEATOTIC LIVER TRANSPLANTATION**

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**Background:** Numerous steatotic livers are discarded for transplantation because of their poor tolerance to ischemia/reperfusion (I/R). TUDCA protects steatotic livers under normothermic conditions but the responsible mechanisms are poorly understood. We examined whether the benefits of tauroursodeoxycholic acid (TUDCA) in steatotic liver grafts might be explained by changes in toll-like receptor 4 (TLR4).

**Material and Methods:** Syngeneic liver transplantation (LT) was performed using steatotic Zucker rats. Allogeneic LT was induced in Sprague Dawley and Wistar rats (were either choline-deficient or standard chow diet, respectively). This strain combination Sprague to Wistar is fully allogenic and results in acute liver transplant rejection.

**Results:** The biochemical and histological parameters of hepatic injury indicated that TUDCA protected steatotic grafts in experimental models of isograft and allograft steatotic liver transplantation. This was associated with TLR4 pathway induction. Indeed, TUDCA increased TRIF protein levels in steatotic isografts and allografts submitted to transplantation. The protein levels of MyD88 were unchanged in all groups. The benefits of TLR4 induction on hepatic I/R damage in steatotic isografts and allografts were evidenced using TLR4 agonists. Recipients transplanted with steatotic allografts without any treatment showed 30% survival at 14 days. The treatment with TUDCA and TLR4 agonist reduced lethality in recipients transplanted with steatotic grafts, and resulted in a 70% survival rate at 14 days in both (allograft and isograft) models.

**Conclusions:** Herein we show that TUDCA may play a beneficial role in the protection of steatotic grafts in experimental models of syngeneic and allogeneic steatotic LT. Our results report new properties of TUDCA in steatotic LT, based on a relationship between TUDCA and TLR4. We also describe the role of the TLR4 pathway in steatotic LT. TUDCA up-regulated the TLR4 pathway, specifically the TRIF pathway, protecting steatotic liver grafts in experimental models of syngeneic and allogeneic steatotic LT. The results point to new possibilities for therapeutic interventions based on TLR4 signaling activation to protect steatotic liver grafts against damage associated with transplantation.

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**INTERFERON GAMMA INDUCIBLE PROTEIN (IP)-10 LEVELS IDENTIFY INDIVIDUALS WITH RAPID FIBROSIS AT 12 MONTHS POST-TRANSPLANTATION FOR HCV**

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**Introduction:** Recurrent HCV (rHCV) infection is universal post-transplantation and is associated with significant morbidity and mortality. Fibrosis (F) stage <2 at 12 months identifies patients with a slow fibrosis progression rate compared to patients with rapid fibrosis progression (F ≥2).

**Methods:** We hypothesised that plasma IP-10 (interferon gamma inducible protein 10) levels can distinguish between slow and rapid fibrosis progression at 12 months and predict development of F≥4 post-transplantation. Predictive ability was assessed using the area under the curve generated by receiver operator characteristic analysis (AUROC), logistic regression and Cox regression analysis.

**Results:** 133 patients (111 male) were included. IP-10 levels were lower in the slow fibrosis group compared to the fast fibrosis group (p < 0.0001). IP-10 correlated with fibrosis stage, necro-inflammatory score, and serum transaminases (<0.0001). IP-10 (AUROC = 0.95, p < 0.0001) and donor age (AUROC 0.73, p = 0.001) were strong predictors of F≥2 at 12 months. Using multivariate logistic regression analysis, IP-10 retained independent significance (p < 0.05). Diabetes (AUC 0.86, p < 0.0001), IP-10 (AUC 0.79, p < 0.0001) and donor age (AUC 0.65, p < 0.04) were strong predictors of F≥4. Multivariate Cox regression identified diabetes, IP-10 and donor age to be independent predictors of F≥4 (p < 0.04). An IP-10 level >163 mg/ml was associated with a shorter time to the development of F≥4 (p < 0.0001).

**Conclusions:** IP-10 is an independent predictor of F≥2 at 12 months and F≥4 in patients with rHCV post-transplantation. IP-10 levels could therefore help identify patients who require early anti-viral treatment.

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**STUDY OF HEPATITIS C VIRUS SUPERINFECTION AFTER LIVER TRANSPLANTATION BY ULTRA-DEEP PYROSEQUENCING**

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**Background and Aims:** Hepatitis C virus (HCV) chronic infection is the main indication for liver transplantation (LT). The limitation of organ supply has led to the use of grafts from extended-criteria donors, such as HCV-positive donors. In these particular cases, superinfection with two different viral populations occurs, leading to the dominance of one strain over the other. In a previous study, we examined early viral kinetics during superinfection by cloning and sequencing, using a limited number of clones (Ramirez S. et al., 2010). Thus, the aim of this study was to analyze HCV superinfection after LT by next-generation sequencing.

**Methods:** We included 6 HCV-infected patients who underwent LT with HCV-infected grafts. Serum samples were collected before (donor and recipient) and after LT (days 1 and 2, week 1, months 1, 4, 6 and 12) and analyzed by ultra-deep pyrosequencing (UDPS) using
the 454 GS-FLX platform (Roche). Viral complexity was measured by mutation frequency and genetic diversity.

**Results:** Successive expansions and contractions of quasispecies were observed, evolving in all cases towards a more homogeneous population, with a relatively low genetic variability. In patients 1, 3 and 5, the donor population outcompeted the recipient virus immediately after LT (day 1), whereas in cases 2, 4 and 6, the recipient virus overtook the donor’s. In all cases, the most complex viral population excluded the other and became dominant. In cases 1, 2, 4 and 6, minority mutants derived from the donor or the recipient were detected at various points after LT regardless of the final result of the in vivo competition. Interestingly, in case 2, viral coexistence lasted even after the first year after LT.

**Conclusions:** Our results show that during superinfection with a different HCV strain in the LT, the viral population with the highest diversity always outcompetes the other and becomes dominant. The exclusion of non-dominant can take place as early as the first day or after several months following LT. However, the excluded virus may remain as a minority population (even after 1 year) and could emerge if there were any changes in the environment.

**567 PROTECTIVE EFFECTS OF NF-kappaB INHIBITOR ON NEUTROPHIL-DEPENDENT ISCHEMIA/REPERFUSION LIVER INJURY IN RATS**

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**Background and Aims:** To investigated the effects of NF-kappa B inhibitor on expression of inflammatory media and infiltration of neutrophils after ischemia/reperfusion injury in rats.

**Methods:** The model of partial hepatic ischemia was established in Wistar rats. The rats were peritoneally injected with either prolen dithiocarbamates (Pro DTC, 15 mg/kg) or sterile saline in 15 min before the ischemia.

**Results:** In the untreated rats, the level of NF-kappa B P65 obviously elevated at 1h and peaked at about 3–6h after reperfusion. The transcription of TNF-α, MIP-2 and ICAM-1 and the release of serum TNF-α and MIP-2 significantly increased 3h after reperfusion. Capillary endothelial cells of the livers strongly expressed ICAM-1 12h after reperfusion. ProDTC treatment significantly decreased the content of NF-kappa B P65 in the liver in concurrence with the expression of TNF-α, MIP-2 and ICAM-1 gene as well as the activity of myeloperoxidase (MPO) at the corresponding time points reperfusion (P < 0.05). Administration of ProDTC resulted in a statistically significant decrease in AST, ALT, LDH (P < 0.05).

**Conclusions:** ProDTC protects the liver from ischemia/reperfusion injury by suppressing NF-kappa B activation and subsequent expression of proinflammatory mediators.

**568 ASCITES IN LIVER TRANSPLANT RECIPIENTS WITH RECURRENT HEPATITIS C IN THE ABSENCE OF ADVANCED FIBROSIS: IMPACT OF CRYOGLOBULINEMIA**

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**Background and Aims:** Experience shows that a subgroup of patients with recurrence of hepatitis C virus (HCV) infection after liver transplantation (LT) develop ascites, even in the absence of cirrhosis. Ascites have a deleterious impact on patients and graft survival. The mechanisms of ascites in these patients are unclear. The aim of this study was to determine whether HCV-associated cryoglobulinemia could be a predisposing factor for ascites in LT recipients with HCV recurrence.

**Methods:** Between 2000 and 2010, eighty-two patients with documented post-transplant HCV recurrence who survived more than one year were studied. There were 60 males and 22 females. Mean age 57±7 years. Pre-transplant refractory ascites and hepatocellular carcinoma were present in 21% and 55%, respectively. At the time of listing, the mean MELD score was 15±7. Serum cryoglobulinemia was systematically tested in all patients at least once during follow up. Protocol liver biopsies were systematically performed after LT at 1, 3 and 5 years or at shorter interval when needed.

**Results:** During the study period, 14 (17%) out of 82 patients with HCV recurrence developed ascites. When ascites occurred, biopsy showed fibrosis stage F0, F1, F2, F3 and F4 (METAVIR) in 7%, 29%, 36%, 21% and 7% respectively. No significant correlation was found between fibrosis stage and ascites. On univariate analysis, ascites was significantly associated with advanced donor age (p = 0.02), pre-transplant refractory ascites (p = 0.02), presence of perisinusoidal fibrosis on post-transplant biopsy (p = 0.02), positive cryoglobulinemia (p = 0.005) and post-transplant diabetes (p = 0.005). On multivariate analysis, logistic regression, only positive cryoglobulinemia was independently and significantly associated with post-transplant ascites (p = 0.005). The relative risk to develop ascites when cryoglobulinemia was positive was 5.9. All patients with ascites were HCV-RNA positive at the end of the study. Five-year survival was significantly lower in patients with ascites (75% vs 85%, p = 0.02). ohne

**Conclusions:** This study shows that, in patients with post-transplant HCV recurrence, the risk to develop ascites is markedly increased when cryoglobulinemia is positive, even in the absence of extensive fibrosis or cirrhosis. These findings suggest that cryoglobulinemia-associated microangiopathy could play a role in the development of ascites in this population.

**569 HUMAN-SCALE TRANSPLANTABLE LIVER GRAFT USING DECELLULARIZED WHOLE-ORGAN SCAFFOLD**

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**Background and Aims:** There is a need for new methods to promote recovery from organ failure and regenerative medicine is an option that should be considered. Recent approaches using synthetic scaffolds and decellularized tissue have achieved a more complex level of tissue organization in organs such as the urinary bladder and trachea, with some success in clinical trials. In this context, the concept of decellularization technology has been applied to produce whole organ-derived scaffolds by removing cellular content while retaining all the necessary vascular and structural cues of the native organ. And there is an urgent need to find its feasibility of human scale-up and biological alterations of the scaffold after implantation.

**Methods:** In this study, we demonstrate that this decellularization technology could be applied in a large animal model to develop a transplantable engineered liver graft. Decellularized liver scaffold was generated by the same procedure as we did in rats using trypsin and tritonX-100, which was connected to portal vein for inflow and inferior vena cava for outflow with artificial vessel grafts. Anti-
coagulants and antibiotics were applied prior to and during the surgery to prevent coagulation in the native matrix scaffold and infection after transplantation. Histological study was performed at different time points through day 7 to evaluate cell infiltration and adhesion around the scaffold.

**Results:** The decellularization technology could be scaled up in size. The liver graft was successfully transplanted in porcine body by vessel anastomosis without any leakage through day 7. Although histological study revealed massive adhesion with infiltration of inflammatory cells including lymphocytes and fibroblasts especially in the edge of the scaffold and the coagulations were not totally avoided in the transplanted graft, the graft was well perfused and preserved in the porcine abdominal cavity without bleeding or absorption.

**Conclusions:** Although it requires improvement and customization with regard to anti-coagulation and further applicable cell sources, we could scale-up and optimize the system to apply this unique technology for clinical applications.

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**IFN-α BLOCKADE PROTECTS THE LIVER FROM ISCHEMIA–REPERFUSION INJURY BY INHIBITING APOPTOSIS VIA REDUCED IN IRF-1 EXPRESSION**

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**Background:** Plasmacytoid dendritic cells (pDC) are comparatively abundant in the liver and are capable to produce large amounts of type-I IFN (IFN-α/β). We have shown that liver I/R injury stimulates hepatic pDC to produce IFN-α, and that pDC depletion protects the liver from I/R injury by reducing IFN-α production. Others have reported that type-I, but not type II, -IFN receptor KO mice are totally protected from liver I/R injury. IFN regulatory factor (IRF) 1 is a transcription factor, induced by type-I and II IFNs, that promotes cell apoptosis. IRF-1 deficiency is known to inhibit liver I/R injury by reducing expression of death receptors and death ligands by hepatocytes. We hypothesized that IFN-α blockade might protect the liver from I/R injury by reducing IRF-1 expression and consequently reducing hepatocytes apoptosis.

**Methods:** 8–10 week old male C57Bl/6 mice were subjected to liver I/R injury. After 6 hr reperfusion, the mice were euthanized and liver injury assessed by serum ALT and histological evaluation. For IFN-α blockade, anti-IFN-α Ab was injected intravenously before induction of ischemia. Expression of IRF-1, death receptors (Fas and DR5) and the death ligand (FasL) was evaluated in the liver by RT-PCR. To ascertain whether IFN-α blockade inhibited IRF-1 expression by hepatocytes in vitro, culture medium from freshly-isolated liver pDC following liver I/R was applied to hepatocytes with/without anti-IFN-α Ab. IRF-1 expression by hepatocytes was determined by western blot.

**Results:** Anti-IFN-α Ab administration significantly reduced serum ALT after liver I/R injury (1745 ±702 vs 770 ±178 IU/L). Anti-IFN-α Ab-treated mouse liver exhibited significantly less necrosis determined by HE staining (4.9±1.2 vs 0.2±1.8%) and less apoptotic hepatocytes by TUNEL staining (40.2±55.4 vs 18.6±16.8%). Anti-IFN-α Ab treatment significantly reduced IRF-1, Fas, DR5 and FasL gene expression in the liver after liver I/R. Liver pDC isolated after I/R induced greater amounts of IRF-1 in hepatocytes than pDC without I/R, and IRF-1 induction were inhibited by anti-IFN-α Ab.

**Conclusions:** IFN-α blockade protects the liver from I/R injury by inhibiting apoptosis as the result of reduced IRF-1 expression. IFN-α blockade may be a promising therapeutic strategy for alleviation of liver I/R injury.
related to hyperammonemia is based on studies of cell cultures, animal models and clinical studies where the actual tissue or plasma concentrations of ammonium range from clinical relevant levels in the micromolar range to more than 5 mM. To assess the significance of the ammonium concentration, we studied the extracellular release of lactate, glutamate, and lactate in cerebral cortex of rat brain slices in a dose–response study.

**Methods:** We applied concentrations of ammonium from 0.15 mM to 10 mM to 29 brain slices in a perfusion chamber with exposure times up to 90 minutes. We measured the extracellular changes in lactate, adenosine and glutamate by the use of enzymatic biosensors inserted into cerebral cortex.

**Results:** We found a consistent reduction in the extracellular lactate concentration ranging from 4 to 400 micromolar independent of the ammonium concentration (R²=0.006, ammonium vs. lactate). The reduction in lactate was not affected by inhibition of the neuronal lactate transporter MCT-2 by adding alpha-cyano-4-hydroxycinnamic acid. We found a positive correlation between the ammonium concentration and the glutamate increase (R²=0.43, p < 0.05) with a marked release of glutamate (up to 55 micromolar) with exposure to 10 mM of ammonium. We also observed a positive correlation between the ammonium level and the change in adenosine (R²=0.68, p < 0.05) where ammonium levels above 500 mM were associated with adenosine release up to 18 micromolar. The peak in glutamate preceded the peak in adenosine release by 18±7 minutes (p < 0.05).

**Conclusion:** Cortical tissue exposed ammonium displayed a linear dose–response-like relationship between ammonium concentration and the changes in glutamate and adenosine. Interestingly, we found that ammonium induced a reduction in the extracellular lactate concentration independent of the ammonium concentration within the studied range. The reduction appeared not to be related to increased neuronal uptake of lactate.

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**573 ATTENUATION OF OXIDATIVE STRESS PROTECTS THE BRAIN IN RATS WITH “ACUTE-ON-CHRONIC” LIVER FAILURE**

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**Background:** Acute-on-chronic liver failure (ACLF) is defined as an acute decompensation of chronic liver disease. Brain edema is frequently observed in hepatic encephalopathy associated with both acute and chronic liver disease. While in acute liver failure, toxic levels of ammonia induce cerebral oxidative stress and brain edema, in chronic liver disease, systemic (not central) oxidative stress and hyperammonemia synergistically cause brain edema. This study investigated the role of both systemic and central oxidative stress and ammonia in relation to brain edema in a rat model of ACLF.

**Methods:** ACLF was induced in male Sprague-Dawley rats by portacaval shunt (PCA), followed 4 weeks later by hepatic artery ligation (HAL). Acute liver failure (ALF) induced by concomitant PCA and HAL, PCA (4 weeks) and SHAM-operated rats were used as controls. ACLF rats were divided into 2 groups that were sacrificed at: 1) coma stage of hepatic encephalopathy (defined as loss of corneal reflex) (ACLF-C) and 2) in parallel with ALF-induced coma (ACLF-P) rats. Brain edema (specific gravimetric technique), ammonia levels (commercial kit) and oxidative stress markers (plasmonic and cerebral reactive oxygen species, fluorescence spectroscopy) were evaluated along with hepatic function (routine biochemistry, haematoxylin-phloxine-saffron histopathology).

**Results:** Coma was delayed by 8h in ACLF compared to ALF rats (p < 0.01). Liver biochemistry markers did not differ between ACLF-C, ACLF-P and ALF rats; liver histopathology showed mild necrosis in ACLF-P, moderate in ALF and severe in ACLF-C. Brain water content was significantly attenuated in both ACLF-C and ACLF-P vs. ALF rats (p < 0.01). Arterial ammonia concentration followed a similar pattern: they were attenuated in ACLF-C: 0.35±0.07 mM and ACLF-P: 0.49±0.14 mM vs. ALF: 1.34±0.09 mM (p < 0.001), but remained high compared to SHAM: 0.06±0.01 mM (p < 0.001). Systemic oxidative stress was present in both ACLF and ALF rats, while cerebral oxidative stress was present only in ALF rats.

**Conclusion:** Brain edema, ammonia levels and oxidative stress are reduced in ACLF rats compared to ALF rats. These findings suggest that during chronic liver failure, compensatory mechanisms that prevent the apparition of brain edema and attenuate oxidative stress during an acute deterioration are developed.
METHODS
Blood cells and plasma and being devoid of side effects permit efficient delivery of siRNA, minimizing interference with blood cells and plasma and being devoid of side effects. Novel lipid-like particles (LPs) develop spontaneous and progressive biliary fibrosis (Popov et al, J Hepatol 2005). Optimized siRNA directed against the transcripts of the major scar tissue protein, procollagen α(I), was encapsulated in C12–200 LPs and specifically delivered to the liver of Mdr2 knockout mice. LPs with siRNA to GFP (green fluorescent protein) served as negative controls. DIR-labeled C12–200 LPs were used for in vivo and ex vivo tracking using near infrared (NIR) and fluorescent imaging. Groups (n=7–8) of 8 week old Mdr2KO mice and their nonfibrotic wild-type controls received 4 injections of procollagen α(I) or control (GFP) siRNA-LPs for 2 weeks (doses of 0.4 and 0.8mg siRNA per kg BW). 24h after the last injection liver collagen was quantified biochemically as hydroxyproline (Hyp) and in liver sections using Sirius red morphometry. Fibrosis related transcript levels were determined by qRT-PCR.

RESULTS
Transcript levels were determined by qRT-PCR and in liver sections using Sirius red morphometry. Fibrosis related 0.4 and 0.8mg siRNA per kg BW). 24h after the last injection to fibrosis and elevated hepatic vascular tone is the primary factor in the development of portal hypertension. Heparin may decrease the expression of its target gene in the fibrotic liver by 80%, which was accompanied by a significant reduction of collagen deposition by 25%. Parameters of hepatic inflammation (ALT and AST) and kidney function (creatinine) remained unchanged after siRNA-LPs injection, indicating the absence of proinflammatory or renal toxic side effects.

CONCLUSION
C12–200 LPs are a specific and safe vehicle to deliver siRNAs to the liver with high efficiency. C12–200 LPs loaded with siRNA to fibrogenic transcripts are a promising approach to efficiently inhibit liver fibrosis progression in vivo.

576 EFFECTS OF ENOXAPARIN ADMINISTRATION ON CCl4, CIRRHOTIC RATS WITH PORTAL HYPERTENSION
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BACKGROUND AND AIMs: Increased hepatic vascular resistance due to fibrosis and elevated hepatic vascular tone is the primary factor in the development of portal hypertension. Heparin may decrease fibrosis by inhibiting thrombin-mediated hepatic stellate cell activation, and reduce hepatic vascular tone by enhancing eNOS activity. Our study aimed at evaluating the effects of acute and one-week enoxaparin administration on hepatic and systemic hemodynamics, liver fibrosis and NO availability in CCl4, cirrhotic rats.

METHODS: Acute study: Enoxaparin 1.8mg/Kg sc or its vehicle (n=10 per group) was administered 24h and 1h before the study to evaluate its effects on hepatic vascular tone. Chronic study: Enoxaparin 1.8mg/Kg per day or vehicle (n=13 per group) were given for one-week to evaluate its effects on hepatic fibrosis and hepatic vascular tone. Mean arterial pressure (MAP), portal pressure (PP) and portal blood flow (PBF) were measured in vivo. Hepatic NO availability (cGMP), liver fibrosis (Sirius Red staining), and HSC activation/apoptosis (α-SMA – immunohistochemistry and western blot – and Desmin by immunohistochemistry) were determined in liver tissue.

RESULTS: No significant changes in hemodynamic parameters and hepatic cGMP levels were observed after acute enoxaparin administration. However, one-week enoxaparin significantly decreased PP (12.2±1.8 vs. vehicle 13.9±1.9mmHg; p<0.02) without significant differences in PBF, MAP or heart rate. Reduction in PP was associated with a significant reduction in liver fibrosis (~26%), liver α-SMA expression (~44% western blot) and content (~76% immunohistochemistry), and liver desmin content (~67%; immunohistochemistry) suggesting HSC deactivation and/or apoptosis. In addition, a significant 3 fold increase in hepatic cGMP levels was observed in enoxaparin-treated rats.

CONCLUSIONS: This study demonstrates that enoxaparin is able to reduce portal pressure in cirrhotic rats by improving the structural component of increased liver vascular resistance. These findings describe potential beneficial effects of enoxaparin beyond the treatment/prevention of portal vein thrombosis in cirrhosis, which deserve further investigation.

577 ASSESSMENT OF ADRENAL FUNCTION IN PATIENTS WITH STABLE DECOMPEN$ATED CIRRHOSIS USING SALIVARY AND TOTAL SERUM CORTISOL
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BACKGROUND: The prevalence of adrenal insufficiency (AI) in patients with decompensated cirrhosis (DC) depends on the methods used to assess AI. Data on the exact factors related with the presence of AI in this group of patients are limited.

AIM: To evaluate the correlation between salivary (SC) and serum total (STC) cortisol and the factors associated with abnormal adrenal response in patients with stable DC.

METHODS: All patients with stable DC admitted between 9/2010 and 9/2012 were included. In each patient, clinical and laboratory data, including glomerular filtration rate (GFR) (using 51Cr-EDTA), were recorded. SC and STC were assessed before (T0) and 1h (T60) following an injection of corticotropin (250μg).

RESULTS: We evaluated 89 consecutive patients (63 men, age 56±10 years). At T0 and T60, SC were 6.7±5 mg/17±10 ng/ml, respectively and STC were 12±6 μg/dl and 25±8 μg/dl, respectively. Correlations between SC and STC were significant for T0 (Spearman r: 0.49, p<0.001) and T60 (Spearman r: 0.55, p<0.001), but only for serum albumin>2.5 mg/dl. The prevalence of AI was higher according to SC than according to SC (55% vs 37%, respectively). The patients with discrepancy between SC-based and STC-based tests for AI diagnosis, compared to those without discrepancy, had significantly lower serum creatinine (0.9±0.2mg/dL vs 2.5mg/dl. The prevalence of AI was higher according to SC than according to SC (55% vs 37%, respectively).

The patients with discrepancy between SC-based and STC-based tests for AI diagnosis, compared to those without discrepancy, had significantly lower serum creatinine (0.9±0.2mg/dL vs 2.5mg/dl. The prevalence of AI was higher according to SC than according to SC (55% vs 37%, respectively).
the only factor significantly associated with the presence of SC-based AI. A cut off point for UK less than 40 mmol/L gave a sensitivity 81%, specificity 46% and NPV 80% for the presence of SC-based AI.

**Conclusions:** The prevalence of AI was higher according to STC than according to SC in patients with stable decompensated cirrhosis. UK could be a useful marker for the presence of SC-based AI.

**578 ALBUMIN INFUSION IMPROVES SYSTEMIC AND RENAL BLOOD FLOW AUTOREGULATION BY RESTORATION OF ENDOTHELIAL DYSFUNCTION**

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**Background and Aims:** Hemodynamic alterations in liver failure are associated with endothelial activation (EA), dysfunction (ED), inflammation and activation of vasoconstrictor systems. Albumin is a multifunctional protein which is reduced in quantity and function in liver failure. Albumin infusion in cirrhotic patients is associated with improvements in systemic hemodynamics and renal blood flow autoregulation but the mechanisms are unclear. This study test the hypothesis that albumin modulates these beneficial effects through restoration of endothelial function.

**Methods:** Patients: Subjects with refractory ascites (n = 12) or acute-on-chronic liver failure with acute kidney injury (AKI, n = 10) received albumin 40–60 gr/d (3–4 days). Hemodynamics (cardiac output – CO, mean arterial pressure – MAP, renal blood flow – RBF), oxidative stress (F2-isoprostanes), EA/ED (von Willebrand factor – vWF, nitrate – NO) and renal function were assessed at baseline and after treatment.

**In vivo:** Analbuminemic and wild-type rats were assessed for markers of ED (ADMA-asymmetric dimethylarginine–), hemodynamics and renal function 6 weeks after sham/bile duct ligation (BDL) surgery.

**In vitro:** Human umbilical vein endothelial cells (HUVECs) were stimulated with lipopolysaccharide (LPS) with or without albumin. We studied markers of EA (E-selectin, VCAM1) and intracellular ROS (reactive oxygen species).

**Results:** Patients: Albumin infusion improved hemodynamics (MAP, CO and RBF) resulting in a shift of the RBF autoregulation curve towards normalization, paralleled by a decrease in oxidative stress markers, vWF expression and an improvement renal function. Restoring endothelial function correlated with restoration in RBF (r²=0.55, p<0.001).

**In vivo:** Analbuminemic-sham rats had higher plasma ADMA levels than wild-type (0.64±0.08 vs 0.38±0.13 mmol/L, p<0.05). BDL increased ADMA levels in both groups (Figure1). Analbuminemic rats had a decreased in MAP post-BDL and higher creatinine compared to wild-type post-BDL (72.43±5.5 vs 33.6±10, p<0.05).

**Conclusions:** The results of this study provide evidence to indicate that albumin exerts its positive hemodynamic effects in cirrhosis through stabilization of the disturbed endothelial function.

**579 ENDOTHELIAL DYSFUNCTION IN CIRRHOSIS AND ITS RELATIONSHIP WITH PORTAL HYPERTENSION AND LIVER FAILURE**

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**Introduction and Aims:** Brachial artery flow-mediated vasodilation (FMD) by ultrasound is the gold-standard for assessing systemic endothelial dysfunction (SED). It has been suggested that SED might play a role in the pathophysiology of the complications of cirrhosis but data are lacking in this field. In this study we assessed the relationship of SED with liver failure, portal hypertension and Doppler indices in cirrhotic patients undergoing measurement of portal venous pressure.

**Methods:** The examinations included a standard abdominal B-mode scan, Doppler ultrasound (US) examinations, vascular US for the presence of carotid plaques and FMD. Portal flow velocity, damping index of the hepatic vein, congestion and resistivity index of hepatic and splanchnic vessels were measured with Doppler US. According to literature systemic endothelial dysfunction was defined as FMD by BAUS >10%.

**Results:** In 38 patients (25 men, 55±16yrs) with cirrhosis (Child–Pugh score 7.2±2.3; MELD 12±5) we assessed on the same day the presence of portal hypertension (mean HVPG 17.6±4.5 mmHg) and FMD by BAUS (mean value 8.0±3.0%). FMD increased in parallel with worsening of liver function as measured by Child–Pugh and MELD score (r=0.39). The proportion of patients with systemic endothelial dysfunction decreased with deterioration of Child class, being higher in patients belonging to Child A class (83%) and lower in Child C decompensated cirrhosis (25%). Moreover, SED decreased progressively with the severity of portal hypertension and a significant difference was found between patients with HVPG <10 mmHg and those with HVPG >10 mmHg (6.4±3.8% vs 8.6±3.3%). Carotid plaques were more prevalent in patients with SED (25% vs 3%). No significant correlation was found between SED and intra- and extra-hepatic Doppler flow parameters.

**Conclusion:** Endothelial dysfunction measured with FMD correlates inversely with the severity of portal hypertension and liver failure. In patients with cirrhosis FMD increases as liver function deteriorates, and it decreases according to cardiovascular risk. Further studies to elucidate role of FMD in cirrhotic patients are warranted as hyperdynamic syndrome of advanced cirrhosis may alter vascular reactivity.
Background and Aims: Serum albumin (SA) is the most important protein involved in the regulation of oncotic pressure. Beside the reduced production, recent evidences have shown that the SA of patients with cirrhosis present post-transcriptional alterations which may likely affect the non-oncotic properties of the protein, such as binding, transport and detoxification of a variety of water-insoluble molecules, metals, drugs, and reactive oxygen species. Thus, we aimed to assess in a large population of cirrhotic patients: 1. the structural changes occurring at the molecular sites responsible for the SA non-oncotic properties, and 2. whether these alterations correlate with disease severity and specific clinical features.

Methods: 143 hospitalized cirrhotic patients (Child–Pugh A/B/C: 37/81/25) and 50 age- and sex-matched healthy controls were enrolled. Blood samples were collected at admission to quantify the amount of SA with impaired N-terminal activity (Ischemia Modified Albumin, IMA) by the ACB-assay. IMA was also normalized for SA concentration (IMAR). The most frequently SA structural alterations, such as cysteinylation, N-terminal truncation, glycosylation and nitrosylation, were also assessed by LC-ESI-MS. Statistical analysis was performed using ANOVA, multivariate regression, ROC curve analysis, Kaplan–Meier and COX regression when appropriate.

Results: IMA and IMAR were significantly higher in cirrhotic patients than controls (p < 0.001) but no correlations were found with disease severity. Interestingly, IMA and IMAR were increased in patients with bacterial infection as compared to non infected (p < 0.001) with a discriminating performance for bacterial infection comparable to PCR as documented by ROC curve analysis. The LC-ESI-MS spectra revealed that specific SA oxidized and truncated isoforms were greatly increased in cirrhotic patients and correlate with disease severity and were associated with specific clinical features (ascitic decompensation and renal failure identified by serum creatinine > 1.5 mg/dl) Interestingly, the oxidized and truncated SA isoforms, considered all together, were independently associated with a poor survival.

Conclusions: Liver cirrhosis induces post-transcriptional SA alterations which correlate with specific clinical features and poor survival. Furthermore, increased IMA was found to be a predictor of bacterial infection comparable to C-reactive protein. Further studies are needed to define whether these changes play a pathogenetic role and their prevention may represent a potential therapeutic target.

Background and Aims: Hepatic venous pressure gradient (HVPG) measurement is the gold standard way to assess portal pressure. Through our experience of more than two thousand transjugular liver biopsies, we have noticed in some patients the presence of another pressure gradient between the right atrium and free hepatic veins (RAHVG) which adds to HVPG and increases absolute PHT.

Methods: 302 consecutive patients who underwent a transjugular liver biopsy with pressure measurement and who had PHT with HVPG ≥ 6 mmHg were retrospectively included in this study. RAHVG was defined by a pressure difference ≥ 6 mmHg.

Results: 140 (46%) patients presented a RAHVG. Age, Child–Pugh’s score, ascites, protein in ascites level, steatosis, sodium plasma level, albumin, bilirubin, γGT, HVPG and wedged hepatic venous pressure were significantly associated with the RAHVG, but only ascites, HVPG and caudate lobe size were independently significant in multivariate analysis. The presence of a RAHVG was a prognostic factor with a worse overall survival (HR = 1.54; 95% CI [1.03–2.32]; p = 0.02) and survival without PHT complication (HR = 1.54; 95% CI [1.02–2.34]; p = 0.03).

Conclusions: The RAHVG is caused by a caudate lobe hypertrophy. It is associated with more severe cirrhosis and worsens the prognosis of cirrhotic patients.
Background and Aims: Sepsis is a common complication of cirrhosis with a high mortality. Cirrhosis is associated with cardiac chronotropic and inotropic dysfunction which is known as cirrhotic cardiomyopathy and might be linked to endotoxemia. The present study was aimed to explore the hypothesis that the inflammatory response induced by administration of low dose of lipopolysaccharide (LPS) exacerbates cardiac chronotropic dysfunction in cirrhotic rats; and if so whether this is associated with altered cardiac toll-like receptor expression.

Methods: Cirrhosis was induced by surgical ligation of the bile duct in male Wister rats. Four weeks after bile duct ligation or sham surgery, the subjects were given intraperitoneal injection of either saline or LPS (0.1 mg/kg). Five hours after LPS injection, the atria were isolated and spontaneously beating rate and chronotropic responsiveness to isoproterenol was assessed using standard organ bath. The expression of toll-like receptor 4 (TLR4) was assessed the atria using immunohistochemistry as well as quantitative RT-PCR.

Results: LPS injection could induce a significant hyporesponsiveness to adrenergic stimulation in sham-operated rats. However, in cirrhotic rats, the chronotropic responses did not change after acute injection of LPS. Immunohistochemical study showed that TLR4 is mainly expressed in the myocardium in control atria and its expression is markedly decreased in myocardial layer following chronic bile duct ligation.

Conclusion: Our data showed that cirrhosis is associated with development of tolerance to cardiac chronotropic effect of LPS in rats and this might be due to altered localization of TLR4 in myocardium.
Cardiac thick-filament structural and functional changes contribute to cirrhotic cardiomyopathy in rats

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Background and Aims: Cirrhotic cardiomyopathy is one of the complications in cirrhosis, but the pathogenic mechanisms, in particular the cellular contractile machinery at the myofilament level, remain to be fully clarified. Cardiomyocyte contraction is the result of thick and thin myofilament sliding and thus contraction/relaxation. Myosin heavy chain is the main structure in the thick filament. The present study aimed to elucidate the role of myosin heavy chain (MHC), T-tubular integrity and Ca\(^{2+}\) transients in cardiac contractile abnormalities in cirrhosis.

Methods: Cirrhosis was induced in male Lewis Brown-Norway rats by bile duct-ligation (BDL). Contractile force of trabeculae was measured using a He-Ne laser beam; cell shortening was quantified using a video sarcomere detector (Ionoptix, Milton, MA); and Ca\(^{2+}\) transients were studied at varied frequency and extracellular calcium concentrations [Ca\(^{2+}\)]. T-tubular integrity was assessed by power spectrum analysis of images of myocytes stained with di-8-ANEPPS and myosin heavy chain (MHC) isoform distribution by gel electrophoresis.

Results: BDL rats showed a mild loss of transverse tubular integrity; a reduced maximum and a reduced rate of rise of the Ca\(^{2+}\) transient (Max F/F0) without a change in the rate of relaxation of the Ca\(^{2+}\) transient; a reduction of both the rate of rise and fall of contraction; a decreased maximal force-generating capacity; a loss of the inotropic effect of increased stimulus frequency; a decreased spontaneous diastolic sarcomere length fluctuation. Myosin heavy chain isoforms were shifted from V1 to V3 in BDL heart. In other words, the predominant alpha-MHC of normal rat hearts that produces stronger contractility was shifted to a dominance of beta-MHC at the myofilament level, remain to be fully clarified. Cardiomyocyte contraction is the result of thick and thin myofilament sliding and thus contraction/relaxation. Myosin heavy chain is the main structure in the thick filament. The present study aimed to elucidate the role of myosin heavy chain (MHC), T-tubular integrity and Ca\(^{2+}\) transients in cardiac contractile abnormalities in cirrhosis.

Conclusion: Cardiomyocyte contractility in cirrhotic rats showed features typical of heart failure including systolic and diastolic prolongation, impaired force-frequency relation, and decreased force-generating capacity. At least part of the mechanism for the above is likely due to a myosin heavy chain isoform shift and abnormal calcium kinetics in the cirrhotic heart.

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The IL-6/GP130 signaling pathway in myeloid cells modulates the inflammatory response during sepsis

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Background and Aim: Ascites and its bacterial infection (SBP) is a frequent and life-threatening complication in patients suffering from liver cirrhosis. After bacterial peritonitis, the mobilization of the regulatory myeloid cells is a major requirement to prevent sepsis. Current research on inflammatory pathways has shown that GP130 in hepatocytes is essential in the modulation of inflammation. Therefore, we investigated the contribution of the GP130 pathway for the skewing of macrophage towards the regulatory M2 phenotype in a model of peritonitis.

Methods: Depletion of GP130 in hematopoietic cell-deficient mice was achieved by bone marrow transplantation (BMC) using MxCre GP130\(^{loxp}\)/ GP130\(^{loxp}\) GFP (cre- BMC-WT; cre- BMC-KO) as donors into WT as recipients. Subsequently, the recipients underwent coecal ligation and puncture (CLP) – a model of bacterial peritonitis. Additionally, bone marrow derived macrophages (BMDM) generated from BMC-WT and BMC-KO mice were stimulated with LPS, LPS/IFN\(\gamma\), IL-4, IL-10/IL-4 or medium. Our data clearly showed (a) reduced expression of Arginase I and Macrophage mannose receptor (MMR) after IL-4 stimulation and; (b) defective IL-6 response after stimulation with LPS/IFN\(\gamma\). The anti-inflammatory phenotype, associated with a reduction of IL-4R\(\alpha\), was rescued by the exogenous addition of IL-10 in vivo and in vitro.

Conclusion: These data identify IL-6/GP130 signaling pathway as a critical component in myeloid lineage of immune cells for host survival and control of inflammation and infection during bacterial peritonitis. The IL-6/GP130 signaling cascade is required for the maintenance of the cytokine responses by regulating the activation of M1 macrophages during bacterial peritonitis and sepsis.
PATIENTS WITH MINIMAL HEPATIC ENCEPHALOPATHY SHOW IMPAIRED MISMATCH NEGATIVITY CORRELATING WITH REDUCED PERFORMANCE IN ATTENTION TESTS

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Background and Aims: Attention impairment is an early event in cognitive impairment of patients with minimal hepatic encephalopathy (MHE). The underlying mechanisms remain unclear. Mismatch negativity (MMN) is an auditory event-related potential which reflects attentional trigger. Patients with schizophrenia show impaired attention and cognitive function which are reflected in altered MMN. We hypothesised that patients with MHE, similarly to those with schizophrenia, should show MMN alterations related with attention deficits.

The aims of this work were to assess whether:

a. MMN is altered in cirrhotic patients with MHE compared to those without MHE;

b. MMN changes in parallel with MHE and/or performance in attention tests in a longitudinal follow up study;

c. MMN predicts performance in attention tests and/or in the Psychometric Hepatic Encephalopathy Score tests.

Methods: We performed MMN analysis, attention and coordination tests in 34 control subjects, 37 patients with liver cirrhosis without MHE and 23 with MHE.

Results: Patients with MHE show reduced performance in selective and sustained attention tests and in visuo-motor and bimanual coordination tests. The MMN wave area was reduced in patients with MHE but not in those without MHE. Reduction of MMN area is associated specifically with reduced performance in attention tests but not with other alterations such as motor coordination. This is supported by multivariate logistic regression analysis showing that only performance in attention tests, but none of the other tests performed, predicts the MMN area.

This is further supported by the results of patients who improved from MHE to non-MHE in the follow up study. They improved in attention tests, resulting in resolution of MHE and normalization of MMN area. In contrast, one patient did not show impaired attention or MMN area in the first study and MHE was due to impaired motor coordination which improvement leads to resolution of MHE in the second study without changes in MMN area.

Conclusions: The data indicate that MMN is useful to identify the attention deficits in patients with MHE.

Reference(s)

B. pseudocatenulatum CECT 7769 ADMINISTRATION DECREASES INFLAMMATION AND BACTERIAL TRANSLLOCATION IN THE LIVER OF MICE DURING CCL4-INDUCED CIRRHOSIS

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Background: An intestinal dysbiosis is associated with bacterial-DNA translocation during development of experimental cirrhosis. The use of probiotics has emerged as an alternative to the use of antibiotics in the control of these episodes, promoting intestinal homeostasis and avoiding the onset of antibiotic-resistant species.

Hypothesis and Aims: B.pseudocatenulatum CECT7765 may decrease liver inflammation, enhancing intestinal homeostasis and preventing bacterial-DNA translocation. The objective is to assess the degree of liver inflammation during the induction of experimental cirrhosis and its relationship with bacterial-DNA translocation in the liver of these animals according to the administration of bifidus.

Animals and Methods: Chronic liver damage was induced in Balb/c mice by oral administration of CCl4. After a 16-week study protocol, animals were distributed in groups for 1-week administration of B.pseudocatenulatum CECT7765 (10E9 CFU/day/oral) or vehicle. Subsequently laparotomies were performed and liver tissue, intestinal content, GLMs and blood samples were collected. Fibrosis and liver inflammation status were quantified by histology and expression of TGFβ-1, Procollagen-a1, TIMP-1 and MMP-2 gene expression, although TNFalpha, TNFRI and TNFRII gene expression was also significantly lower in the liver of animals receiving bifidus while IL10R IL-10 gene expression showed a significant increase in this group during development of cirrhosis. Lipid peroxidation (MDA levels) was confirmed in all animals by histology and preventing bacterial-DNA translocation. The objective is to assess the degree of liver inflammation during the induction of experimental cirrhosis and its relationship with bacterial-DNA translocation in the liver of these animals according to the administration of bifidus.

Results: 50 animals were included in the study. Liver damage was confirmed in all animals by histology and profibrogenic gene expression, although bifidus-treated animals significantly decreased severity of liver injury. TNFalp, TNFRI and TNFRII gene expression was also significantly lower in the liver of animals receiving bifidus while IL10R IL-10 gene expression showed a significant increase in this group during development of cirrhosis. Linal micobiota was quantified and bacterial-DNA translocation rate was assessed in liver, GLMs and blood.

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POSTERS

591 BACTERIAL DNA MEASUREMENTS IN PATIENTS WITH CIRRHOSIS UNDERGOING TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT (TIPS) INSERTION
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Background and Aims: Bacterial translocation may be associated with abnormal immune activation leading to haemodynamic alterations and a poor outcome in patients with cirrhosis. Presence of bacteria in lymph nodes, bacterial products, and bacterial DNA (bDNA) have been proposed as markers of bacterial translocation. In this study, we investigated bDNA and its relation to markers of inflammation in the portal and hepatic venous circulations in patients with cirrhosis undergoing TIPS insertion.

Methods: In 28 cirrhotic patients undergoing TIPS insertion, we analysed blood samples for bDNA and markers of inflammation.

Results: bDNA-levels were similar in the hepatic and portal vein (median 6.07 (3.06–10.61) versus 6.51 gene copies (3.15–11.52), p = 0.80). bDNA levels in hepatic and portal veins correlated significantly (r = 0.62, p = 0.002). In patients receiving antibiotics, bDNA tended to be lower, reaching significance in the hepatic vein (p = 0.025). Dividing patients into groups according to bDNA, we found no significant difference with respect to markers of inflammation. Markers of inflammation in general did not differ between the hepatic and portal veins. However, SUPAR and VEGF were higher in the hepatic vein (p = 0.031, and 0.003, respectively).

Conclusions: Bacterial DNA was measurable in the hepatic and portal vein blood and the levels correlated significantly but without a significant transhepatic gradient. bDNA tended to be lower in patients treated by antibiotics compared to untreated patients. The absence of a transhepatic gradient suggests that in patients with advanced liver disease, no major hepatic elimination of bDNA occurs. In contrast previous reports, bDNA was not related to markers of inflammation.

592 CARDIAC CIRRHOSIS CONTRIBUTES TO THE WATER RETENTION IN PATIENTS WITH HEART FAILURE
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Objectives: Liver cirrhosis in heart failure patients is considered a bystander and water accumulation is thought to mainly originate from forward failure, arterial underfilling with activation of the renin angiotensin system (RAAS). We here assess cardiac fibrosis in patients with heart failure by sequential assessment of liver stiffness (LS) and analyse the cardiac and hepatic determinants of water retention.

Methods and Patients: 100 patients with acute decompensated heart failure were retrospectively analysed. In an additional prospective validation cohort, various serum hormones of water status, liver tests, echocardiography and LS were sequentially measured in 39 patients with acute heart failure at the days of admission and release.

Results: 44% of patients in the prospective cohort showed morphological signs of liver cirrhosis. In confirmation, LS exceeded 8 kPa in ca. 70% of the retro- and prospective cohort (66.6% vs 70.8%). LS was the best predictor of liver cirrhosis (AUROC 0.998) although it decreased during diuretic therapy in 88% with a mean of 3.58 kPa. Weight loss correlated best with the decrease of LS (r = 0.759, p < 0.0001), signs of cirrhosis or right heart failure (e.g. TDI’s) but not forward failure (ejection fraction). Likewise, presence of cirrhosis correlated significantly with LS (r = 0.844, p < 10^-6), size of right ventricle and atrium (r = 0.796, p = 2x10^-6 and r = 0.635, p < 0.005) and GGT (r = 0.622, p < 0.005) but no forward failure. Major discriminating factors between cirrhotic and non-cirrhotics were LS, right atrium size, presence of ascites and GGT. Hormones of water regulation such as copeptin and aldosterone were all higher in cirrhotics (28.7 vs. 11.6 pmol/l and 103.4 vs. 24.0 pmol/l) despite the intake of aldosterone antagonists. Interestingly, water retention and LS were significantly correlated with the C1q/TNF related protein (C1R/T) (r = 0.692, p < 0.005 and r = -0.803, p < 0.01) a novel and important upstream regulator of aldosterone.

Conclusion: Cardiac cirrhosis/congestion seems to be a major factor for the water accumulation in patients with heart failure leading to further increase of the RAAS. Our data provide novel mechanistic rationale for the treatment of patients with heart failure with aldosterone antagonists and the effective surveillance via LS measurements.

593 LENALIDOMIDE AMELIORATES THE PORTAL HYPERTENSIVE SYNDROME IN NON-CIRRHOTIC AND CIRRHOTIC PORTAL HYPERTENSIVE ANIMALS
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Introduction: We aimed to investigate the influence of lenalidomide, a immunomodulatory and anti-angiogenic derivative of thalidomide, on portal hemodynamics, angiogenesis and inflammation in cirrhotic and non-cirrhotic portal hypertensive rats.

Methods: Male Sprague Dawley rats underwent either partial portal vein ligation (PPVL; isolated portal hypertension), bile duct ligation (BDL; cirrhotic portal hypertension), or sham-operation (SO). Treatment with lenalidomide (250 mg/kg) or vehicle (VEH) was given via gavage for seven days prior to hemodynamic measurements. In PPVL hemodynamic studies were performed after seven days, in BDL 28 days after surgery measuring portal pressure (PP), mean arterial pressure (MAP), and portosystemic collateral blood flow (PSCBF). Splanchic and hepatic tissues were analyzed for mRNA (RT-PCR) and protein expression (western blot) of angiogenic and inflammatory markers.

Results: In PPVL portal pressure was significantly reduced by LENA treatment (15.2 ± 0.9 vs. 12.7 ± 0.5 mmHg; p = 0.02), while in BDL animals no significant effect of lenalidomide on portal pressure (13.9 ± 2 vs. 12.4 ± 2 mmHg; p = 0.128) was observed. Comparing VEH- to LENA-treated animals, PSCBF was significantly decreased both in the PPVL (86 ± 7% vs. 27 ± 7%; p = 0.001) and in the BDL...
(72±19% vs. 52±11%; p = 0.040) rats. Protein expression of VEGFR2 was significantly reduced in splanchic tissue (p = 0.049) and in the liver (p = 0.006) of BDL animals. No significant differences in splanchic or hepatic protein expression were observed for VEGF (p = 0.304, p = 0.118), TNFα (p = 0.215, p = 0.164) or PDGFβ (p = 0.43, p = 0.18). In BDL rats, splanchic (p = 0.007) and hepatic (p = 0.048) PI GF mRNA expression was significantly reduced by LENA treatment. In BDL, a reduction of VEGF-mRNA levels in splanchic tissue (p = 0.012) and the liver (p = 0.082) was observed. LENA treatment did not influence TNFα or PDGFβ mRNA expression in BDL rats, neither in splanchic tissue nor in the liver. In PPVL animals splanchic protein expression of CD31 was significantly decreased (p = 0.003), while TNFα was increased (p = 0.044).

**Conclusion:** Lenalidomide ameliorates portal hypertensive syndrome in cirrhotic and non-cirrhotic portal hypertensive rats by decreasing proinflammatory and antiangiogenic signaling. Therefore, lenalidomide has some potential as a novel therapeutic option in cirrhotic patients with portal hypertension.

### 594 VON WILLEBRAND FACTOR LEVELS AND VARIATIONS IN THE VON WILLEBRAND FACTOR GENE INFLUENCE LIVER STIFFNESS: THE ROTTERDAM STUDY

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**Background and Aims:** Hypercoagulability is considered to be one of the causative factors of liver fibrogenesis. Elevated von Willebrand factor (VWF) levels are known to increase the risk of thrombosis, but the relationship with liver fibrosis is unknown. Aim of the current study was to investigate the association between VWF levels, its genetic determinants and liver stiffness measurements (LSM) in a population-based cohort and in a subgroup of participants with non alcoholic fatty liver disease (NAFLD).

**Methods:** This study was based on the Rotterdam study, a large population-based cohort among subjects aged ≥55 years. Transient elastography was used to assess hepatic fibrosis. NAFLD was diagnosed with abdominal ultrasound. VWF antigen levels were determined in plasma with an enzyme-linked immunosorbent assay. Ten polymorphisms known to strongly affect VWF levels were studied. Genotyping was performed with the Infinium HumanHap 550K chip.

**Results:** Reliable LSM and genetic data were available in 1037 participants (age 74.1±5.6 years; 50.7% males). Median LSM was 5.1 kPa (IQR 4.2–6.4). Median VWF antigen level was 1.11 IU/ml (IQR 0.86–1.42). NAFLD was diagnosed in 331 participants (31.9%). VWF levels were associated with LSM in the total cohort and in a NAFLD subgroup (p = 0.001 and 0.007 respectively). rs9390459, rs687621 and rs1063857 were associated with VWF levels in the total cohort after correction for age and sex in an additive genetic model (p-values 0.034/0.001/0.004 respectively). For genotype AA versus AG/GG at two polymorphisms located in the VWF gene, rs216321 and rs2283333, higher LSM were observed (p = 0.012 and 0.017 respectively). After adjustment for age, sex, NAFLD, spleen size, HOMA-IR and ALT, this relationship remained significant for rs2283333 (p = 0.046). A trend towards significance was observed for genotype AA versus AG/GG at rs216321 after adjustment for these factors (p = 0.097). In participants with NAFLD a third polymorphism located in the VWF gene, rs1063857, was significantly associated with LSM in a multivariate additive genetic model (p = 0.019).

**Conclusions:** In this population-based cohort and in a NAFLD subgroup VWF levels and three polymorphisms located in the VWF gene were independently associated with LSM. These results suggest that VWF levels may play a causal role in liver fibrogenesis.

### 595 ALKALINE PHOSPHATASE IS A PROTECTIVE ENZYME DURING LIVER FIBROGENESIS

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**Background and Aim:** Serum alkaline phosphatase (AP) levels serve as a marker for many liver diseases. Recent studies indicate that AP may act as a protective enzyme by dephosphorylation of LPS because dephosphorylation blocks toxicity of this product. Gut-derived LPS is known to aggravate liver damage and fibrosis and we hypothesized that higher levels of AP may represent a physiological response upon higher levels of this toxin during fibrosis. We therefore studied hepatic expression levels and effects of intestinal AP during fibrogenesis.

**Methods:** Intestinal AP knock-out C57BL/6 mice (iAP KO) were examined at 6 weeks of age and compared to age-matched wild-type. Liver fibrogenesis was induced by CCl4 administration to Balb/c mice for 1 day (acute studies) or 8 weeks (chronic studies). The latter group received saline or calf-intestinal AP (5 units, i.v.), from week 6 to 8 (3x/week, n = 6/group).

**Results:** iAP KO mice displayed higher intrahepatic expression levels of fibrogenic markers (PAR-1, Collagen I) paralleled by an increase in macrophages of the pro-fibrotic M2 phenotype relative to WT. So, lack of intestinal AP stimulates fibrogenesis within the liver. We subsequently examined intrahepatic AP expression in more liver damage induced by CCl4-induced acute liver damage and after chronic CCL4 administration, characterized by liver fibrosis. Results showed a gradual increase in intrahepatic AP-activity from normal to fibrotic animals. Histochemical analysis revealed that this AP-activity is able to dephosphorylate the lipopolysaccharide (LPS). We further explored the role of AP by injecting iAP to mice with CCL4-induced fibrosis. Data show a reduced hepatic AP activity, paralleled by significant lower expression levels of desmin and significant lower accumulation of M2 macrophages in iAP-treated mice compared to control.

**Conclusions:** Based on the increased hepatic fibrogenic activity in intestinal AP-KO mice, and the attenuated fibrogenesis in fibrotic mice receiving intestinal AP, we conclude that iAP attenuates fibrosis. The enhanced expression of AP in fibrotic livers and the demonstration that this AP activity is able to dephosphorylate LPS, suggests that endogenous AP serves as a protective enzyme after liver damage. Enhanced AP activity during disease may therefore reflect a physiological response to LPS.

### 596 TARGETING OF LYSYL OXIDASE-LIKE-2 (LOXL2) PROMOTES REVERSAL OF LIVER FIBROSIS VIA INHIBITION OF COLLAGEN CROSS-LINKING AND FIBROTIC MATRIX STABILIZATION

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**Background and Aims:** We have previously shown that transglutaminase-independent progressive collagen cross-linking and stabilization retards liver fibrosis reversal and presents a promising target for antifibrotic therapies. Here, we tested whether collagen cross-linking can be successfully inhibited to halt and/or
reverse fibrosis in vivo by an antibody directed against LOXL2, an inducible protein with lysyl oxidase activity.

**Methods:** Advanced liver fibrosis was induced in C57Bl6 mice by repeated injections of thioacetamide (TAA). Novel anti-LOXL2 therapeutic antibody (AB0023mAB, 30 mg/kg) or control antibody (M64, 30 mg/kg) was administered i.p. twice a week (n = 10–16 per group) during fibrosis progression (delayed treatment, from week 6 to 12 of TAA) or during recovery from TAA (fibrosis reversal, 4–12 weeks). Non-selective lysyl oxidase inhibitor beta-aminopropionitrile (BAPN, 100 mg/kg/day i/p) was administered for comparison. Collagen crosslinking was assessed ex vivo by a step-wise collagen extraction/fractionation method. Liver fibrosis was evaluated by histology, biochemical determination of collagen and analysis of profibrogenic gene expression by QRT-PCR.

**Results:** Immunohistochemical analysis revealed that LOXL2 was virtually absent from healthy liver but strongly induced in TAA liver fibrosis, with predominant localization within fibrotic septae. Delayed anti-LOXL2 treatment of pre-established, advanced liver fibrosis (week 6 through 12 of TAA) inhibited fibrotic matrix stabilization, with a 30% reduction in the highly cross-linked collagen fraction. Histological signs of bridging fibrosis improved, with a 25% decrease in net collagen deposition in LOXL2-treated group as assessed biochemically via hydroxyproline (p = 0.025).

When LOXL2 was inhibited during the recovery from fibrosis, profound acceleration of remodeling of fibrotic septa was observed, with thinning and splitting of collagen fibrils histologically, and a 36% decrease in hepatic collagen levels (p = 0.021) at early recovery time-point (4 weeks). In contrast, no significant effect on collagen cross-linking, fibrosis progression or reversal could be detected using histological or biochemical methods in control antibody- or BAPN-treated mice.

**Conclusions:**
1. Antibody-mediated LOXL2 inhibition effectively suppressed collagen cross-linking during experimental liver fibrosis progression in vivo,
2. LOXL2 inhibition rapidly and potently accelerates hepatic fibrosis resolution in a recovery model from TAA-injury,
3. Feasibility of antibody targeting of LOXL2 to prevent and reverse liver fibrosis should be evaluated in future clinical trials.

**597 METHAPYRILENE TREATMENT IS A RENAISSANCE ALTERNATIVE TOBILE DUCT LIGATION AS A MODEL OF REPRODUCIBLE AND REVERSIBLE PERIPORTAL FIBROSIS IN THE RAT**

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**Background and Aims:** Hepatic fibrosis is largely responsible for the significant morbidity and mortality caused by liver disease and cannot currently be treated therapeutically1. Experimental models of hepatic fibrosis are therefore required in order to develop and test the efficacy of novel anti-fibrogenic agents. Bile duct ligation (BDL) is the most commonly used experimental model of hepatic perportal fibrosis. However BDL has several limitations; it is a severe procedure requiring practically irreversible surgery; the extent of damage cannot be modulated and it is associated with a high mortality rate. Methapyriline (MP, N,N-Dimethyl-N-(2-pyridinyl)-N-(2-thienylmethyl)-1,2-ethanediame hydrochloride) causes periportal necrosis in rats when given acutely2. This work therefore aimed to investigate if chronic MP administration could be used as an alternative to BDL as a rat model of hepatic perportal fibrosis.

**Methods:** Male rats were orally dosed with 150 mg MP/kg body weight tri-weekly for 3 weeks, 6 weeks or 3 weeks followed by 3 weeks vehicle. Histology and immunohistochemistry were then used to measure markers of chronic liver disease compared to those seen after BDL.

**Results:** The administration of MP, similar to BDL, resulted in significant perportal inflammation and collagen deposition compared to controls. The number of perportal myofibroblasts and portal tract fibroblasts were also significantly increased as gauged by α-smooth muscle actin and vimentin staining respectively. Periportal cytokeratin-19 staining was significantly higher than controls after MP treatment and BDL indicating that MP administration, like BDL, activated a ductular reaction. The severity of MP induced liver damage could be enhanced by dosing MP for a longer time period and all markers of chronic liver disease measured were reversible. Importantly, MP administration resulted in no mortality.

**Conclusions:** These results demonstrate that chronic MP causes perportal fibrosis with pathology qualitatively similar to that seen after BDL. It therefore offers a refined alternative to BDL as a model of perportal fibrosis; its use would reduce animal distress; resolve the issue of BDL and surgical-associated complications and mortality whilst allowing moderation of fibrosis severity.

Acknowledgements: Funded by the NC3Rs.

cirrhotic patients. Thus, increasing n6/n3 PUFA ratio to levels observed in Child–Pugh C class patients resulted in up-regulation of NOX2-generated oxidative stress, while the opposite was detected lowering n6/n3 PUFA ratio at levels observed in controls. These data warrant further study to evaluate if n-3 supplementation may reduce inflammation and eventually liver disease progression.

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A KEY ROLE FOR TISSUE FACTOR IN ACTIVATION OF COAGULATION IN CIRRHOSIS

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Background and Aims: Cirrhosis patients have a dysregulated coagulation system and are prone both to hemorrhage and thrombosis. The mechanisms responsible for thrombosis have not been defined. The aim of our study was to determine the role of tissue factor (TF), the major cellular initiator of coagulation, in thrombosis in cirrhosis.

Methods: First, we measured plasma levels of coagulation activation markers [thrombin-antithrombin complexes (TAT) and D-dimers], microparticles, microparticles TF activity, and plasma interleukin-6 in 46 cirrhosis patients (Child–Pugh A, n = 12; Child–Pugh B, n = 10; Child–Pugh C, n = 24) and in 9 healthy controls. Second, we examined the role of TF in coagulation activation by performing bile duct ligation in wild-type (WT), low TF mice (expressing 1% of WT TF levels in all tissues), and mice deficient for TF in myeloid cells (TF<sup>−/−</sup>LysM<sup>Cre+</sup> cells). Results: Cirrhosis patients had higher plasma TAT [4.5 (2.2–41.3) vs. 2.9 (0.9–4.8) ng/mL; p = 0.006], D-dimers [6429 (141–19093) vs. 144 (86–358) ng/mL; p < 0.001], microparticle TF activity [0.121 (0.000–0.542) vs. 0.009 (0.000–0.037) pg/mL; p < 0.001] and interleukin-6 [15 (0–1628) vs. 0 (0–21) pg/mL; p = 0.001] levels compared to healthy controls. TAT and D-dimers levels correlated with cirrhosis severity (MELD, Child–Pugh), microparticle TF activity and interleukin-6 levels. Total microparticle levels were not different between cirrhosis patients and controls. Bile duct ligated mice (day 12) had features similar to cirrhosis patients with increased levels of TAT [9.3 (6.8–12.0) vs. 6.0 (4.3–8.8) ng/mL; n = 10 vs. 15; p = 0.003], microparticle TF activity, white blood cell TF activity and plasma interleukin-6 compared to sham mice. Low TF mice had reduced TAT plasma levels [10.7 (7.0–18.8) vs. 8.2 (5.8–11.8) ng/mL; n = 22 vs. 13; p = 0.005] compared with WT controls. In contrast, TAT plasma levels were not different between TF<sup>−/−</sup>LysM<sup>Cre+</sup> mice and their littermate controls (n = 14 per group; p = 0.613).

Conclusion: Cirrhosis, and primarily severe cirrhosis, is associated with coagulation activation. Bile duct ligation in mice is a suitable model to study mechanisms of this coagulation activation. Non-myeloid TF activates coagulation in this model and may also contribute to thrombosis in cirrhosis patients.

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NEBIVOLOL TREATMENT INCREASES SPLANCHNIC BLOOD FLOW AND PORTAL PRESSURE IN CIRRHOTIC RATS VIA MODULATION OF NO SIGNALING

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Introduction: We evaluated the effects of nebivolol, a third-generation beta-blocker capable of increasing NO-bioavailability on portal pressure, and on splanchic and systemic hemodynamics in a cirrhotic portal-hypertensive rat model.

Methods: Male Sprague Dawley rats underwent sham operation (SO) or bile duct ligation (BDL). When cirrhosis was fully developed, the animals were orally treated with low-dose (5 mg/kg) or high-dose (10 mg/kg) nebivolol (NEBI) or vehicle (VEH) for seven days. Heart rate (HR), mean arterial pressure (MAP), portal pressure (PP), and superior mesenteric artery blood flow (SMABF) were measured. Portosystemic collateral blood flow (PSCBF) was quantified by radioactive microspheres. Hepatic and splanchnic NO<sub>x</sub> levels and GSH/GSSG ratios (RedOx state) were measured.

Results: BDL-VEH rats developed features of a portal hypertensive syndrome as shown by increased HR, PP, GSH/GSSG, while MAP was decreased compared to SO-VEH rats. Nebivolol significantly reduced HR both in SO (p < 0.001) and BDL (p < 0.001) animals. BDL-NEBI rats had significantly higher PP (15.5 vs. 12.6 mmHg; p = 0.006) and SMABF (5.3 vs. 3.7 ml/min/100 g; p = 0.016) than BDL-VEH animals. The increase in PP and SMABF was documented both in low-dose and high-dose BDL-NEBI animals. The nebivolol-induced reduction in HR significantly correlated with the increase in SMABF (R = 0.878; p < 0.001). While no significant beneficial effects on hepatic RedOx state were observed, splanchnic NO<sub>x</sub> levels were clearly increased by NEBI treatment (BDL-VEH: 22.75 ± 0.66 μM vs. BDL-NEBI-5: 25.82 ± 0.55 μM vs. BDL-NEBI-10 28.06 ± 1.63 μM; p < 0.05). Along with the dose-dependent increases in splanchnic NO<sub>x</sub> levels observed in BDL-NEBI-5 and BDL-NEBI-10 rats, a corresponding increase in SMABF and PP was recorded. Splanchnic GSH/GSSG ratios were slightly increased in BDL-NEBI-10 rats compared to BDL-NEBI-VEH rats (p < 0.1), but not in BDL-NEBI-5 animals. Nebivolol treatment did not affect PCBFS in SO and BDL animals.

Conclusion: Nebivolol increases portal pressure in cirrhotic animals by increasing splanchnic blood flow without affecting porto-systemic shunting. These effects are dose-dependent and involve NO-signaling pathways. Thus, our data suggest that nebivolol should not be considered as a novel betablocker for treatment of cirrhotic patients with portal hypertension.

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mir-133a MEDIATES TGF-b-DEPENDENT DE-REPRESSION OF COLLAGEN-SYNTHESIS IN HEPATIC STELLATE CELLS DURING LIVER FIBROSIS

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Background and Aims: miRNAs are novel regulators of organ fibrosis. miR-133a plays a role in cardiac and muscle remodeling, but its function in the liver is unclear. We therefore aimed at evaluating a possible function of miR-133a in hepatofibrogenesis.

Methods: miR-133a levels were measured in whole liver samples from different murine hepatic fibrosis models and human liver tissue from patients with liver cirrhosis. The cell-specific regulation of miR-133a was assessed in FACS-sorted hepatic cell subpopulations. Murine and human primary hepatic stellate cells (HSC) were isolated and treated with different cytokines to evaluate upstream regulators of miR-133a. Moreover, GRX-1 mediated NO-signaling pathways. Thus, our data suggest that nebivolol should not be considered as a novel betablocker for treatment of cirrhotic patients with portal hypertension.
with transforming growth factor (TGF)-b resulted in a significant downregulation of miR-133a in these cells. In turn, overexpression of miR-133a in primary murine HSC led to decreased expression of collagens. In addition, miR-133a serum levels were increased in patients with chronic liver disease and indicate the presence and progression of liver cirrhosis.

**Conclusions:** Evidence is presented for a novel antifibrotic functional role of miR-133a in hepatofibrogenesis. miR-133a may thus represent a target for diagnostic and therapeutic strategies in liver fibrosis.

### 602 HEMODYNAMIC EFFECTS OF A COMBINED ACUTE TREATMENT WITH A NON-SELECTIVE BETA BLOCKER PLUS DROXIDOPA IN CIRRHOTIC RATS

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**Background:** Droxidopa, an oral norepinephrine precursor, improves hemodynamic and renal alterations of portal hypertensive rats without changing portal pressure. Non-selective beta blockers (NSBB) decrease portal pressure and prevent variceal bleeding in cirrhotic patients. Droxidopa could be effective for the renal alterations of cirrhotic patients on NSBB therapy.

**Objective:** To evaluate the acute effects of a combined treatment with a NSBB (carvedilol or propranolol) plus droxidopa on the hemodynamic and diuresis of bile duct ligated (BDL) cirrhotic rats.

**Methods:** Three acute studies (oral single doses):

1. Droxidopa (20 mg/kg) (Chelsea Therapeutics Inc.) or vehicle was administered to BDL rats treated with carvedilol (2.5 mg/kg) or vehicle one hour before (n=7, n=7, n=6, n=7).

2. Droxidopa (25 mg/kg) or vehicle was administered to BDL rats treated with propranolol (25 mg/kg) or vehicle two hours before (n=8, n=8, n=7, n=7).

After treatments, hemodynamic parameters were registered for 2 hours and diuresis volume was collected.

**Results:** BDL rats treated with carvedilol plus droxidopa showed no changes in mean arterial pressure (MAP) and portal pressure (PP) compared to vehicles; superior mesenteric blood flow (SMABF) and resistance (SMAR) were similar to animals treated only with carvedilol or with droxidopa alone, respectively. In contrast, treatment with propranolol combined with droxidopa decreased PP (value at 90 minutes: 15.22 ± 0.50 mmHg) compared to vehicle (17.20 ± 0.47 mmHg, p < 0.016) and to droxidopa alone (17.49 ± 0.83 mmHg, p = 0.046). Moreover, propranolol plus droxidopa combination decreased SMABF and increased SMAR in an additive way compared to each drug separately, while the increase in MAP observed in animals treated with droxidopa alone was only partially blocked. Pretreatment with carvedilol blunted the diuretic effect of droxidopa, while 2h diuresis in the animals treated with propranolol plus droxidopa was similar to animals receiving only droxidopa.

**Conclusion:** While combining droxidopa with carvedilol has no beneficial additive effects, the acute oral administration of propranolol plus droxidopa reduces portal pressure maintaining and improving hemodynamic and renal benefits of droxidopa treatment in cirrhotic rats. Adding droxidopa to standard treatment with propranolol may improve the hemodynamic alterations in patients with advanced cirrhosis and balance the possible adverse effects of each drug in these patients.
pressure (MAP), heart rate (HR), and portal pressure (PP) were performed 1–35 days afterwards. Hemoglobin (Hb), white blood cell counts (WBC) and platelet counts (PLT) were analyzed from femoral arterial blood. Megakaryocyte-density was evaluated in PAS-staining of sternum specimens and in bone marrow smears.

**Results:** PP was higher in PPVL (16.9 mmHg; p = 0.001) and in BDL (15.3 mmHg; p < 0.001) than in SO animals (8.8 mmHg). Accordingly hyperdynamic circulation was more pronounced in PPVL (MAP: 94 mmHg; p = 0.108, HR: 328 bpm; p = 0.131) and in BDL (MAP: 78 mmHg; p = 0.002, HR: 317; p = 0.292) than in SO animals (MAP: 108 mmHg, HR: 303 bpm).

Hb was significantly lower in PPVL and BDL (13.8 ± 0.7 and 11.4 ± 1.4 g/dl) than in SO (14.8 ± 0.7 g/dl; p < 0.001) rats. While WBC and PLT were similar in SO and PPVL animals, BDL rats had significantly higher WBC (19.8 ± 12.9 vs. 4.8 ± 2.0 G/L; p < 0.001) and PLT (1471 ± 144 vs. 1120 ± 151 G/L; p = 0.001). Megakaryocytes were regularly distributed throughout the bone marrow and exhibited regular morphology. Spleenocytes had no significant effects on hemodynamics or hematological parameters in SO rats, whereas PPVL-SPLECT rats showed lower PP (16.9 ± 2.9 vs. 19.6 ± 3.2 mmHg; p = 0.048) and higher Hb levels (14.4 ± 1.5 vs. 13.8 ± 0.7 g/dl; p = 0.082). Hemodynamic effects of splenectomy were most pronounced in BDL rats, causing a significant PP decrease (14.0 ± 1.3 vs. 15.3 ± 2.1 mmHg; p < 0.010) and MAP increase (91 ± 21 vs. 78 ± 16 mmHg; p = 0.017). Hb increased in BDL-SPLECT animals compared to BDL animals without splenectomy (12.9 ± 0.9 vs. 11.4 ± 1.4 g/dl; p = 0.094), as did WBC (261 ± 113 vs. 19.8 ± 12.9; p = 0.034) and PLT (1710 ± 411 vs. 1471 ± 144 G/L; p = 0.108).

**Conclusions:** Spleenectomy decreased portal pressure and ameliorated hyperdynamic circulation, especially in cirrhotic portal hypertensive rats. PPVL and BDL led to a decrease of Hb levels, while when combined with splenectomy, Hb levels remained high. Only BDL rats showed a significant raise of WBC and even PLT, which further increased when splenectomized.
(p=0.04) and IL-10 after LPS (p=0.006) and LTA (p=0.002) stimulation was also higher in patients with TLR4 polymorphisms than in wild-type patients.

**Conclusions:** The presence of D299G and/or T399I TLR4 polymorphisms was associated with a higher frequency of previous hepatic encephalopathy and a distinctive pattern of cytokine production in patients with cirrhosis. This pattern is characterized by lower spontaneous cytokine production and higher responsiveness after stimulation than wild-type patients. These results suggest that TLR4 polymorphisms could influence the development of complications in cirrhosis.

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**DEFECTIVE MONOCYTE PHAGOCYTOSIS MAY EXPLAIN SUSCEPTIBILITY TO SEPSIS AND BE A TARGET FOR IMMUNOTHERAPY IN ACUTE-ON-CHRONIC LIVER FAILURE**

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**Background:** Acute-on-chronic liver failure (ACLF) is characterised by rapid decompensation in liver function and multi-organ failure. Patients with ACLF have a marked susceptibility to infection and sepsis is a major cause of mortality. Monocyte dysfunction is associated with infection in other inflammatory pathologies. We postulate that defects in monocyte function account for the predisposition to infection in ACLF.

**Methods:** Ex-vivo phagocytic function of CD14+ monocytes was determined using FITC-labelled E.coli uptake in ACLF (n=12), decompensated chronic liver disease (DCLD, n=8), non-decompensated chronic liver disease (CLD, n=9), acute liver failure (ALF, n=14) and 37 healthy controls (HC). Phagocytosis was further evaluated in all 3 monocyte subpopulations (CD14+CD16−, CD14++CD16+, CD14lowCD16++) using pHrodo-labelled E.coli and lineage-specific gating strategy in 5 ACLF and 5 HC. Ex-vivo immunophenotypic analysis of monocyte subsets was performed using flow cytometry in all patient groups. Serum cytokine arrays (TNFα, IL-1β, IL-2, IL-4, IL-5, IFN-γ, IL-12, IL-10, IL-13) were determined using MSD®.

**Results:** Phagocytosis was markedly reduced in ACLF compared to stable CLD, DCLD and HC (Figure 1), and to a similar magnitude to that detected in ALF. The defect was most marked at day 1 versus day 3–5 following admission (69% vs 91%; p=0.06). Analysis of monocyte subsets reveals impairment of phagocytosis in all subpopulations compared to HC (Figure 2). Compared to HC, monocytes show significant reductions in scavenger receptor (CD163), antigen presentation and co-stimulatory molecule (HLA-DR and CD86) expression. Inflammatory cytokine profiling in ACLF demonstrates levels of pro-inflammatory mediators similar to that of HC (IFN-γ: 0.34 vs 0.08; [p=0.97], IL-1: 0.0 vs 0.3; [p=0.1], IL-12: 0.20 vs 0.07; [p=0.42]) and raised anti-inflammatory/Th2 type mediators (IL-13, 0.01 vs 3.4; p=0.02).

**Conclusion:** In ACLF, we demonstrate defects in monocyte function characterised by impaired phagocytosis and an anti-inflammatory phenotype that may account for the increased susceptibility to infection. Reversal of these functional defects may represent a promising novel target for immunotherapy in patients with ACLF.
**Background:** In most cells, ER stress triggers the classic UPR ER E-mail: manuweiss@yahoo.fr University, Paris, France

LPS stimulates the classic UPRER. Indeed, LPS induced both components of the 3 UPR signaling pathways (XBP1S, XBP1U, ATF6A, EIF2K1, EIF2K2 and EIF2S1) and major UPR target genes such as HSPA5, HSP90 and EDEM. LPS also enhanced induction of all cytokine genes by stimulating MAPK and NF-kappaB pathways and induced a battery of cytokine genes and proteins. Finally, LPS did not inhibit the tunicamycin-induction of the classic UPR³.

**Conclusion:** LPS-stimulated PBMCs from patients with cirrhosis have an induction, and not the expected inhibition, of the classic UPR³. This may play a role in enhanced LPS-induced innate immune response which characterizes cirrhosis.

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**610 PROGNOSTIC POTENTIAL OF CARDIAC AND PROINFLAMMATORY MARKERS IN CIRRHOSIS**

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**Background and Aims:** Inflammation and cardiac dysfunction seem to play an important role in the development of extrahepatic complications leading to increased mortality in patients with cirrhosis. A number of novel cardiac markers such as proANP, copeptin, and high-sensitivity troponin T (hs-TnT) and proinflammatory markers including soluble urokinase-type plasminogen activator receptor (suPAR) and high-sensitive C-reactive protein (hs-CRP) have been shown to be related to these complications. We aimed to investigate if cardiac and proinflammatory markers are related to markers of severity of liver disease, cardiac and hemodynamic changes, and survival.

**Methods:** 193 cirrhotic patients (Child class: A=46; B=97; C=50) had a full hemodynamic investigation performed with measurement of splanchnic and systemic hemodynamics and measurement of circulating levels of proANP, copeptin, hs-TnT, hs-CRP, and suPAR.

**Results:** SuPAR, hs-CRP, and hs-TnT were significantly different throughout the Child classes (Table, p < 0.01; p < 0.01; p < 0.02). All markers except copeptin correlated with Child score (p < 0.01). ProANP and suPAR correlated to indicators of disease severity in cirrhosis including hepatic venous pressure gradient (HVPG) (r = 0.24 and r = 0.34; p < 0.001) and systemic vascular resistance (r = -0.24 and r = -0.33; p < 0.001). Cardiac (proANP, hs-TnT, p < 0.01) and proinflammatory (hs-CRP, suPAR; p < 0.05) markers were associated with mortality in a univariate Cox analysis, however the strongest predictors of mortality in a multivariate Cox analysis were hs-TnT, ascites and HVPG (reg.coeff.: 0.34, p < 0.001; 0.16, p < 0.001; 0.06, p = 0.04).

**Conclusion:** Markers of cardiac dysfunction and inflammation are significantly associated with disease severity, degree of portal hypertension and survival in cirrhosis. In particular, hs-TnT and suPAR seem to contain prognostic information.

<table>
<thead>
<tr>
<th>Child Class</th>
<th>A (n=46)</th>
<th>B (n=97)</th>
<th>C (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median IQR</td>
<td>Median IQR</td>
<td>Median IQR</td>
</tr>
<tr>
<td>hs-TnT (ng/L)</td>
<td>3.0</td>
<td>3.0; 4.7</td>
<td>3.0</td>
</tr>
<tr>
<td>ProANP (pmol/L)</td>
<td>513</td>
<td>366; 666</td>
<td>495; 882</td>
</tr>
<tr>
<td>Copeptin (pmol/L)</td>
<td>4.7</td>
<td>3.2; 11.9</td>
<td>3.9; 12.6</td>
</tr>
<tr>
<td>SuPAR (ng/mL)</td>
<td>5.5</td>
<td>4.7; 9.0</td>
<td>9.9*</td>
</tr>
<tr>
<td>hs-CRP (mILU/mL)</td>
<td>0.25</td>
<td>0.14; 0.47</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Significant differences: *from class A (p < 0.02), **from class A and B (p < 0.01).
**02c. CIRRHOSIS AND ITS COMPLICATIONS: BLEEDING**

### 611 PORTAL HYPERTENSIVE GASTROPATHY IN LIVER CIRRHOSIS IN CHILDREN

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**Introduction:** Portal hypertensive gastropathy (PHG) and gastric antral vascular ectasia (GAVE) syndrome are recently characterized entities that may be associated with gastrointestinal bleeding in patients with and without liver cirrhosis. Up to 65% of patients with portal hypertension of cirrhosis will develop PHG, but it can also occur in the setting of non-cirrhotic portal hypertension. In patients with portal hypertension, PHG is often associated with the presence of esophageal and/or gastric varices. Mechanisms involved in the pathogenesis of PHG have not been fully elucidated.

**Aim of study:** To assess the existence of PHG in children with liver cirrhosis and to make correlations between PHG and clinical parameters and evolution.

**Material and Methods:** The study group consisted of 52 patients with liver cirrhosis (mean age 13 years), diagnosed and monitored during 8 years (2004–2011) in the Pediatric Clinics of Iasi. Upper digestive endoscopy was performed in all patients. Portal hypertensive gastropathy lesions have been described according to current criteria. Presence of Helicobacter pylori was also assessed.

**Results and Discussions:** PHG 1st grade was found in 36 patients and 2nd grade in 16 patients. There was no appearance of acute erosive gastritis or bleeding. Appearance of ‘snake skin’ gastric mucosa, PHG characteristic lesions arises in the natural history of portal hypertension in 10 cases, in a mean period of 48 months. Helicobacter pylori has been found in 24 patients, with higher prevalence in patients with 1st grade PHG (p = 0.003). Statistical correlations were made, appearance of gastric mucosa suggestive of portal-hypertensive gastropathy positively correlated with the presence of ascites and low albumin levels (p = 0.011 and 0.023, respectively).

**Conclusions:** PHG is a common finding in upper endoscopy in children with liver cirrhosis, requiring careful evaluation in order to predict disease outcome and risk of gastrointestinal bleeding. Helicobacter pylori infection needs specific treatment, carefully conducted in the context of the main disease.

### 612 EARLY TIPS IN PATIENTS WITH ACUTE VARICEAL BLEEDING AND THE EFFECT ON THIRTY DAY AND SIX MONTH MORTALITY RATES – A SINGLE CENTRE EXPERIENCE


**Methods:** All TIPS performed at the RLUH between December 2010 and August 2012 were reviewed. All patients fulfilling criteria were included. Data including time of bleed, index endoscopy, TIPS, aetiology of liver disease, Child–Pugh and MELD scores, abstinence rates, and mortality rates were obtained from the RLUH computer records and patient records.

**Results:** Forty-seven TIPS were performed at the RLUH during the time period specified. 31 patients had early TIPS as management for variceal haemorrhage following index endoscopy, 58% (n = 18) were from an ITU setting. The mean age was 50.9 and the aetiology of underlying liver disease was recorded as alcohol 68.8% (n = 22), alcohol and hepatitis C 21.9% (n = 7) and other 9.4% (n = 3). Results are displayed in table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean MELD</td>
<td>16.4 (7–30)</td>
<td>–</td>
</tr>
<tr>
<td>Mean Child Pugh</td>
<td>16.2 (7–12)</td>
<td>–</td>
</tr>
<tr>
<td>Median Bleed to TIPS time (hrs)</td>
<td>13.59 (0.1–148.00)</td>
<td>–</td>
</tr>
<tr>
<td>Mean Pre TIPS portal pressure gradient (mmHg)</td>
<td>22.8 (10–36)</td>
<td>–</td>
</tr>
<tr>
<td>Mean Post TIPS portal pressure gradient (mmHg)</td>
<td>7.9 (2–13)</td>
<td>–</td>
</tr>
<tr>
<td>30 Day Mortality</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>6 Month Mortality</td>
<td>19.3</td>
<td></td>
</tr>
<tr>
<td>Rebleed</td>
<td>3</td>
<td>9.7</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>5</td>
<td>16.1</td>
</tr>
</tbody>
</table>

**Conclusion:** The use of early TIPS improves mortality with manageable side effects. Ongoing alcohol use post TIPS is a major cause of 6 month mortality. Early TIPS for variceal bleeding may be considered as a first line treatment option.

### 613 USEFULNESS OF ACTIVE BLEEDING AS PROGNOSTIC FACTOR IN CHILD–PUGH B CIRRHOTIC PATIENTS WITH ACUTE VARICEAL BLEEDING. IS EARLY TIPS JUSTIFIED?


**Background and Aims:** Active bleeding has been reported as prognostic factor in UGB secondary to esophageal varices in cirrhotic patients. Recently, the use of an early transjugular intrahepatic portosystemic shunt (TIPS) has been proposed in Child–Pugh B (CPB) cirrhotic patients with active bleeding during endoscopy. The aim of our study is to determine short and long term outcome of a cohort of CPB cirrhotic patients consecutively admitted to our hospital with acute variceal bleeding (AVB).

**Patients and Methods:** A cohort study. During 2006 and 2011, 299 episodes of UGB secondary to esophageal varices were admitted, 48 CPA (16.6%), 153 CPB (52.9%) and 88 CPC (30.4%). We included in our study 102 consecutive CPB cirrhotic patients with a first episode of AVB in the study period. We collected demographic, analytical and bleeding episode variables. We defined active bleeding as the presence of spurting or oozing from esophageal varices on endoscopy.

**Results:** Treatment failure, mortality at 42 d. and actuarial survival at 1 year were 4/35 (11.4%), 4/35 (11.4%) and 64.7% in the group without active bleeding (NS). When we excluded patients that could hardly be treated with TIPS (>75y, HCC>Milan and creatinine>3 mg/dL) these figures were 3/20 (15%), 1/20 (5%) and 78.5% in the group without active bleeding (NS). Age, portal thrombosis, presence of hepatocellular carcinoma and ascites were statistically associated with 42 d. mortality although presence of ascites was the only independent prognostic factor of 42 d. mortality (OR 13.65; 1.40–133.21).

**Conclusions:** Active bleeding at the time of endoscopy is not significantly associated to failure of treatment, death at 6 weeks or
survival at one year in Child–Pugh B cirrhotic patients with AVB. The presence of ascites is associated with mortality at 42 d. It is necessary to better define the few Child–Pugh B cirrhotic patients that may benefit with an early TIPS.

614 HEMODYNAMIC MONITORING OF CHRONIC NON-SELECTIVE BETA-BLOCKER THERAPY AS PRIMARY PROPHYLAXIS OF VARICEAL HEMORRHAGE IN CIRRHOTIC PATIENTS: A META-ANALYSIS

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Background and Aims: Variceal bleeding in cirrhotic patients is a major cause of morbidity and mortality. Primary prophylaxis can be performed by administering non-selective beta-blocker therapy (NSBB) and is monitored by heart frequency, although this is unrelated to portal pressure. Monitoring of hemodynamic response to NSBB is still under debate. Our aim was to assess the predictive value of hemodynamic monitoring using hepatic venous pressure gradient (HVPG) in primary prophylaxis for variceal bleeding.

Methods: We conducted a systematic search in PubMed, Embase (OVID version), Web of Science, COCHRANE Library and meeting abstracts. Randomized controlled trials and case series concerning pharmacological primary prophylaxis, in relation with HVPG response and variceal hemorrhage, were included for analysis. Initially a random effects model was fitted, which resulted in a zero estimate between the study variances and a Cochrane effect analysis was executed with the Mantel-Haenszel method for heterogeneity which was far from significant. Therefore a fixed gradient (HVPG) in primary prophylaxis for variceal bleeding.

Results: 1000 studies were found and six studies, consisting of 308 patients, were selected based on selection criteria. One study that met the selection criteria was not used in our analysis due to the small number of patients and lack of bleeding events (de-Madaria). No significant heterogeneity (P > 0.70) was found between trials. According to the Mantel-Haenszel method, patients who were hemodynamic responders (HVPG ≤12 mmHg or reduction of ≥20% from baseline) to NSBB had a lower RR for variceal bleeding (0.13, 95% CI: 0.06–0.29) as compared to non-responding patients.

Conclusions: Hemodynamic responders to NSBB for primary prophylaxis of variceal bleeding have a low risk of bleeding as compared to non-responders. Hemodynamic monitoring in primary prophylaxis has potential for risk assessment in clinical practice and requires further assessment of clinical impact.

615 EARLY USE OF TIPS DECREASES REBLEEDING AND IMPROVES THE SURVIVAL IN CIRRHOTIC PATIENTS OF EVL ULCER BLEEDING: A PROSPECTIVE STUDY

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Introduction: Endoscopic variceal ligation (EVL) is the recommended method to manage esophageal variceal bleeding with lower variceal ulceration and rebleeding rates compared to sclerotherapy. However, a proportion of patients do develop EVL induced ulcers and present with massive bleeding. In these cases, endoscopic therapy is difficult and often unsuccessful and definitive methods, such as TIPS need to be evaluated.

Aim: To determine the effectiveness of reduction in portal pressure by polytetrafluoroethylene-covered transjugular stenting (TIPS) for band-induced esophageal ulcer bleeding.

Method: Following admission within 24 hours, consecutive patients with cirrhosis and EVL induced bleeding ulcers were prospectively treated using standard therapy (endoscopy and vasoactive drugs; Group 1, n=15) or standard therapy plus TIPS (Group 2, n=22). Primary end-point was survival at 6 wks. Secondary end-points were early and late rebleeding.

Results: Out of 488 cirrhotic patients who underwent EVL from 2010–11, 36 (7%) (Median age 42 (range 32–72), 90% males) developed bleeding EVL ulcer. These patients were enrolled and offered either of the two treatments. Baseline parameters including age, presence of ascites, HE, HRS, platelets, PT (INR), HVPG, Child and MELD score were similar in both the groups. After follow-up of 6 wks, 10 patients (62%) in group 1 and 5 (25%) in group 2 died (p < 0.05). The incidence of failure to control bleed/early rebleed was seen in 9 patients (56%) in group1 and in 1 (5%) in group 2 (p < 0.05). Similarly, the incidence of late rebleeding was seen in 5 patients (45.4%) in group 1 while in 1 (5%) in group2 (p <0.05). The median time to death was 2.5 (0.5–12) days and 13 (2–21) days in the two groups (p <0.05).

Conclusion: Around 7% patients develop life threatening EVL induced ulcer bleeding. Portal pressure reduction with the TIPS is significantly superior in the control of bleeding, preventing rebleeding and reduction in mortality.

616 ECONOMIC EVALUATION OF EARLY TIPS PROCEDURES WITH EPTFE COVERED STENT-GRAFTS CONFIGURED FOR TIPS COMPARED TO ENDOSCOPIC PROCEDURES TO MANAGE ACUTE VARICEAL BLEEDING

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Aims: To establish the resource and cost savings from the early use of transjugular intrahepatic portosystemic shunts (TIPS) procedures with ePTFE covered stent-grafts configured for TIPS (SG) compared to endoscopic procedures and pharmaceuticals in high risk patients (Child–Pugh class B/C) with acute variceal bleeding.

Background: Endoscopic therapies are currently the primary treatment for bleeding varices, with TIPS used when endoscopic treatment fails or when patients are not amenable to endoscopic intervention. Trials have shown clinical benefit (lower mortality, fewer re-bleeds and lower hepatic encephalopathy (HE)) from the

Figure: Forest plot, Mantel–Haenszel meta-analysis.

Conclusions: Hemodynamic responders to NSBB for primary prophylaxis of variceal bleeding have a low risk of bleeding as compared to non-responders. Hemodynamic monitoring in primary prophylaxis has potential for risk assessment in clinical practice and requires further assessment of clinical impact.
earlier use of TIPS in patients with persistent bleeding. There are currently no published cost-effectiveness analyses of this earlier use.

**Methods:** A Markov economic model was developed to measure the incremental resources and costs of early TIPS with SG, compared to endoscopic band ligation (EBL) plus pharmaceuticals, with TIPS as rescue therapy. Clinical data came mainly from published studies including an RCT (Garcia-Pagan 2010), whilst healthcare costs were from UK national databases. Events & costs were modelled over two years.

**Results:** Using early TIPS with SG compared to EBL plus pharmaceuticals was estimated to save £1,655 per patient over 2 years. The total treatment costs were £6,455 for TIPS and £8,110 for EBL, providing a net saving of £1,655 per patient. Early TIPS and subsequent re-interventions cost £4,332 more than the EBL arm. However, savings were accrued from fewer EBL procedures and pharmaceuticals (saving £3,223); fewer episodes of recurrent bleeding (saving £2,475) and reduced rate of severe HE (saving £290). Modelling 100 patients, mortality was reduced in the early TIPS arm, 28 patients compared to 63 in the EBL plus pharmaceuticals arm. Sensitivity analyses showed the results were sensitive to device costs, frequency of EBL procedures and the relative rates of severe HE per patient. Using TIPS with SG earlier to manage variceal bleeding was cost saving under all sensitivity analyses.

**Conclusion:** The model showed that early utilisation of TIPS with ePTFE covered stent-grafts configured for TIPS was cost saving and improved survival compared to EBL and pharmaceuticals for high risk patients (Child–Pugh class B/C) with acute variceal bleeding.

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**FREE SERUM CORTISOL LEVELS AS A PREDICTIVE FACTOR FOR SURVIVAL IN PATIENTS WITH LIVER CIRRHOSIS AND ACUTE VARICEAL BLEEDING**

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**Background and Aim:** Critical illness-related corticosteroid insufficiency has been reported in patients with cirrhosis and variceal bleeding (VB) based on synaechen testing and total cortisol assays. In cirrhosis, total serum cortisol (T) has been considered inaccurate and free serum cortisol (FC) has been suggested as the optimal test in assessing adrenal function. Salivary cortisol (SC) has been considered a surrogate marker for FC. We evaluated FC and SC in patients with cirrhosis and portal hypertension. ET-1 influences mechanisms of cirrhosis and portal hypertension. ET-1 plays major role in pathophysiological mechanisms of cirrhosis and portal hypertension. ET-1 influences portal pressure and development of complication by inducing intrahepatic and portal-systemic collateral vasconstriction which may lead to gastrointestinal variceal hemorrhage. It was also reported that patients and animal models with liver cirrhosis and portal hypertension have been observed with elevated insulin concentrations, insulin resistance, altered blood glucose adjustment capabilities and development of diabetes mellitus. The investigations on diabetes mellitus and glucose manipulation have indicated that exposure to high glucose concentrations enhanced vasocnstriction of different vascular beds. Besides, glibenclamide, a hypoglycemic agent and a highly selective ATP-sensitive K+ channels blocker, may cause vasoactive effect in different vascular beds.

**Aim:** This study was designed to survey the influences of diabetic condition, glucose concentration and hypoglycemic agents on the responsiveness of the intrahepatic vascular bed to ET-1 in cirrhotic rats.

**Methods:** Sprague-Dawley rats were received common bile duct ligation to induce cirrhosis and allocated to control or diabetic (streptozotocin 60 mg/kg) groups. Basic hemodynamics were measured and the in situ liver perfusion study was performed to different concentrations of ET-1 (10−8 M) when the intrahepatic vascular bed was incubated with Krebs solution, Krebs solution with 45 mM D-glucose and Krebs solution with 45 mM D-glucose and 1 mM glibenclamide.

**Results:** Body weight had significantly decreased and blood glucose had obviously elevated in diabetic rats compared to control rats. In situ liver perfusion showed that the perfusion pressure changes to ET-1 were much higher in diabetic group than in control group. Glucose perfusion did not significantly influence intrahepatic ET-1 responsiveness. Glucose plus glibenclamide markedly reduced intrahepatic vascular contractile response to ET-1 both in normoglycemic and hyperglycemic cirrhotic rats.

**Conclusions:** The current study suggests that diabetes enhances intrahepatic vascular responsive to ET-1 in cirrhotic rats. In patients with cirrhosis and diabetes, alteration of intrahepatic vascular responsive to ET-1 should be considered when the patients are receiving glibenclamide therapy.
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THE ROAD TO A SUCCESSFUL TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT (TIPS) SERVICE: EXPERIENCE OF A UK TEACHING HOSPITAL
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Methods: Consecutive patients undergoing attempted TIPS from 2004–2009 were identified and demographics, disease aetiology, synthetic function, technical and clinical success defined. We subsequently instituted the following changes to our TIPS service: 1. an elective TIPS anaesthetist list; 2. use of VIATORR® PTFE-covered stents; 3. two interventional radiologists per procedure.

Outcomes were re-analysed from 2009–2012.

Results: From 2004–2009, 36 patients (7.2 cases/year) were identified (63.8% male; mean (SD) age 49.8 ± 14.7 years) Child’s A 22%/B 61%/C 17%; aetiology ALD 50%, NASH 17%, mixed aetiology 8%, PBC 6%). Indications for TIPS were variceal haemorrhage (68.2%), refractory ascites (27.3%) and portal hypertensive gastropathy (2.8%). TIPS technical success was 22/36 (61.1%). In those with refractory ascites (27.3%) and portal hypertensive gastropathy 6%). Indications for TIPS were variceal haemorrhage (68.2%), ascites (29.7%) and hydrothorax (1.6%). TIPS technical success was 22/36 (61.1%). In those with successful TIPS, clinical success (resolution of bleeding or ascites) occurred in 13/22 (59.1%). Early complications occurred in 6/22 (27.2%) and 12-month survival was 9/20 (45%) (mean follow up of surviving patients 18 11.6) months). From 2004–2009, 64 patients (21.3 cases/year) underwent TIPS (69% male; mean (SD) age 53 (± 12) years; Child’s A 13%/B 44%/C 44%; aetiology ALD 61%, NASH 9%, HCV/ALD 8%). Indications were variceal haemorrhage (68.8%), ascites (29.7%) and hydrothorax (1.6%). TIPS technical success was 62/64 (96.8%, p < 0.0001) with clinical success in 53/60 (88.3%, p = 0.009). 10/62 (16.1%) had early complications (4 encephalopathy; 2 infections (1 resulting in death); 2 pulmonary oedema; 2 failure to control bleeding). A further 12/59 (20.3%) developed late encephalopathy. 12 month survival increased to 29/44 (65.9%, p = 0.17) (mean follow up of surviving patients 18 (± 11.6) months).

Conclusions: We have demonstrated that technical and clinical outcomes, in patients undergoing TIPS, can be improved by establishing a coherent multidisciplinary approach including dedicated hepatology assessment, combined with adequate interventional radiology and anaesthetic support. This co-ordinated approach is essential to deliver an effective service at a time when demand for TIPS is likely to increase[1].

Reference(s)

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IS THE DIAGNOSIS OF IDIOPATHIC NON-CIRRHOTIC PORTAL HYPERTENSION BE CONSIDERED IN PATIENTS WITH PRIMARY ANTIBODY DEFICIENCIES?
E-mail: ilapenta@hotmail.it

Background and Aim: Idiopathic Non-Cirrhotic Portal Hypertension (INCPH) has been reported in association with immunodeficiencies. The aim of this study was to describe the prevalence of portal hypertension (PH) in a population of patients with primary immunodeficiencies (PID) without known cause of liver disease.

Methods: 122 patients with PID (106 Common Variable Immunodeficiency, 5 X-Linked Agammaglobulinemia, 2 Hyper IgM syndrome) regularly followed-up were included in the analysis (Table 1). Five patients with concomitant HCV or HBV infection were excluded. Esophageal varices were searched by upper endoscopy. Spleen and portal diameters were evaluated by ultrasonography (US). Blood samples was collected by patients and immunophenotyping was performed with a combination of fluoro-chrome labeled monoclonal antibodies. FACS analysis were performed on a FACScalibur flow cytomter using the CellQuest and FlowJo software. Clinical manifestations were retrospectively evaluated by clinical files.

Results: Two patients had oesophageal varices: none bled. 30 patients (25%) had portal vein ≥13 mm and splenomegaly; 41 pts (36%) had isolated splenomegaly. Portal vein enlargement was highly correlated to splenomegaly and platelet counts (Table 2 & Figure 1). Compared to the 87 patients without sings of PH, the patients with PH had similar age and length of follow up; increased frequency of bronchectasis (p = 0.007), gastroenteritis (p = 0.02), chronic lymphoid hyperplasia (p = 0.002); cytopenia (p = 0.03); a more severe defect of immunoglobulins production (p = 0.01) decreased frequency of CD4 naïve (p = 0.01), increased of CD4 memory (p = 0.007) and late CD8 effector T cells (p = 0.02) indicating a more severe immunodeficiency. In contrast the frequency of autoimmune manifestations were similarly distributed in the two groups. Hypothetically, in the patients with a more severe PID, bacterial infection of the gut and subsequent obstruction of small portal veins due to endoheilitis may be involved in the etiology of PH.

Table 1
<table>
<thead>
<tr>
<th></th>
<th>n=66</th>
<th>Bilirubin (mg/dl)*</th>
<th>0.5±0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>49±15</td>
<td>GGT (mg/dl)*</td>
<td>28±26</td>
</tr>
<tr>
<td>Pts with XLA*</td>
<td>5</td>
<td>INR</td>
<td>1±0.08</td>
</tr>
<tr>
<td>Pts with Hyper IgM syndrome*</td>
<td>2</td>
<td>RBC (cell/mm³)*</td>
<td>4,700,000±9,430</td>
</tr>
<tr>
<td>Pts with CVID*</td>
<td>106</td>
<td>Hb (g/dl)*</td>
<td>13.4±6</td>
</tr>
<tr>
<td>Splenomegaly*</td>
<td>41</td>
<td>WBC (cell/mm³)*</td>
<td>6,300±2,400</td>
</tr>
<tr>
<td>Portal vein enlargement*</td>
<td>30</td>
<td>PLT (cell/mm³)*</td>
<td>214,000±90,330</td>
</tr>
<tr>
<td>Esophageal varices*</td>
<td>2</td>
<td>IgG (mg/dl)*</td>
<td>267±175</td>
</tr>
<tr>
<td>GOT (mg/dl)*</td>
<td>24±12</td>
<td>IgA (mg/dl)*</td>
<td>27±36</td>
</tr>
<tr>
<td>GPT (mg/dl)*</td>
<td>23±18</td>
<td>IgM (mg/dl)*</td>
<td>42±79</td>
</tr>
</tbody>
</table>

*Mean±SD; *No. of patients.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Pts with portal hypertension (n=30)</th>
<th>Pts without portal hypertension (n=87)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal vein diameter*</td>
<td>15±2</td>
<td>10±1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hepatomegaly*</td>
<td>17 (57%)</td>
<td>26 (30%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Liver diameter*</td>
<td>17±2</td>
<td>15±2</td>
<td>0.008</td>
</tr>
<tr>
<td>Spleen diameter*</td>
<td>16±5</td>
<td>12±2</td>
<td>0.001</td>
</tr>
<tr>
<td>Pts with cytopenia*</td>
<td>14 (47%)</td>
<td>23 (26%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Pts with leucopenia*</td>
<td>7 (23%)</td>
<td>17 (20%)</td>
<td>NS</td>
</tr>
<tr>
<td>Pts with thrombocytopenia*</td>
<td>12 (40%)</td>
<td>13 (15%)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Portal enlargement.

*Mean±SD; *No. of patients (%).

Figure 1. Regression curves.
CONCLUSIONS: Signs of PH, i.e. esophageal varices or portal vein enlargement are frequently detected in patients with PID without viral or drug induced liver disease. Patients with PH seem to represent a subgroup with a more severe PID. Sings of PH should be actively searched in the patients affected by PID.

621 IMPROVING RISK PREDICTION IN ACUTE VARICEAL BLEEDING WITH OBJECTIVE VARIABLES: THE ROLE OF MELD

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Background and Aims: Acute variceal bleeding (AVB) in cirrhosis has a high mortality (15–20%). Previously described prognostic models are seldom used, in part due to the lack of external validation. In addition, most of them include subjective variables (Child, active bleeding), which are inconsistently evaluated. An objective and robust estimation of the risk of death is needed, especially since the demonstration that early-TIPS improves survival in high-risk patients.

This study aimed at improving the risk prediction in patients with AVB, by testing the performance of recently described models in a contemporary series of patients. A prediction rule with purely objective variables was developed to select high-risk patients.

622 MASS-LIKE ESOPHAGEAL VARICES (F3) ARE DIFFERENT FROM TORTUOUS VARICES (F2) IN CLINICAL OUTCOME AND EUS FINDINGS

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Background and Aims: Esophageal varices are simply defined by small or large ones based on their size (5 mm). However, 3-grade system (F1, F2, and F3) also has been used in Eastern countries. We investigated difference between F2 and F3 varices in terms of their clinical outcomes and endoscopic ultrasonographic (EUS) findings.

Methods: Patients with large esophageal varices defined by AASLD and no history of previous endoscopic therapy were included. F2 was defined as tortuous or beaded varices. F3 was defined as asymmetrically large mass-like varices. Perforating veins, esophageal collateral veins, and cardiac submucosal venous plexus were evaluated using a 20 MHz-miniature ultrasound probe during conventional endoscopic examination. After endoscopic band ligation (EBL), clinical outcome including variceal bleeding and aggravation of varices was assessed.

Results: In total, 47 patients were included. Twenty patients (43%) had F3 varices. There were no differences between F2 and F3 groups in age, gender, causes of cirrhosis, proportion of Child-Turcott-Pugh class A, severe red color signs (p > 0.05). On EUS examination, perforating veins that connect esophageal collateral veins with varices were more common in F3 than in F2 varices (75% vs. 44%, p = 0.036). Size of large esophageal collateral veins were similar between two groups (4.1 ± 1.7 mm in F2 vs. 4.0 ± 2.1 mm in F3, p = 0.898). EBL was performed in 45 patients and 24 patients were followed up more than 1 year. During 1 year, acute variceal bleeding was noted in 3 cases (12.5%). Bleeding varices or enlarged varices that required therapeutic intervention was noted in 6 out of 8 patients (75%) with F3 varices and 4 out of 16 patients (25%) with F2 varices (p = 0.032).

Conclusions: F3 varices have more perforating veins and higher risk for recurrence than F2 varices. It should be considered that large esophageal varices be classified as F2 (tortuous) and F3 (mass-like).
Background and Aim: Rebleeding after an initial oesophageal variceal haemorrhage remains a significant problem despite therapy with band ligation +/- non-selective β-blockers. Carvedilol is a vasodilating non-selective β-blocker with alpha-1 receptor and calcium channel antagonism. It has been shown to have greater portal hypotensive effects than propranolol and has shown clinical benefit in the prevention of a first variceal bleed. Our aim was to compare oral carvedilol with band ligation in the prevention of rebleeding following a first variceal bleed.

Methods: Patients who were stable 5 days after presentation with a first variceal haemorrhage and had not been taking (or had contraindications to) β-blockers, were randomised to oral carvedilol (62.5 mg daily then 12.5 mg daily after one week if tolerated) or a band ligation programme. Patients were followed up at clinic after one week, monthly, then 3-monthly.

The primary end-point was variceal rebleeding, on intention-to-treat analysis.

Results: 63 patients were randomised, 32 to carvedilol and 31 to banding. Fifty-six (89%) patients had alcohol related liver disease. There was no difference in baseline mean age (51yrs ±10.9 and 50yrs ±13.0) or median Childs Pugh score (9, IQR 6–11 and 9, IQR 8–11) for patients randomised to carvedilol or banding respectively. Mean follow-up was 23 months. Compliance was 72% and 90% for carvedilol and banding respectively (p=0.14) and there was no difference in the number of serious adverse events between the two groups. Variceal rebleeding occurred during follow-up in 12 (37.5%) and 9 (29.0%) patients in the carvedilol and banding groups respectively (p=0.72), with mortality 25.0% and 51.6% respectively (p=0.058). This interim analysis indicates that to show a significant difference in rebleeding, 482 patients would be required in each group.

Conclusions: These results suggest that carvedilol is not clearly superior to band ligation in the prevention of variceal rebleeding. However there appears to be a survival benefit for patients taking this drug compared with those undergoing banding, which requires further exploration.

Methods: We analyzed all patients who received a SEMS between February 2012 and October 2012 for acute EVB failing VBL. Data on sex, age, procedural details, rebleeding and clinical course were collected prospectively, anonymized, and analyzed. Endoscopic interventions were exclusively performed by endoscopists experienced in therapeutic hemostatic interventions. VBL using a 6-Shooter Multi-band Ligator® was the principal modality to obtain hemostasis. Insertion of the SEMS (Sx-Ella Danis, fully covered, Ø 30/25/30 × 135 mm, ELLA-CS) was considered if endoscopic hemostasis failed and if the patient was not considered candidate for TIPS at that moment.

Results: Five patients (M/F 3/2; median age 58 (range 48–78)) received a SEMS for uncontrollable EVB. None of them was candidate for TIPS at time of bleeding due to severe comorbid illnesses (i.e. sepsis, metastasized cancer) (Table 1). Successful initial hemostasis was achieved with SEMS in all five patients (Figure 1), and sustained hemostasis in four of them (80%). One patient experienced a rebleeding 7 days after SEMS placement, which was considered due to suboptimal wall pressure of the SEMS at the level of the gastroesophageal junction. Stents were removed in 2 patients after >14 days and remained in situ till death in the 3 other patients (range 6–214 days). No SEMS-related complications occurred during follow-up.

Conclusion: SEMS can be a definite treatment for uncontrollable oesophageal variceal bleeding in patients with a limited life expectancy and those (currently) unsuitable for TIPS. Inclusion of such patients in future trials on the applicability of SEMS for variceal bleeding is recommended to increase the generalizability of results.

625 MACROPHAGE ACTIVATION IS A PROGNOSTIC PARAMETER FOR VARICEAL BLEEDING AND OVERALL SURVIVAL IN PATIENTS WITH LIVER CIRRHOSIS


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Background and Aims: Soluble CD 163 (sCD163) is shed in the blood circulation by activated macrophages, correlates strongly with the hepatic venous pressure gradient (HVPG) and is thereby a good indicator for portal hypertension. It is unknown if sCD163 correlates with the risk of variceal bleeding and overall survival (OS) in patients with liver cirrhosis. We performed a prospective study to investigate if sCD163 serum levels correlate with the risk of variceal bleeding and with OS in cirrhotic patients.

Methods: Patients with liver cirrhosis were prospectively enrolled and followed until death or last contact. At the day of inclusion into the study blood samples were taken and the sCD163 serum levels were assessed by ELISA (Enzyme-linked immunosorbent assay). The time until the end points death and variceal bleeding were assessed and the risks of death or variceal bleeding were calculated with uni- and multivariate Cox regression analyses.

Results: High sCD163 levels (>4100ng/ml) were associated with variceal bleeding and death independently from the MELD (model of end stage liver disease) score, age and gender. Furthermore, high sCD163 levels were associated with gastrointestinal bleeding independently from the variceal stage and red spots.

Conclusions: The sCD163 serum level is a new independent non-invasive risk factor for death and variceal bleeding in cirrhotic patients.
ELEVATED LEVELS OF VON WILLEBRAND FACTOR ARE ASSOCIATED WITH MAINTAINED NORMAL PRIMARY HEMOSTASIS IN THROMBOCYTOPENIC PATIENTS WITH LIVER CIRRHOSIS

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Background and Aims: During primary haemostasis platelets adhere, mediated by von Willebrand factor, to subendothelial structures. Thrombocytopenia is commonly seen in patients with liver cirrhosis, while vWF levels are elevated. The presence of concomitant thrombocytopathy is still discussed. We aimed to evaluate the platelet function analyzer PFA-100 in cirrhotic patients to detect the presence of thrombocytopathy as well as to investigate the influence of elevated von Willebrand factor on primary haemostasis in thrombocytopenic patients.

Patients and Methods: In 57 patients who were evaluated for liver transplantation due to end-stage liver disease the PFA-100, platelet count, haematocrit, vWF-activity and vWF-antigen were determined. Closure time for the PFA-100 was measured using standard cartridges containing collagen-ADP (COL-ADP) or collagen-epinephrine (COL-Epi). For detection of thrombocytopathy the PFA-100 was evaluated in patients with a platelet count >150/μl and a haematocrit >27%. The influence of increased vWF on PFA-100 closure times was evaluated in patients with thrombocytopenia (defined as a platelet count <150/μl). Patients taking antiplatelet drugs or NSAIDs were excluded.

Results: Of the 57 patients 13 were classified as Child–Pugh A, 26 as Child–Pugh B and 18 as Child–Pugh C. Mean (±SD) platelet count was 115/μl (±67), haematocrit 33.8% (±6.4), vWF-antigen 393.8% (±209.2) and vWF-activity 314.1% (±174.6). Median closure times for COL-Epi were 176 sec (±61) and 159 sec (±73) for COL-ADP. There were pathological results for the PFA-100 in 29 of 57 (50.9%) patients for COL-Epi and in 29 of 48 (60.4%) patients for COL-ADP. In 11 patients without thrombocytopenia a prolonged closure time was found in 5 (COL-Epi) and 3 (COL-ADP) patients; in 3 of them both test were pathologic. Thrombocytopenic patients with a normal PFA-100 after induction with COL-Epi had significant higher levels of vWF-antigen than patients with a pathological PFA-100 (494.8±269.7% vs. 351.4±129.5%, p = 0.039).

Conclusion: There is evidence for thrombocytopathy in patients with end-stage liver disease and normal platelet count. Increased levels of vWF in patients with liver cirrhosis might compensate for a reduced platelet count to maintain normal primary haemostasis. In thrombocytopenic patients substitution of vWF might therefore be an interesting approach to improve primary haemostasis.
for HCC are available only for patients with early stage tumors, so it is necessary to discover a precise method for detecting early stage HCC. MicroRNAs (miRNAs) play important roles in gene regulatory networks, and aberrant miRNA expression has been observed in human hepatocarcinogenesis. This study compared the expression of miRNA in surgically resected HCC tissues and surrounding non-tumor tissue from 23 patients with early stage HCC. We also compared the expression of miRNA in serial stages of HCC in a mouse HCC model. This study looked for miRNAs that can be used as biomarkers of HCC.

**Methods:** miRNAs were isolated from mouse liver tissues, surgically resected HCC tissues, and surrounding non-tumor tissues from 23 HCC patients and used to synthesize cDNA. We designed primers for 50 miRNAs, which were selected based on published microarray studies. We used SYBR Green quantitative RT-PCR (qRT-PCR) assays to compare the expression of miRNAs in HCC and surrounding non-HCC tissues in humans and in the mouse HCC model.

**Results:** Six miRNAs were highly up-regulated in early stage HCC; miR-17–5p, 24, 25, 107, 221, and 222 were significantly up-regulated in 65, 61, 74, 65, 83, and 78% of the HCC samples, respectively. In more than 90% of the HCC samples (21 of 23 samples), at least one of miR-221 and miR-25 was up-regulated, as compared with adjacent non-tumor tissues. miR-17–5p, 24, 25, 221, and 222 were also significantly up-regulated in the mouse HCC model. In addition, miR-222 was progressively up-regulated with the stage of HCC in this model.

**Conclusions:** We identified miRNAs that are significantly up-regulated in early HCC. More than 90% of the patients with an early stage HCC had elevated expression of either miR-221 or miR-25, suggesting that the combination of these two microRNAs might be an effective biomarker for detecting early HCC. In addition, miR-222 is suitable for determining the stage of HCC.

**629 ANALYSIS OF AFP, CYTGENETIC ABERRATIONS AND MTHFR GENE POLYMORPHISM IN HEPATOCELLULAR CARCINOMA (HCC) PATIENTS**

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**Background and Aims:** Hepatocellular carcinoma (HCC) is one of the most prevalent human cancers, ranking the fifth most common worldwide, with a high incidence in developing country. The usefulness of alpha-fetoprotein (AFP) as a tumor marker is well known. Conventional cytogenetic studies have established prognostic scores such as MELD- and CLIP score, liver function parameters and overall survival (OS). The concentration of the soluble IL-33 receptor sST2 significantly differed between the 3 cohorts with highest levels in patients with liver cirrhosis without HCC (p < 0.0001) and lowest levels in healthy controls (p < 0.0001). Patients with liver cirrhosis without HCC showed almost twice as high sST2 levels as patients with HCC. In contrast, IL-33 levels were similar between the cohorts.

In patients with HCC sST2 levels were significantly associated with OS (p = 0.002) and significantly correlated with the CLIP- and the MELD-score, AFP, AST and CRP. IL-33 levels did not correlate with OS, the CLIP- or the MELD-score or liver function parameters in either of the groups.

**Conclusion:** In the present study the serum concentration of the soluble IL-33 receptor sST2 was associated with prognosis of HCC indicating a potential role of the IL-33/sST2 signal transduction in HCC pathogenesis. We therefore consider sST2 a new prognostic marker in HCC.
to compare clinical, biological, pathological features as well as prognosis of HCC in patients with or without MS.

**Material and Methods:** 925 consecutive patients with HCC were included between 01/2005 and 03/2012. Clinical, biological, pathological, radiological data were recorded and compared between patients with and without MS (NCEP ATP III). Follow-up included CT-Scan or RMI every 3/6 months (median follow-up 16.5 months).

**Results:** MS was present in 29% of patients who were older (68.3 vs 64.48 years; p < 0.001) and often male (90% vs 82%; p = 0.02). Underlying liver disease was less advanced and HCC was developed more often on non-cirrhotic livers (28.8 vs 21.5%; p = 0.01). Diagnosis is delayed in the MS group, with a low screening rate (28 vs 40%; p < 0.001).

Overall survival (OS) was similar in the 2 groups (2.68 years (MS) vs 3.23; p = 0.44). Recurrence free survival (RFS) after curative treatment was similar in the 2 groups (2.18 years (MS) vs 2.49; p = 0.509) but late recurrences (>2 years) were more frequent in the MS group.

A subgroup analysis was performed, comparing patients with MS only (n = 80), with those with alcoholic disease (n = 271) or hepatitis C (n = 106). HCC occurred more often on non-cirrhotic livers in the MS group (49%) vs 15% (HCV) and 13% (OH) (p = 0.01). Overall survival was similar for MS group and HCV group (36 months) but inferior for alcoholic disease group exhibiting a more severe underlying liver disease (22 months). No difference was observed between the 3 groups for the RFS after curative treatments.

**Conclusion:** One third of patients diagnosed with HCC had MS, frequently associated with an excessive alcohol intake. In 9% of cases, MS was the only risk factor found. HCC with MS occurred more often on non-cirrhotic livers. In spite of a less severe hepatic disease in the MS group, OS was not better and the rate of recurrence, especially late recurrences (>2 years) are high.

**632 AETIOLOGY OF CHRONIC LIVER DISEASE INFLUENCES CLINICAL PRESENTATION AND OUTCOME OF HEPATOCELLULAR CARCINOMA: A SINGLE CENTER COHORT STUDY**

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**Background and Aims:** The epidemiologic patterns of chronic liver disease (CLD) and hepatocellular carcinoma (HCC) differ by geographic area; in Italy HCC incidence increased in recent years. We studied the impact of CLD aetiology on HCC patients’ clinical presentation and outcome.

**Methods:** From year 2000 to 2010 490 consecutive HCC-patients were diagnosed and followed-up at our Hepatology Unit. CLD aetiology was HBV in 66 (13.5%), HCV in 314 (64.1%), HBV/HCV in 7 (1.4%) and non viral (alcohol/NASH/NALFD) in 103 (21%). Occult HBV (HBsAg+/anti-HBc+) was present in 32.4% of HCV− and 42.6% of HCV+ patients. Age, sex, baseline features, therapy and outcomes were studied.

**Results:** In Table 1 we report significant (Chi-square/ANOVA) different variables by CLD-aetiology. Age and profiles of HBV/HCV/non-viral HCC-patients differed significantly. In Alcohol/NASH/NALFD patients prevalence of single HCC <5 cm (46.2%) was similar to that of HBV (45.5%); in spite of less frequent diagnosis on screening; shorter survival was influenced by end-stage cirrhosis (23.1%) and extrahepatic diseases (23.1%). HBV-patients were younger and showed higher HCC-related death rate possibly because oral anti-HBV treatment delayed progression of cirrhosis. Survival independent factors (Cox-regression) were Child–Pugh, baseline HCC stage, vascular involvement and treatment.

**Table 1.**

<table>
<thead>
<tr>
<th>Age (mean, years)</th>
<th>Overall</th>
<th>HBsAg+</th>
<th>HCV+ HCC− Anti-HBc+</th>
<th>HCV+ HCC+ Anti-HBc−</th>
<th>Non-viral</th>
</tr>
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<tbody>
<tr>
<td>67.0</td>
<td>61.9</td>
<td>68.8</td>
<td>68.0</td>
<td>66.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>74.0%</td>
<td>91.0%</td>
<td>69.5%</td>
<td>63.2%</td>
<td>81.7%</td>
</tr>
<tr>
<td>Cirrhosis (prevalence)</td>
<td>96.2%</td>
<td>93.5%</td>
<td>98.9%</td>
<td>96.1%</td>
<td>95.2%</td>
</tr>
<tr>
<td>Liver function: Child–Pugh A</td>
<td>77.5%</td>
<td>81.0%</td>
<td>83.0%</td>
<td>82.0%</td>
<td>63.7%</td>
</tr>
<tr>
<td>Liver decompensation</td>
<td>20.6%</td>
<td>16.7%</td>
<td>11.6%</td>
<td>17.6%</td>
<td>13.6%</td>
</tr>
<tr>
<td>HCC Diagnosis at screening</td>
<td>54.8%</td>
<td>54.1%</td>
<td>68.8%</td>
<td>71.4%</td>
<td>34.5%</td>
</tr>
<tr>
<td>HCC Stage: Unifocal ≤5cm</td>
<td>54.5%</td>
<td>45.5%</td>
<td>60.0%</td>
<td>60.1%</td>
<td>46.2%</td>
</tr>
<tr>
<td>HCC treatment (yes)</td>
<td>79.6%</td>
<td>86.3%</td>
<td>75.6%</td>
<td>84.6%</td>
<td>69.4%</td>
</tr>
<tr>
<td>Cause of death: HCC</td>
<td>64.9%</td>
<td>85.2%</td>
<td>70.0%</td>
<td>57.7%</td>
<td>53.8%</td>
</tr>
<tr>
<td>Post-referral to MS</td>
<td>75.4%</td>
<td>77.7%</td>
<td>71.9%</td>
<td>77.5%</td>
<td>67.1%</td>
</tr>
</tbody>
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**Conclusion:** Clinical profile and outcome of HCC patients differs significantly according to the aetiology of the associated CLD. Decision making in both clinical trials and practice should take into account the different profiles when comparing different HCC therapeutic strategies.

**633 A NOVEL APPROACH TO IMAGE ANALYSIS FOR HEPATIC ONCOLOGY DIAGNOSIS BASED ON FRAC TAL GEOMETRY. PRELIMINARY RESULTS**

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**Introduction:** Biological and medical systems are predominantly irregular, complex and non-linear, since cannot be quantified by classical geometry approach. Novel mathematical algorithms can expand the information content of medical images, providing an objective measurement to reduce subjectivity in the perception and interpretation. The aim of the study was to investigate capabilities of fractal diagnostic value of diagnostic imaging.

**Methods:** Fractal Dimension (FD) is a statistical quantity that gives an indication of how completely a fractal appears to fill space, zooming down to more finer scales. We proposed a method of medical images analysis obtained from a wide range of sources – radiology imaging. The fractal parameters of these images were calculated for 7 patients with liver lesions for ultrasound, CT, MRI images and generated 3D vector and voxel models by patented method, ‘covering’ the parts of these expertly segmented images by two-dimensional geometric shapes (squares, rectangles, triangles, circles, ellipses) and three-dimensional (cubes, simplices, balls, ellipsoids, pyramids) with applying iteration method, which involves finding the appropriate (i-th) value approaching the value of FD.

**Results:** FD was estimated as 1.67 for hepatocellular carcinoma case; 1.72 – for cholangiocarcinoma; 1.45–1.56 for complex cysts; and 1.15–1.35 for metastases. We consider that only three-dimensional reconstruction from expertly segmented images allows to perform accurate analysis. The most informative description of self-similarity is fractal analysis, conducted with the maximum number of steps. However, objective analysis is limited by resolution of diagnostic equipment, is possible only under visual control by expert. The application of automated and semi-automated image analysis leads to control the process, correctly selecting the areas for research, preselecting a suppositive fractal structure.

**Conclusion:** Fractal analysis of medical images is a promising non-invasive sophisticated approach, it should become highly informative indicator of pathological formations using nonlinear mathematic parameters of structure, gives insights into tumor morphology and can become a useful tool for analyzing tumor
634 OCCULT AND OVERT HBV CO-INFECTIONS INDEPENDENTLY PREDICT POSTOPERATIVE PROGNOSIS IN HCV-ASSOCIATED HEPATOCELLULAR CARCINOMA

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Objective and Background: The roles of chronic hepatitis B virus (HBV) co-infection (CI) in carcinogenesis of hepatitis C virus (HCV)-associated hepatocellular carcinoma (HCC) remained controversial. To gain new insights into this issue, we investigated the postoperative prognostic value of HBVCI in HCV-associated HCC.

Methods: A study cohort of 115 liver tissues obtained from the noncancerous parts of surgically removed HCV-associated HCCs were subjected to virological analysis in a tertiary care setting. Assayed factors included clinicopathological variables, tissue amounts of viral genomes, genotypic characterization of viruses, as well as the presence of overt (serum HBsAg positive) or occult (serum HBsAg negative but tissue HBV-DNA positive) HBVCI. Cox proportional hazard model was used to estimate postoperative survivals.

Results: Of the 115 patients, overt and occult HBVCIs were detected in 35 and 16 patients, respectively. Multivariate analysis revealed that tumor size >3 cm (adjusted hazard ratio (AHR), 2.079 [95% confidence interval, 1.149–3.761]), alpha-fetoprotein >8 ng/mL (AHR, 5.976 [2.007–17.794]) albumin ≤4 g/dL (AHR, 2.539 [1.399–4.606]), ALT >50 U/L (AHR, 1.086 [1.006–1.172]), presence of occult HBVCI (AHR, 2.708 [1.317–5.566]), and absence of overt HBVCI (AHR, 2.216 [1.15–4.269]) were independently associated with unfavorable disease-free survival. Patients with occult HBVCI had a shorter disease-free (P=0.002), a shorter overall survival (P=0.026), a higher bilirubin level (P=0.003) and a higher prevalence of precore G1896A mutation (P=0.006) compared with those with overt HBVCI.

Conclusion: Occult and overt HBVCI served as independent predictors for postoperative survival in HCV-associated HCC.

635 HIV VIRAL LOAD INDEPENDENTLY PREDICTS SURVIVAL IN HIV-INFECTED PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)

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Background: High HIV viral load in HIV/HCV-coinfected patients is associated with faster hepatic fibrosis progression. It is unknown if HIV viral load also affects other aspects of liver disease. This study examines the influence of HIV viral load on outcome of hepatocellular carcinoma (HCC).

Methods: HIV-infected patients with HCC were retrospectively identified from 1992–2012 in 39 centers in North and South America, Europe, and Australia. Each HCC diagnosis was confirmed using the 2005 AASLD criteria.

Results: Among 223 HIV-infected patients with HCC, 221 had results of HIV RNA testing done at time of HCC diagnosis and are analyzed here. HIV RNA was undetectable (<400 copies/mL) in 149 patients (67%). Compared to the 72 patients with any detectable HIV RNA (400+ copies/mL), they were similar in age (mean 52 years), sex (94% male), etiology of HCC (HCV 77%; HBV 22%), and alcohol consumption (excessive in 36%). However, patients with HCV RNA <400 copies/mL were more frequently diagnosed after screening (66% vs. 40%, P<0.001), had a lower mean Child-Turcotte-Pugh score (6.7 vs. 7.4, P=0.013) and more frequently received effective HCC therapy (67% vs. 39%, P<0.001). However, Barcelona-Clinic-Liver-Cancer (BCLC) stages were similar (advanced stages C/D in 54% vs. 58%, P=0.52). The HIV RNA undetectable group had significantly better overall survival (median, 16.2 vs. 5.0 months, P<0.001, log rank). In multi-variable Cox regression analysis, log HIV RNA independently predicted survival (hazard ratio, 0.84, 95% C.I., 0.72–0.99; P=0.033) after controlling for age, HCC screening, HCC therapy, BCLC staging, AST/ALT ratio, and AFP level. By contrast, CD4+ cell count, portal vein thrombosis, extrahepatic metastases, multiple tumors, excessive alcohol use were not independently associated with survival in multi-variable analysis.

Conclusion: In HIV-infected patients with HCC, higher HIV viral load is independently associated with higher mortality.

636 QUANTITATIVE EVALUATION OF INK4A PROMOTER METHYLATION USING PYROSEQUENCING AND CIRCULATING CELL-FREE DNA FROM PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Background and Aims: Promoter hypermethylation of the inhibitor of cyclin-dependent kinase 4 (INK4A) has been reported in 70–80% tumor specimens of hepatocellular carcinoma (HCC). Several studies have also demonstrated increases in cell-free methylated INK4A DNA in plasma/serum specimens of HCC patients. The published studies generally use assays that are based on methylation specific PCR (MSP), a method that cannot tell exactly which CpG site is methylated and levels of methylation at nucleotide resolution. MSP is also subject to PCR allele dropout due to the existence of epi-alleles caused by different methylation of CpG sites. Pyrosequencing is a highly quantitative method that generates a percentage of methylation at a given CpG site. Several CpGs can be analyzed simultaneously. PCR primers can be designed to anneal to sequences that do not have CpG site, therefore, PCR amplicons can include all the epi-alleles to increase sensitivity of the methylation assay. The aim of this study is to examine INK4A promoter methylation by pyrosequencing using circulating cell free DNA from patients with HCC and control benign liver lesions.

Methods: Genomic DNA was extracted and purified from serum specimens of 70 patients (33 HCC, 37 chronic liver diseases). Methylation levels of seven CpG sites in region +148 to +182 in exon 1 of the INK4A gene, corresponding to −72 bp to −37 bp upstream of transcriptional starting site (TSS), were measured by pyrosequencing (PyroMark Q24, Qiagen).

Results: Background methylation rarely passed 5% under the experimental condition. Methylation in control non-HCC serum ranged from 0 to 4% in most cases, and 0–52% in HCC cases. The area under receiver operating characteristic (ROC) curve was 0.80. The sensitivity/specificity values were 70%/79% and 48%/94% at 5% and 7% lower limit of quantification (LOQ), respectively.

Conclusions: INK4A promoter methylation is robustly detected by pyrosequencing in serum specimens from patients with HCC, supporting further investigation of this biomarker.
Primary liver cancer (PLC; ranked sixth in incidence and third in mortality worldwide) is a cancer grouping composed of hepatocellular (HCC) and intrahepatic bile duct carcinomas. HCC make up the bulk of PLC and are highly malignant, usually diagnosed at late stages and often have very poor prognosis with limited treatment options. Thus, there is a need to identify early diagnostic biomarkers of HCC risk. Also, it is plausible that metabolic imbalances, reflective of obesity, diabetes, and lifestyle habits play a central role in HCC etiology. Thus, a NMR metabonomic study was undertaken in a case–control study nested within EPIC, a large prospective cohort of over 520,000 subjects from 23 centers in 10 Western European countries. Detailed dietary/lifestyle data and biological samples (stored under liquid nitrogen) were collected at enrolment from the majority of participants. After an average of 7.6 years of follow-up, 112 first primary incident HCC cases were identified and matched to control subjects (1:2; by age, sex, study centre). Serum samples from matched cases and controls were analyzed by high-field 1H NMR spectroscopy with the following objectives: (i) to identify early predictive biomarkers of HCC by following a metabonomic approach and (ii) to identify metabolomic profiles representative of distinct dietary or lifestyle patterns. Although the protocols of the EPIC study were not specifically designed with metabolomic analyses in mind, extensive pilot testing has shown that EPIC serum samples are suitable for this type of analysis. High-resolution one-dimensional 1H NMR (NOESY and CPMG) spectral profiles were recorded at 800MHz. Additional 2D 1H–1H (Jres, TOCSY) and 1H–13C (HSQC) data were recorded for metabolite assignments. We present here the first results of the metabonomic analysis of the EPIC HCC nested case–control study that enabled the identification of both pre-diagnostic biomarkers as well as those related to exposures associated with disease risk, may have relevant public health impact and enhance understanding of HCC cancer aetiology, particularly in HCC cases arising in the absence of hepatitis B or C infections.

Dairy products, calcium and risk of hepatocellular carcinoma in the European prospective investigation into cancer and nutrition (EPIC) study

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Primary liver cancer is the sixth most common malignancy and the third most common cause of cancer death worldwide, but very little is known about the role of dietary factors in its etiology. Of interest are milk and dairy foods, whose consumption has been shown to be related to cancer risk at other sites. Dairy foods are rich sources of calcium and vitamin D, which may be cancer protective. But, their increased consumption may also lead to higher circulating levels of IGF-1, a growth factor possibly related to increased risk of hepatocellular carcinoma (HCC), the most common type of primary liver cancer.

Objective: To investigate the association between milk and dairy foods, and calcium intakes with incident HCC (Ninc=191) in EPIC, a large prospective cohort in 10 Western European countries. A nested HCC case and matched control sub-set of the cohort was assessed for hepatitis B/C virus infections status.

Methods: A total of 477,206 participants were followed up for an average of 11 years. Diet was assessed by country-specific validated questionnaires. For cohort analyses, the Cox proportional hazard model was used to estimate hazard ratios (HRs) and 95% confidence intervals (95%CI). For nested case–control analyses, conditional logistic regression was used to calculate odds ratios (ORs) and 95%CI. All models were adjusted for relevant confounders.

Results: In the cohort study, a significant positive association for total dairy foods (highest vs. lowest tertile, HR=1.58, 95%CI: 1.08–2.33; Ptrend=0.022), total dietary calcium (HR=1.74, 95%CI: 1.14–2.68; Ptrend=0.009), and dairy calcium (HR per 200 g/day 1.11, 95%CI: 1.02–1.20) were observed with HCC risk after multivariable adjustment. Non-significant associations were observed for milk, cheese and yoghurt. Similar associations were observed in the nested case–control study among hepatitis-free individuals.

Conclusions: Results from this large cohort study with collection of data and biological samples prior to diagnosis suggest that higher consumption of total dairy foods and calcium from dairy sources are associated with increased HCC risk, further emphasizing a role for diet in HCC etiology. Higher circulating IGF-1 level due to more dairy food intake may be a possible biologic explanation for these observations, but requires further study.

Cytokeratin 18 as a potential marker predicts early local recurrence of hepatocellular carcinoma after curative local ablation

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Introduction and Aim: Evidence has now accrued that hepatocellular apoptosis plays a central role in chronic liver disease including hepatocellular carcinoma (HCC). Cytokeratin 18 (CK18) fragments have been examined as potential tumor markers in many different types of epithelial cell carcinomas, including HCC. Some HCC is highly invasive with a high frequency of recurrence following surgery and thermal ablation is common, problematic, and poorly understood. Therefore we evaluated serum level of CK18 as a prognostic marker Predicts both early recurrence of HCC after curative local ablation and macroscopic vascular invasion.

Methods: A case control study was done on 108 patients with HCC of various Barcelona Clinic Liver Cancer (BCLC) Staging underwent percutaneous local ablation and followed up for 28 months from June 2009 till September 2012 for local recurrence, compared to 110 Control subjects classified into 80 cirrhotic patients proved by liver biopsy and 30 healthy control. We excluded conditions with high apoptosis e.g. extra hepatic malignancy, severe infections and any systemic disorders Serum level of CK18 M30-Apoptosense analyzed in all subjects provided written informed consent.

Results: study showed that CK18 was significantly elevated in HCC patients versus control groups (p<0.05). There was no significant difference in CK18 pre and post successful local ablation (percutaneous ethanol injection, radiofrequency ablation
and microwave ablation) \( p = 0.15 \), whereas there was significant increased basal CK18 level in patients with recurrent local lesion versus nonrecurrent \( p < 0.01 \). Interestingly there was marked significant increased in serum CK 18 with macroscopic vascular invasion level versus those without \( p < 0.001 \).

**Conclusion:** CK18 M30 could be used as a prognostic marker. Predicts early local recurrence of HCC and macroscopic vascular invasion but it was of no value in detection successful ablation.

### 640

**EPIDERMAL GROWTH FACTOR GENETIC POLYMORPHISM PREDICTS RISK OF HEPATOCELLULAR CARCINOMA IN EGYPTIAN PATIENTS WITH HCV (GENOTYPE 4)-RELATED CIRRHOSIS**

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**Background:** Epidermal growth factor (EGF) and its receptor play critical roles in cell proliferation and tumorigenesis. EGF is one of the candidate gene for HCC. The EGF gene is mapped to chromosome 4 \( (4 q 25) \) and it consists of 24 exons. A functional polymorphism in the EGF gene has been linked to increased cancer susceptibility in Egypt, the growing incidence of HCC, which is nearly doubled over the last decade is parallel with that Egypt is plugged with highest prevalence of HCC in the world, ranging from 6 to 28%.

**Aim of the study:** This study aimed to investigate the association between the EGF +61A/G polymorphism and the risk for hepatocellular carcinoma (HCC) in HCV-related cirrhotic Egyptian patients.

**Subject and Methods:** We analyzed 133 (HCC) \( ( \text{group I}) \) & 105 HCV-related cirrhotic patients without any focal lesion \( ( \text{group II}) \). All patients were attending Tropical Medicine department in Mansoura university hospital were subjected to clinical examination, routine liver function tests, abdominal ultrasound (US), Triphasic computed tomography \( ( \text{CT}) \), \( \alpha \)-feto protein \( ( \text{AFP}) \), and PCR HCV & genotyping of HCV. The diagnosis of HCC was verified histologically, or based on the finding of typical radiological features in two image examinations \( ( \text{US & CT}) \) or by a single positive imaging technique associated with AFP two image examinations \( ( \text{US & CT}) \) or by a single positive

**Results:** The EGF +61 genotypes frequencies in HCC \( ( \text{group I}) \) were AA \( (24.8\%) \), AG \( (32.3\%) \), and GG \( (42.9\%) \) and in HCV-cirrhotic \( ( \text{group II}) \) were AA \( (57.1\%) \), AG \( (34.3\%) \), and GG \( (8.6\%) \). The carriage for allele G of EGF +61A/G single nucleotide polymorphism \( \text{SNP} \) was significantly associated with development of HCC compared with HCV-related cirrhotic group \( \text{OR} = 4.0404, 95\% \text{CI} 2.3275 - 7.014, p = 0.0001 \).

**Conclusion:** Our data suggest an increased risk to develop HCC in Egyptian patients with HCV carrying the G allele of EGF +61A/G SNP.

### 641

**CD142, VEGF AND MICROVASCULAR DENSITY MVD-CD34 EXPRESSION IN HEPATOCELLULAR CARCINOMA OF PATIENTS WITH CIRRHOSIS AND CORRELATION WITH TUMOR GROWTH AND PROGRESSION**

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**Background and Aim:** Finding the molecular markers associated with angiogenesis may help identify patients at increased risk for recurrence and metastasis of HCC. The aim of this study is to investigate the level of CD142, VEGF, and MVD-CD34 expression in HCC and surrounding cirrhotic liver tissue and their relationship to tumor growth and progression.

**Materials and Methods:** This study included forty six patients with HCC arising on top of cirrhosis. These patients underwent liver transplantation or partial hepatectomy during the period 2010–2011. Immunohistochemical staining for CD142, VEGF and MVD-CD34 antibodies was performed in HCC tissue specimens and paracarcinomatous cirrhotic tissue. The quantitation of the microvessels identified by anti CD34, VEGF expression identified by anti VEGF monoclonal antibody, and CD142 expression identified by anti TF monoclonal antibody. Each monoclonal expression was identified in both HCC tissue, and the surrounding cirrhotic tissue.

**Results:** CD142 and VEGF showed significantly increased expression in HCC compared to LC, and showed increased expression from grade I to grade II to grade III, but no significant difference in their expression between grade III and grade IV. There is highly significant association between CD142 and VEGF expression between LC and different grades of HCC \( (p < 0.001) \). MVD-CD34 was decreased significantly from LC to HCC and increased significantly from grade I to II to III to grade IV HCC \( (p < 0.001) \). The MVD-CD34 was significantly higher in tumors with high immunoreactivity for CD142 than in tumors with low immunoreactivity for CD142 (median, 53.26 vs 37.01/HPF, \( p < 0.02 \)).

**Conclusion:** Expression of the angiogenic factors CD142, VEGF and MVD-CD34 is increased in HCC relative to LC and correlated with tumor aggressiveness. CD142 has been recognized to be capable of inducing angiogenesis through up-regulation of VEGF. Expression of these factors may be useful as prognostic indicators in patients with HCC.

### 642

**ASSOCIATION BETWEEN EPIDERMAL GROWTH FACTOR GENE 61A>G POLYMORPHISM AND AGE AT THE DIAGNOSIS OF HEPATOCELLULAR CARCINOMA: IMPLICATIONS FOR SURVEILLANCE**

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**Background and Aims:** Carriage of the variant allele of the 61A>G single-nucleotide polymorphism \( \text{SNP} \) in the epidermal growth factor gene \( (rs4444903) \) is a risk factor for hepatocellular carcinoma \( \text{(HCC)} \). To identify the best strategy by which rs4444903 genotyping might be applied to risk stratification in HCC, we studied a cohort of HCC patients presenting at the liver clinic of an academic hospital.

**Methods:** One-hundred two patients \( (M=78, 76\%; \text{mean age, 69 years}) \) received a diagnosis of HCC and were clinically monitored along a median follow-up period of 14 months. Non-mutually exclusive causes of liver disease included hepatitis B \( (N = 8) \), hepatitis C \( (N = 53) \), and alcohol \( (N = 49) \). Genotyping for rs4444903
was performed on whole blood specimens by a PCR-based restriction fragment length polymorphism assay, as previously described (Tanabe KK et al., JAMA 2008).

**Results:** At presentation, BCLC stage and Child class were A (N=51), B (N=29), C (N=18), D (N=4), and A (N=72), B (N=26), and C (N=4), respectively. The median number of tumor nodules was 1, and the largest median nodule size was 3.2 cm. The population was in Hardy-Weinberg equilibrium for rs4444903 (A/A: N=34, A/G: N=50, G/G: N=18, variant allele frequency = 0.42). The age at diagnosis was younger in carriers of the variant allele, either analyzed genotypically (A/A vs. A/G vs. G/G, 74 vs. 69 vs. 67 years, p = 0.029) or by a dominant model (A/A vs. G/G, 74 vs. 69 years, p = 0.021). Gender, aetiology, presentation modality, follow-up duration, BCLC stage, Child class, nodule number, major nodule size, and serum alpha-fetoprotein had no association with rs4444903. At the end of follow-up, 38 patients had died (median survival time = 48 months). At univariate analysis, survival probabilities were related to BCLC stage, major nodule size, Child class, and portal vein thrombosis presence and but not to rs4444903 alleles. The only independent predictors of survival at presentation were tumor size and number of tumor nodules.

**Conclusions:** Carriers of the variant G allele at the rs4444903 SNP present with HCC on average five years earlier than carriers of the wild-type allele, suggesting that G carriers might benefit from entering a surveillance program at younger age.

**643 HEPATOCELLULAR CARCINOMA ON NON-FIBROTIC LIVER (F0/F1): EPIDEMIOLOGY, CLINICAL AND PATHOLOGICAL FEATURES, PROGNOSIS. STUDY OF 141 CASES. COMPARISON TO HCC ON FIBROTIC (F2–F3) OR CIRRHOTIC LIVER**

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**Introduction:** HCC on non fibrotic liver (nHCC) is uncommon and risks factors, clinical feature as well as prognosis remain poorly known. The aim of this study was to analyse a cohort of 141 cases of nHCC compared to HCC on fibrotic or cirrhotic liver.

**Patients and Methods:** From a prospective database of 1009 consecutive patients diagnosed with HCC since 2005, 141 cases of nHCC (histologically proved F0–F1) were identified and compared to 93 patients F2–F3, and 470 cirrhotic patients CHILD A. Epidemiological, clinical, biological, pathological data were recorded. Follow-up included CT-Scan or MRI every 3 to 6 months.

**Results:** Usual risk factors of HCC (alcohol, hepatitis B/C, metabolic syndrome (MS)) were found in 2/3 of nHCC. MS was present in 37% of F0/F1 vs 50% (F2/F3) and 33.5% (F4). Pathological features of NASI were observed in F2/F3 patients but not in F0/F1 patients. Diagnosis is delayed in nHCC due to the lack of screening (7.8%). Tumors were larger at diagnosis (74 mm F0/F1 vs 50 F2/F3 vs 35 F4 p < 0.05). The rate of patients eligible to curative treatment is higher in nHCC (68% vs 43%) leading to a better overall survival (OS) (4.01 years F0/F1 vs 2.14 F4 p = 0.02). After curative treatment, time to recurrence (TTR) and OS are similar in both groups (1.31 years vs 1.06 p = 0.88 and 60 months vs 54 respectively). Patients F0–F1 without known risk factors (34%), were younger (62.4 vs 65.7 years old F4) with a different sex ratio (female 44% vs 14.9% F4) and tumors massively expressed the glutamine synthase (86.4% of patients). This group exhibited a better prognosis in OS (6.35 years vs 2.13 F4 Child A) and TTR (2.03 years vs 1.06 F4).

**Conclusion:** Usual risk factors of HCC are observed in 2/3 of nHCC including MS in 1/3 of cases. Prognosis is better in n-FHC frequently eligible to curative treatment. However, after curative treatment, OS and TTR are similar to those observed in patients with cirrhosis. In the subgroup of nHCC without identified risk factor, tumors occur in a younger and female population, and the prognosis is better.

**644 SURVEILLANCE PROGRAM FOR DIAGNOSIS OF HCC IN LIVER CIRRHOSIS: ROLE OF ULTRASOUND ECHOPATTERNS**

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International Guidelines suggest the ultrasound surveillance for early detection of HCC in liver cirrhosis (LC) patients, but 40% of nodules <2 cm escape. Retrospective studies proposed the large coarse hypoechoic nodular pattern as a predictor of HCC. Our aim was to evaluate with a longitudinal study the existence of an ultrasound pattern suggestive of HCC in LC patients in surveillance. Since January 2006, 350 LC patients (CP A-B8) underwent the ultrasound screening, median follow up 55 months (range 12–70); 15% developed HCC. In all patients liver function tests and alpha-fetoprotein were dosed. LC was diagnosed by histology (10%), Fibroscan (30%) and according to clinical, US, endoscopic and laboratory data in the remaining. HCC was diagnosed according to AASLD guidelines. Etiology was HCV (78%), HBV (6%), alcohol (5%), cryptogenic (8%), mixed or other (3%). Ultrasound were performed by two experienced sonographers (k of concordance >0.8). Ultrasound patterns were classified: 1) homogeneous (H), 2) bright liver (BL), 3) coarse (C), 4) small coarse hypoechoic nodular (SHP), 5) large coarse hypoechoic nodular (LHP), 6) nodular hypeerecic (NH) (Gastroenterology 1995;108:1778–84). Kaplan–Meier method, log-rank-test, Cox proportional-hazards analyses were used. At enrolment 75% of patients presented C, 8% SHP, 6% BL, 5% NH and 2% H patterns. The risk of developing HCC was significant only in patients with LHP (hazard ratio 3.1; P < 0.003). Kaplan–Meier estimates of HCC cumulative risk in relation to the enrolment US findings showed: cumulative risks of HCC were 60% in patients with LHP, 22% in C, 20% in NH, 18% in SHP, 0% in H and BL patterns. A log-rank test of the curves of echopatterns showed significant differences (P < 0.04).

In our long term follow up of a cohort of LC the LHP pattern resulted as major risk factor for HCC as 45% of patients with LHP at enrolment developed HCC (cumulative risk 60%). This observation, if validated in wider case studies and/or correlated to histological or molecular markers, could raise the question of eventually modifying the timing of follow up in this subset of patients.

**645 OUTCOME OF HEPATITIS B AND C VIRUS ASSOCIATED HEPATOCELLULAR CARCINOMA OCCURRING AFTER RENAL TRANSPLANTATION**

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**Background and Aims:** Chronic hepatitis B (HBV) and C (HCV) virus infections are important causes of morbidity and mortality in kidney transplant recipients (KTR). Immunosuppressive agents enhance HBV and HCV replication, leading to decreased patient survival due to progressive liver disease, including cirrhosis and hepatocellular carcinoma (HCC). The aim of this study was to assess the incidence and outcome of HCC in KTR.

**Methods:** We performed a case–control study in patients with chronic HBV and/or HCV infection who underwent kidney transplantation (KT) between 1976 and 2011 and subsequently
developed HCC. Patients’ characteristics and outcomes were compared to a control group of HBV and/or HCV positive patients matched for age and gender who did not have KT.

Results: Among 2944 KTR, 330 had hepatitis B and/or C. Fourteen developed HCC, an incidence of 4.24%. All patients were Caucasian, and 86% were male (93% in the control group, p = ns). Mean age at HCC diagnosis was 52.6±2 years (53.2±1.5 in controls, p = ns). Mean time between transplantation and HCC diagnosis was 16.7±2.7 years. Six HCCs were related to HBV, 6 to HCV, and 2 to co-infection with HBV and HCV. Immunosuppressive therapy was comparable in HBV, HCV and HBV+HCV patients. All patients had corticosteroids. Sixty-four percent of patients received induction treatment and were on triple therapy including calcineurin inhibitors and antimetabolites. At diagnosis, 71% of patients met Milan criteria (65% in the control group, p = ns). Treatment modalities were comparable between the two groups. Patient survival 2 years after HCC diagnosis was 43% in KTR, compared to 76% in the control group (p = 0.03). There was no significant difference in overall survival between HBV- and HCV-infected KTR with HCC.

Conclusions: HCC occurs with an incidence of 4.24% in HBV and/or HCV infected patients after KT. Survival after HCC diagnosis is significantly worse compared to a control group of non-transplanted patients with HBV and/or HCV, matched for age and gender, and with similar tumour characteristics.

### POSTERS

#### 646 APPLICABILITY OF THE BARCELONA CLINIC LIVER CANCER (BCLC) THERAPEUTIC STRATEGY AND IMPACT ON SURVIVAL IN A SINGLE TERCENTRE CENTRE

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Background and Aims: The BCLC classification of hepatocellular carcinoma (HCC) divides patients into stages with a specific treatment-oriented schedule and has proved to be useful in the management of patients with HCC. However, the recommended treatment is not always applicable in real clinical practice, due to patient and tumor dependent factors. Our aim was to evaluate the causes of non-applicability and impact on survival.

Methods: We registered baseline characteristics and causes for deviation from BCLC treatment allocation for cirrhotic patients treated between January 2005 and August 2012. Survival data was analyzed using the Kaplan–Meier method.

Results: One hundred and twenty two (66.6±6 years, 84% male) consecutive HCC patients with cirrhosis (63% due to alcohol) were registered. Tumor was detected during surveillance in 64% of patients; Child–Pugh A (65%), B (23%) and C (12%); and BCLC A (0 included) (53%), B (16%), C (12%) and D (19%). In 36 (29%) patients first treatment option was not feasible (81% BCLC A) due to technical reasons (n = 29; 63%, tumor location; 23%, comorbidity; 7%, severe ascites; and 7% low portal blood flow) or refusal to undergo recommended treatment (n = 7). Five year mean survival of BCLC A patients who did not followed BCLC recommendations (TACE-Transcatheter arterial chemoembolization – instead of curative treatments) was not significantly different (51 months, CI95% 26–76) than survival of patients treated according to the BCLC recommendations with resection, liver transplantation or ablation (63 months, CI95% 40–86) (Log-Rank P = 0.25), even after censoring follow-up at time of transplants. Groups were not different attending to age, sex, size or number of nodules, Child–Pugh or surveillance detection (P > 0.05).

Conclusions: One third of our patients were not treated with the first line option recommended by the BCLC system. However, in BCLC A patients this limitation did not impact on survival at 3 and 5 years. Our data suggests that TACE might be a valuable alternative option in BCLC A patients with non-applicability curative treatments.

#### 647 IMMUNOHISTOCHEMICAL REAPPRAISAL OF HEPATOCELLULAR ADENOMAS: A SINGLE INSTITUTION EXPERIENCE OF 61 PATIENTS IN THE UNITED STATES

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Background: Hepatocellular adenomas (HCA) are rare benign liver tumors subclassified by immunohistochemistry (IHC) into three subtypes: HNF1α-inactivated (H-HCA), β-catenin-activated (β-HCA) and inflammatory HCA (I-HCA). Tumors without abnormal expression of these markers are designated as unclassified HCA (U-HCA). Here we report our experience of resected HCA based on this classification in a single institution in the United States (US).

Design: HCA between 1990–2012 were retrieved from files. Histological assessment and IHC for liver fatty acid binding protein (LFABP), glutamine synthetase (GS), β-catenin (βC), serum amyloid A (SAA), and C reactive protein (CRP) were performed.

Results: Sixty-one patients were identified. Eleven patients had more than one lesion. Twenty cases (33%) showed loss of LFABP staining and were classified as H-HCA, among those 4 tumors (20%) were steatosis free. Twenty-seven cases (44%) expressed CRP and SAA and were considered I-HCA. One case showed nuclear βC and GS expression without abnormal expression of the other markers (2%) and was considered β-HCA. One case had features of I-HCA and β-HCA (positive for CRP, SAA, GS and nuclear βC); and contained a focus of hepatocellular carcinoma. Two cases showed features of both H-HCA and I-HCA (negative for LFABP, positive for SAA and CRP). Ten (16%) cases did not show abnormal expression of any marker tested and were designated as U-HCA. In patients with multiple HCAs, the immunoprofile was consistent among the different lesions. Table 1 summarizes the staining patterns.

Conclusion: IHC markers classify the majority of hepatocellular adenomas in the study as H-HCA (33%) and I-HCA (44%), while 16% fall into the U-HCA category, and a minority (2%) as β-HCA. Overlap
Higher PPV is found for tumors larger than 2cm in cirrhotic liver. The HCC diagnostic power in non-cirrhotic and non-BC patients; no matter their liver were cirrhotic or non-cirrhotic liver. Moreover, the higher PPV was only observed in those patients.

Table 1. Summary of HCA staining patterns

<table>
<thead>
<tr>
<th>HCA type</th>
<th>Staining Patterns (*nuclear staining)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-HCA</td>
<td>LFABP (-), IC (-), GS (-), SAA (-), CRP (-)</td>
<td>20/61 (33%)</td>
</tr>
<tr>
<td>I-HCA</td>
<td>LFABP (+), IC (-), GS (-), SAA (+), CRP (+)</td>
<td>27/61 (44%)</td>
</tr>
<tr>
<td>β-HCA</td>
<td>LFABP (+), IC (+), GS (+), SAA (-), CRP (-)</td>
<td>1/61 (2%)</td>
</tr>
<tr>
<td>H-HCA/β-HCA</td>
<td>LFABP (-), IC (+), GS (-), SAA (+), CRP (+)</td>
<td>2/61 (3%)</td>
</tr>
<tr>
<td>U-HCA</td>
<td>LFABP (+), IC (-), GS (+), SAA (-), CRP (-)</td>
<td>1/61 (2%)</td>
</tr>
</tbody>
</table>

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VALIDATION OF 2010 AASLD GUIDELINE IN THE DIAGNOSIS OF HEPATOCELLULAR CARCINOMA FOR NON-CIRRHOTIC LIVER

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Background and Aims: The 2010 AASLD guideline modified the noninvasive criteria to diagnose hepatocellular carcinoma (HCC) in cirrhotic liver if tumor size larger than 1 cm with typical dynamic imaging study. Patients with chronic hepatitis B who may not have fully developed cirrhosis could be applied. However, the definition is somewhat vague. We aim to validate the 2010 AASLD guideline in the diagnosis of HCC in non-cirrhotic liver, with hepatitis B or C patients.

Methods: Total 648 patients with liver tumor post surgical resection from 2005–2010 were reviewed. The hepatitis B or C status was determined by their blood specimen. The pathologic fibrosis score was verified by METAVIR scoring classification, and cirrhosis was diagnosed as score 4.

Results: Among the 648 patients, there were 569 HCC patients. The accuracy of diagnosis of liver cirrhosis pre-operatively was around 70%. The 2010 AASLD guideline for HCC diagnosis sensitivity: 99.1%, specificity: 36.7%, positive predictive value (PPV): 91.9%, negative predictive value (NPV): 85.3%, and accuracy: 91.5%. No statistic difference of sensitivity existed in AASLD guideline between cirrhotic and non-cirrhotic liver. However, higher PPV (P<0.001), lower specificity (P=0.048) were found in cirrhotic than non-cirrhotic liver. Moreover, the higher PPV was only observed in those of tumor size more than 2 cm, rather than tumor size of 1–2 cm. There was also no difference of sensitivity, specificity, PPV, and NPV between HCC related to hepatitis B, hepatitis C, hepatitis B+C, and non-BC patients; no matter their liver were cirrhotic or non-cirrhotic.

Conclusion: The 2010 AASLD guideline exhibited an excellent sensitivity in HCC diagnosis. The HCC diagnostic power in non-cirrhotic patients, as well as hepatitis C patients was also acceptable. Higher PPV is found for tumors larger than 2 cm in cirrhotic liver.
650 ANGIO-CT FINDINGS OF 115 SMALL HEPATOCELLULAR CARCINOMAS OF 61 PATIENTS DURING FOLLOW-UP OF 659 PATIENTS WITH CHRONIC LIVER DISEASES CLINICAL FEATURES OF EARLY CANCERS

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Background: In 2009 international consensus group defined early cancers as vaguely nodules with stromal invasion (invasion into portal areas) without arterial vascularization. Although this consensus is reasonable, we don’t know clinical features of early cancers, e.g. how to diagnose early cancers or how early cancers develop. Stromal invasion means decreasing of portal areas and portal blood flow. Therefore we’ve investigated clinical features using Angio-CT system since 2000.

Methods: We’ve followed 659 patients with chronic liver disease and detected 115 small HCCs of 61 patients. We’ve performed Angio-CT for these 115 HCCs, and observed until early cancers changed to typical HCCs.

Results: 107 HCCs showed low attenuation on CTAP and high attenuation on CTHA (typical HCC). Only 8 nodules showed low on CTAP and not high on CTHA (early cancer). 2 of 8 showed low on CTAP and not high on CTHA were resected. Resected specimens showed typical early cancers. One was biopsied by needle and showed well-differentiated carcinoma. Others were observed until changed to typical HCCs by periodic Angio-CT examinations. Observation periods were 0.8–5.5 years. These 8 early cancers developed via multistep mechanism. However these early cancers increased slowly in size and did not metastasize.

Conclusions: 1. Early cancers developed via multistep mechanism, however fewer than expected.
2. Small needle biopsy alone could not diagnose early cancers exactly. Low attenuation on CTAP and not high attenuation on CTHA is useful to diagnose early cancers.
3. We need a new consensus how to manage early cancers.

651 MICA PLAYS AN OPPOSITE ROLE IN HEPATOCARCINGENESIS BETWEEN HEPATITIS B AND HEPATITIS C

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Background and Aims: Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is a major risk factor for developing hepatocellular carcinoma (HCC). We recently reported that a SNP rs2596542 located in the MHC class I polypeptide-related chain A (MICA) promoter region was significantly associated with the risk for HCV-induced HCC (C-HCC) and also with serum levels of soluble MICA (sMICA) by genome-wide association study. MICA plays an important role in innate tumor surveillance. In this study, we focused on the possible involvement of MICA in HBV-induced HCC (B-HCC).

Methods: The MICA SNP rs2596542 was analyzed in 407 B-HCC cases and 5,657 controls, and in 1,394 C-HCC cases and 5,486 controls.

Results: The genetic association analysis revealed a significant association with an SNP rs2596542 (G/A); a G allele increased the risk of B-HCC (P = 0.029 with odds ratio of 1.19), on the other hand, an A allele increased the risk of C-HCC (P = 4.2×10⁻¹³ with odds ratio of 1.39). We also found a significant elevation of sMICA in both B-HCC and C-HCC. Moreover, a G allele of SNP rs2596542 was significantly associated with increased sMICA levels in both B-HCC (P = 0.009) and C-HCC (P = 1.4×10⁻¹³). Interestingly, B-HCC patients with the high serum level of sMICA (>5 pg/ml) exhibited poorer prognosis than those with the low serum level of sMICA (≤5 pg/ml) (P = 0.008).

Conclusions: Although MICA increases on cells under stresses including infection and malignant transformation and could be targeted by NK cells, sMICA could block NK cells. Our results indicated the opposite role of MICA variant and sMICA between B-HCC and C-HCC, and thus MICA could be an attractive therapeutic target for both B-HCC and C-HCC.

652 VIROLOGICAL RESPONSE TO ENTECAVIR IS ASSOCIATED WITH LOW PROBABILITY OF DEVELOPING HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS B PATIENTS WITH CIRRHOSIS

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Background and Aims: The aim of this study was to assess the risk for the development of hepatocellular carcinoma (HCC) according to the underlying liver status and virological response (VR) to entecavir (ETV) in chronic hepatitis B patients with cirrhosis.

Patients and Methods: A total of 324 patients with cirrhosis were treated with ETV for ≥6 months and were followed up (mean duration 36.0 months) for the occurrence of HCC. Patients who developed HCC within 6 months were excluded. VR was defined as HBV DNA undetectability (HBV DNA <2000 copies/ml until June, 2008 and <20 IU/ml after July, 2008).

Results: Two hundred and twenty (67.9%) patients had compensated cirrhosis and remaining (32.1%) patients had decompensated cirrhosis. The 5-year prevalence of HCC was 28.5%. Univariate analysis showed that increasing age (p = 0.002), male gender (p = 0.008), diabetes mellitus (p = 0.012), hepatic encephalopathy (p = 0.017) were significant risks for development of HCC. Low platelet and low serum albumin showed a trend to be risks for developing HCC (p = 0.089 and p = 0.090, respectively). VR was associated with lower HCC risk (p = 0.000). Cox regression analysis showed that age over 50 (p = 0.000, RR 2.906) and male gender (p = 0.005, RR 2.887) were independent risks for the development of HCC. Patients with VR had low probability for development of HCC (p = 0.000, RR 0.506).

Conclusions: Antiviral treatment with ETV does not completely eradicate the risk of developing HCC in patients with cirrhosis. However, VR to ETV is associated with a lower probability of developing HCC. Older age and male gender were significant independent risk factors for developing HCC in ETV-treated chronic hepatitis B patients with cirrhosis.

653 IMPACT OF HIGHLY EFFECTIVE ANTIVIRAL THERAPY ON THE BURDEN OF HEPATOCELLULAR CARCINOMA IN HEPATITIS C CIRRHOSIS

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Background and Aim: With highly effective direct acting antiviral agents (DAAs) in mature stages of development that afford nearly
universal sustained virological response (SVR), it is eagerly hoped that morbidity and mortality from hepatitis C virus (HCV) will soon disappear. At present, cirrhosis, the predominant risk factor for hepatocellular carcinoma (HCC), is increasing among patients with HCV as they become older and the duration of infection longer. We estimate the impact of DAAs with high SVR rates on the incidence of HCC in the population.

Methods: Since the extent to which the risk of HCC decreases in patients with established cirrhosis achieving SVR remains poorly defined, we conducted a simulation experiment in which a cohort of 50-year-old subjects (n = 1,000) with compensated HCV cirrhosis (MELD=6) is followed for 20 years. In a viremic subject, the MELD score, HCC and mortality from end stage liver disease (ESLD) would increase progressively. Once SVR is achieved, MELD would stop increasing and the proportion at risk of HCC decrease over time.

Results: The Table summarizes 20-year outcomes in the 50-year-old cohort based on SVR eliminating HCC risk in 3%/year.

<table>
<thead>
<tr>
<th></th>
<th>SVR=0%</th>
<th>SVR=50%</th>
<th>SVR=100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>%HCC</td>
<td>24%</td>
<td>26%</td>
<td>27%</td>
</tr>
<tr>
<td>%Death from ESLD</td>
<td>72%</td>
<td>57%</td>
<td>42%</td>
</tr>
<tr>
<td>Persons at risk of HCC</td>
<td>5.80%</td>
<td>6.21%</td>
<td>6.63%</td>
</tr>
<tr>
<td>%No longer at HCC risk</td>
<td>0%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>Average annual HCC incidence per year</td>
<td>4.1%</td>
<td>3.4%</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

When no DAA is applied (SVR=0%), HCC would occur in 24% after 5,805 person-years of follow-up. As SVR is increased to 50% and 100%, the proportion of HCC would increase paradoxically to 26% and 27%, respectively, because the number of subjects who remain at risk increases as a result of a dramatic reduction in ESLD deaths. In sensitivity analyses, the incidence of HCC was most influenced by the rate at which the risk of HCC is decreased after SVR, whereas reduction in mortality was dependent upon the SVR rate.

Conclusion: Introduction of highly effective DAA may lead to minimal reduction or even paradoxical increase in the burden of HCC in subjects who are already cirrhotic.

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A NEW CLASSIFICATION OF PRIMARY LIVER CARCINOMAS BASED ON THEIR POSSIBLE CELLULAR ORIGIN
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Introduction: Primary liver cancers (PLCs) are categorized by WHO into three groups: hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and combined HCC-CC. However, the definition of combined HCC-CC is rather confused, and keratin (K)19-positive HCC is not considered a distinct entity even though several studies confirm it has a worse prognosis than K19-negative HCCs. Clinical outcome was worse in muc-ICCs followed in order of increasing survival time by mixed hepatobiliary tumors, K19 positive-HCCs, and K19 negative-HCCs.

Conclusion: Categorization of PLCs into K19-negative and positive HCCs, mixed hepatobiliary tumors, and mixed-ICCs, may be a relevant and useful classification as they showed completely different clinicopathological and radiological aspects. Similar gene-expression profiling in the mixed hepatobiliary tumors and K19-positive HCCs suggest the same cellular origin.

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SERUM MICROPARTICLES (MP) AS A NOVEL TOOL TO IDENTIFY HEPATOCELLULAR CARCINOMA (HCC)
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Background and Aims: We recently showed that subpopulations of serum/plasma microparticles (MPs) serve as novel diagnostic tools to stage and grade chronic hepatitis C (CHC) and nonalcoholic steatohepatitis (NASH) (Kornek M et al., Gastroenterology 2012). Moreover, we demonstrated that MPs derived from activated or apoptotic T cells induce a fibrolytic phenotype in activated hepatic stellate cells (HSC) via membrane fusion (Kornek M et al., Hepatology 2011). Here, we studied if circulating MPs may help to identify patients with hepatocellular carcinoma (HCC) or who are at risk of developing HCC. We therefore performed a pilot study to identify and characterize circulating HCC-associated MPs from patients with CHC with or without HCC in a blinded approach.

Methods: MPs were isolated from human serum samples of patients with CHC by negative selection and characterized by flow cytometry using MP-specific markers. MPs were identified by flow cytometry using specific markers.

Results: MP profiling was done after differential ultracentrifugation using FACS analysis for the presence and quantity of CD133+ MPs and HCC derived MPs. MPs were isolated from human serum samples of patients with CHC and cirrhosis (n = 35), 17 of them having a histological proven HCC, without knowing their HCC status. Sera from twelve healthy patients served as normal controls. MP profiling was done after differential ultracentrifugation using FACS analysis for the presence and quantity of CD133+ MPs and HCC derived MPs.

Results: Proven HCC was associated with a marked increase of CD133+ and HCC derived MPs successfully separated CHC patients without HCC from those with HCC and from healthy controls. The calculated cut-off values were 2.38% for CD133+ MPs and 0.12% for HCC derived MPs. HCC derived MPs were increased 2-fold (198.7%, p < 0.005) in true HCC patients compared to HCC negative CHC patients. CD133+ MPs were significantly increased by 141.9% in HCC positive vs. HCC negative CHC samples (p < 0.05). Their accompanied AUROC values, sensitivity and specificity scores indicated a high diagnostic accuracy (CD133+ MPs: AUROC 0.8571, sensitivity: 71.4%,
specificity: 82.4%; HCC MPs: AUROC 0.7617, sensitivity: 68.8%, specificity: 93.8%).

Conclusions:
1. Patients with HCC were characterized by a striking elevation of CD13+ and HCC derived MP.
2. MP monitoring for HCC is a novel tool to noninvasively assess the presence and possibly extent of HCC.

Studies were supported by a DFG-fellowship to MK and grant R21 DK075857–01A2 to DS

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A COMBINED BILE AND URINE PROTEOMIC TEST INCREASES DIAGNOSTIC ACCURACY OF CHOLANGIOCARCINOMA IN PATIENTS WITH BILIARY STRICTURES OF UNKNOWN ORIGIN

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Background: Detection of cholangiocarcinoma (CC) remains a diagnostic challenge particularly in patients with primary sclerosing cholangitis (PSC) who are at risk for CC development. We recently established diagnostic peptide marker models in bile and urine to detect both local and systemic changes during CC progression. Our aim was to combine both models to reach a higher diagnostic accuracy of CC in patients with unknown biliary strictures.

Methods: On the basis of bile and urine proteomic analysis by capillary electrophoresis mass spectrometry, a case–control phase II study on 87 patients (36 CC including 5 with CC on top of PSC, 33 PSC and 18 other benign disorders) was initiated to elucidate the potential of a combined bile and urine test for CC diagnosis. A logistic regression model based on both proteomic tests was developed to improve the accuracy of CC diagnosis compared to single bile or urine proteomic test application. Moreover, the tumour marker CA19–9 and bilirubin was combined with both proteomic tests to potentially increase accuracy.

Results: Receiver operating characteristics analysis of proteomic CC-classification of the 87 study patients revealed AUC values of 0.85 in case of bile (odds ratio: 6.2) and 0.93 in case of urine (odds ratio: 14.0). From all tested clinical laboratory parameters only model sub-california1–9 demonstrated acceptable discrimination performance with an AUC above 0.75. A logistic regression model composed of the bile and urine proteomic classification factors lead to an AUC of 0.96, and 92% sensitivity and 84% specificity at the best cut-off. Only three of the 36 CC patients were false negative and two of the 33 PSC patients were false positive classified. Inclusion of CA19–9 and bilirubin values to the logistic regression model was of minor benefit as indicated by small correlation coefficients and insignificant P values for these markers.

Conclusion: A logistic regression model combining the classification factors of bile and urine proteome analysis enables CC-diagnosis with an accuracy of 93.8%.

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THE EFFECT OF COFFEE CONSUMPTION ON THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN HEPATITIS B VIRUS ENDEMIC AREA

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Introduction: Coffee consumption is inversely related to the risk of liver cirrhosis or hepatocellular carcinoma (HCC). However, the protective effect of coffee drinking against the risk of HCC was not established in HBV-prevalent region.

Aims: To elucidate the relationship between lifetime coffee consumption and the risk of HCC development under the consideration of replication status of HBV.

Methods: A hospital-based case–control study was performed in 1,364 subjects. A total of 258 HCC patients, 480 health-check examinees (control 1, HCE), and 626 patients with chronic liver disease other than HCC (control 2, CLD) were interviewed on smoking, alcohol and coffee drinking using a standardized questionnaire. HBV envelope antigen (HBeAg) status and serum HBV DNA levels were measured in patients infected with HBV.

Results: After adjustment for age, gender, body mass index, presence of hepatitis virus (except for HCE) and lifetime alcohol drinking/smoking, a high lifetime coffee consumption (≥20,000 cups) was an independent protective factor against HCC, in each analyses using healthy and risky control groups, respectively (HCE group, OR 0.53, 95% CI 0.31–0.89; CLD group, OR 0.57, 95% CI 0.37–0.87). However, the high coffee consumption did not affect the HCC risk in patients with HBV (OR 0.60, 95% CI 0.33–1.08) after adjustment for HBeAg status, serum HBV DNA level and antiviral therapy.

Conclusions: A high lifetime coffee consumption was negatively associated with a HCC development. However, this exposure difference of coffee with the HCC group was reduced in chronic hepatitis B patients by the dominant role of viral replication.

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microRNA PROFILE IN CHRONIC HEPATITIS B RELATED HEPATOCELLULAR CARCINOMA: OVER EXPRESSION OF microRNA-224 IN HEPATOCELLULAR CARCINOMA TISSUE CAN PREDICT RECURRENCE AFTER CURATIVE RESECTION

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Background and Aims: We investigated aberrant microRNA (miRNA) expression in chronic hepatitis B (CHB) related hepatocellular carcinoma (HCC) by comparing miRNA expression of HCC tissue and non-HCC tissue to reveal which miRNAs are related in pathogenesis of HCC development. We also investigated the association between miRNA profiles and tumor characteristics or clinical outcome.

Method: Paired tissues (HCC and non-HCC) from resected liver specimens were obtained from 22 patients who underwent curative surgical resection. Microarray was performed to screen miRNAs showing different expression between HCC and non-HCC tissues. Quantitative real time polymerase chain reaction (PCR) was performed to validate the miRNA microarray data. By using miRBase, target signaling pathways of miRNA were predicted.

Results: In microarray, miR-532–3p, miR-1066–5p, miR-224, miR-93–5p were up regulated in HCC tissue, while miR-139–5p was down regulated in HCC tissue. miR-7–5p, miR-885–3p were up regulated in HCC with microvascular invasion compared with HCC without microvascular invasion. miR-574–5p and miR-224 were up regulated in recurrent HCC than non-recurred HCC after curative resection, while miR-194–3p and miR-192–5p were down regulated in recurrent HCC. The most common predicted target signaling pathway of aberrantly expressed miRNAs in HCC was Wnt signaling pathway. In real time PCR, only miR-139–5p showed aberrant expression in HCC tissues (fold change: 0.147, p = 0.015), and only miR-224 was significantly up regulated in recurrent HCC after curative resection than non-recurred HCC (fold change: 9, p = 0.048). When fold change of 2.5 (vs. non-tumor) was defined as cut-off value, sensitivity and specificity of miR-224 in predicting
recurrence were 75% and 88.6%, respectively. In our experiments, miR-224 showed superiority in predicting recurrence than the presence of microvascular invasion, large tumor size, multiple tumor and high serum alpha-feto protein levels.

**Conclusion:** In CHB related HCC, miR-139–5p was down regulated in HCC and overexpression of miR-224 in HCC can predict recurrence after curative resection.

**659 HIGH HUMAN LEUKOCYTE ANTIGEN G (HLA-G) EXPRESSION IN HEPATOCELLULAR CARCINOMA (HCC)**

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**Background and Aims:** Human leukocyte antigen G (HLA-G) is a non-classical major histocompatibility complex class I molecule, which may down regulate immune system cells functions. The aim of this study was to evaluate the expression of HLA-G in hepatocellular carcinoma (HCC) and non-tumor liver specimens, and stratify it according to histological tumor stage (Edmondson-Steiner Classification).

**Methods:** The expression of HLA-G was studied in 27 HCC samples and its surrounding liver tissue. A 5-m-thick sections were placed on tosylsulfonyl chloride-pretreated slides for HLA-G immunohistochemical assay, using two HLA-G monoclonal antibodies: 5A6G7 (EXBIO, Vestec, Czech Republic) against soluble HLA-G5 and HLA-G6 isoforms and 4H84 (kindly provided by Mc-Master, San Francisco, CA), which recognizes all soluble and membrane-bound HLA-G molecules, both diluted at 1:50. Paraffin-embedded sections of liver tissue obtained from spontaneous abortions at approximately 3 months at 12 gestational weeks were used as positive control. The result was initially divided into positive or negative. Then, it was classified according to intensity of staining (mild, moderate or intense) and semi-quantitative analysis (negative, <25% staining cells; 25% to 50% staining cells; and tissue specimens with >50% staining cells).

**Results:** HLA-G expression was observed in 96.3% (26/27) of primary HCC lesions, and in 100% (23/23) of corresponding adjacent liver tissue. However, the frequency of moderate or intense HLA-G staining was significantly higher in adjacent tissue (86.9% (20/23)) compared to HCC sample (30.8% (8/26)) (P = 0.001). With respect to staining cells percentage, the majority of HCC positive samples presented >75% staining cells [92.3% (24/26)], with only one sample presenting 50%. All surrounding tissues showed >75% staining cells (22/22). Regarding HCC stage, moderate and intense HLA-G expression was more frequent in stages I and II compared to stages III and IV (P = 0.0302).

**Conclusions:** HLA-G was expressed in the majority of HCC samples and its surrounding tissue, with more intense staining in tumor free tissue and more differentiated cancer. Further studies are necessary to clarify the role of HLAG-expression in the setting of HCC, and its applications for liver cancer prognosis.
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ULTRASONOGRAPHIC (US) SCREENING OF HEPATOCELLULAR CARCINOMA (HCC) IN COMPENSATED CIRRHOSIS DUE TO HEPATITIS C VIRUS (HCV) IMPROVES SURVIVAL: A MODELING APPROACH

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Background: US screening in cirrhotic patients is recommended by experts to detect and treat HCC at an early stage. In the absence of randomized studies, a modeling approach was performed to measure the impact on life expectancy (LE) of HCV-related HCC patients (HCV/HCC).

Methods: A Markov model was developed to simulate the progression of HCV/HCC from diagnosis until death. Simulated patients were distributed and treated according to the BCLC classification at diagnosis. Given that, in real-life cohorts, 47% of HCV/HCC have access to HCC-screening, and 58% of patients are aware of their HCV-status, we estimated that 81% of them entered into a screening program (58% × 42% × 0.5 = 47%). Efficiency for an early HCC-diagnosis (BCLC 0/1A) depends on modalities of HCC-screening. In a multicenter French cohort, modalities of HCC-screening were able to diagnose early HCC in 42% of cases (real-life efficiency). In a randomized controlled trial testing rigorous application of US HCC-screening, early HCC was diagnosed in 87% of cases (CHC-2000, Hepatology 2011) (optimal efficiency). The model estimates LE for a cohort of HCV/HCC, aware of their HCV-status, according to five scenarios: S1) no access to HCC-screening; S2) current access rate to HCC-screening (81%) with real-life efficiency for an early HCC-diagnosis (42%); S3) increase in HCC-screening rate (97%) with real-life efficiency for an early HCC-diagnosis (42%); S4) optimal efficiency for an early HCC-diagnosis according to modalities applied in a randomized trial (87%) with current HCC-screening rate (81%); S5) S3+S4.

Results (Figure): Current HCC-screening increases LE by 18 months compared to a scenario without HCC-screening (S2 vs. S1). Compared to current HCC-screening, LE would increase by 3.5 months with improvement in HCC-screening rate (S3 vs. S2), 26 months with improvement in early HCC-diagnosis efficiency (S4 vs. S2), 30.8 months with improvement in HCC-screening rate and in early HCC-diagnosis efficiency (S5 vs. S2). Assuming a more conservative improvement in efficiency for early HCC-diagnosis (sensitivity analysis: 70% vs. 87%), LE would still increase by 16.8 months.

Conclusions: Our study shows the benefits of HCC-screening on LE in HCV/HCC. It shows the necessity to improve modalities of HCC-screening close to those achieved in randomized clinical trials.

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WORLDWIDE TREND OF HEPATOCELLULAR CARCINOMA: SYSTEMATIC ANALYSIS AT FIVE-YEAR INTERVALS FROM 1990 THROUGH 2010

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Background: Hepatocellular carcinoma (HCC) is among the top 10 causes of cancer death worldwide. While previous reports on this malignancy indicated a potential upward trend in mortality and morbidity from this disease, a systematic analysis of global data was lacking. Our study fulfills this need. We offer the results of our data analysis, providing HCC epidemiologic indicators by age and sex from 187 countries presented at five-year intervals from 1990 through 2010.

Methods: Our data collection on incidence, mortality and morbidity of HCC was obtained from multiple sources: vital registration, verbal autopsy, surveillance reports, and cancer registries for the two-decade period of the study. We calculated incidence by modeling mortality-to-incidence ratio. Gaussian process regression was used to provide point estimate of death with confidence intervals for each age-sex-country-year category. We corrected the calculated mortality to fit the overall mortality at each category.

Results: Global age-standard mortality per 100,000 populations was increased from 12.3 (95% CI: 10.3–14.7) to 12.6 (95% CI: 10.8–14.7), while the upward trend was noted in 13 of 21 GBD regions, in the rest of regions the trend was downward. The corresponding incidence also increased from 11.3 (95% CI: 9.5–12.6) to 12.1 (95% CI: 10.5–14.1). The mortality rates varied greatly within and between regions.

Conclusion: Mortality from HCC, already a major cause of cancer death, is globally on the rise. Factors contributing to the trend include infection with HCV and HBV, the latter occurring prior to the initiation of vaccination campaigns and mass screening of blood products. Additionally, longer survival in those living with liver cirrhosis may have increased the risk of developing HCC. Identification and screening of patients infected with viral hepatitis B and C as well as those with chronic liver disease through non-invasive techniques may have led to increased case detection over the study period.

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THE ROLE OF HISTOLOGY IN THE PROGNOSIS OF LIVER ADENOMATOSIS

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Background and Aims: Adenomatosis of the liver is an extreme rare disorder characterized by multiple adenomas in an otherwise normal liver. Its risk of bleeding or transformation into HCC is not well defined. Solitary hepatocellular adenomas (HCAs) are
more common and recently several prognostic molecular features have been described. The risk of bleeding of HCAs is around 18% and of malignant transformation 4%. Two-thirds of HCAs with malignant transformation are β-catenin-activated. We investigated whether liver adenomatosis has the same risk factors to develop complications.

Methods: Study population of 36 patients, seen between 1990–2012 (2 pts excluded with glycogen storage disease). Several liver biopsies or resection specimens were available in 21 pts. Baseline characteristics: female/male ratio = 35/1; age: 38 yrs (17–65); oral contraception (OC) 32/34; duration of OC use: 20 yrs (6–35); Diabetes: 8/36 (21%) and BMI >= 25: 17/31 (55%).

Results: Median follow up is 63 month (4–170). Histology: HNF1α inactivated n = 6 (29%), β-catenin-activated n = 3 (14%), inflammatory n = 8 (38%) and mixed (19%); HNF1α + inflammatory (n = 1), HNF1α + β-catenin-activated (n = 1), inflammatory + β-catenin-activated (n = 2). Complications: bleeding (excluding intratumoral): 3/36 (8%) and malignant transformation: 4/36 (11%). The median minimal size of the nodule with malignant transformation (HCC) was 9cm (8.4–9cm). All of them were β-catenin-activated. Treatments and outcome: regression (after stop OC): 6/36 (17%) with complete regression in 1 pt; surgical resections (due to bleeding or suspicion of HCC): 19/36 (53%); LT (due to HCC): 2/36 (6%); none of the pts died.

Conclusion: Our series confirms a strong female predilection and a link with the metabolic syndrome. Regression after stop OC was observed in 17% (with one complete regression). The risk of malignant transformation was 11% and was only seen in nodules >8cm. Different nodules can have different phenotype markers. Malignant transformation was only seen in β-catenin activated lesions. The management of liver adenomatosis needs to be adapted to the histological phenotype of the lesions.

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THE INCREASE IN POOR LIFESTYLE FACTORS IS A RISK FOR HEPATOCELLULAR CARCINOMA IN PATIENTS WITH HEPATITIS C VIRUS INFECTION

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Background and Aim: Obesity, diabetes mellitus, alcohol intake and smoking are well known to influence the clinical course of hepatitis C virus (HCV) infection, but little is known about how they are associated with carcinogenesis. We investigated the relationship between these lifestyle factors and age at onset of hepatocellular carcinoma (HCC) in HCV-infected patients.

Patients and Methods: Out of 857 patients with HCV antibody-positive HCC initially treated between 1990 and 2011, we included 780 without uncontrollable ascites and with complete data for evaluation. Their clinical characteristics were: male/female ratio, 542/238; mean age at HCC onset, 67.5 years (range, 39–92); and tumor stage (I/II/III/IV) and Child–Pugh class (A/B/C) at HCC onset, 172/284/237/82 and 564/202/14, respectively. We recorded their alcohol and smoking habits, body mass index (BMI) and presence/absence of diabetes, and analyzed factors associated with age at onset of HCC using the Kaplan–Meier method and Cox proportional hazards models.

Results: The mean age at onset of HCC without/with alcohol intake ≥50 g/day, smoking ≥1 pack/day, obesity (BMI ≥30), and diabetes was 69.0±8.1/64.0±8.7, 69.4±8.1/65.5±8.7, 67.5±8.6/59.7±7.0 and 68.0±8.8/66.0±7.9 years, respectively. There were significant differences between age at onset with and without each factor.

Conclusion: Lifestyle factors were related to carcinogenesis in HCV-infected patients. The number of poor lifestyle factors was related to cancer onset at a young age. These results suggest that lifestyle interventions must be performed before HCC arises to prolong life expectancy in patients with HCV.
NON-INVASIVE DIAGNOSIS AND PROGNOSIS OF HEPATOCELLULAR CARCINOMA (HCC): PERFORMANCE OF PIVKA-II IN A WESTERN COHORT OF PATIENTS

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PIVKA-II (Protein Induced by Vitamin K absence or Antagonist-II) is routinely used for HCC diagnosis in Asian countries, the serum level of which being correlated with tumor aggressiveness. The objectives of the study were to evaluate the diagnostic value of PIVKA-II in a Western series of HCC and assess correlations with clinicopathologic data and prognostic factors of HCC including microvascular invasion (mVI).

Patients and Methods: Independent open-labeled exploratory study including serum samples of 107 patients with HCC treated by liver resection or transplantation and 37 patients with chronic liver diseases without HCC. PIVKA-II serum level was measured using CLEIA PIVKA-II assay kit (Lumipulse G PIVKA-II, Fujirebio).

Results: HCC group included 107 patients (100 men, mean age 56±9 years) with 87% having cirrhosis mainly related to chronic viral infection (58%). Control group was composed of 37 patients (20 men, mean age 56±9 years) with 38% having cirrhosis. HCC mean size was 30±20 mm, with 47% of them being <2 cm. Tumors were multiple in 40% and well-differentiated in 47% of cases. mVI was present in 49 HCC (46%). AFP was higher in HCC than in controls (302±136 ng/ml vs 9±2 ng/ml, p<0.01). PIVKA-II was significantly higher in HCC than in controls (969±218 mAU/ml vs 18±3 mAU/ml, p<0.01) and significantly higher in poorly-differentiated (1498±398 mAU/ml vs 401±125 mAU/ml, p<0.01). PIVKA-II was also significantly higher in HCC with mVI than in those without mVI (1683±431 mAU/ml vs 380±145 mAU/ml, p=0.005). The AUROC curve of PIVKA-II for diagnosis of HCC was 0.91 (95% CI: 0.88–0.94). For prediction of mVI, the AUROC of PIVKA-II was 0.71 (95% CI: 0.61–0.81), respectively. Since mVI was correlated with tumor size, platelets count, PT and PIVKA-II in univariate analysis, we constructed a combined model including these factors that increased the performance for prediction of mVI in HCC diagnosis with an AUROC of 0.80 (95% CI: 0.76–0.84).

Conclusions: This study shows the higher performance of PIVKA-II in the non-invasive diagnosis and prognosis of HCC in a western series and its potential for predicting mVI. The mVI predictive model will be validated in an independent prospective study.

Grant: EIDIA/Fujirebio/Innogenetics.

PROSPECTIVE VALIDATION OF IMAGING CRITERIA FOR HEPATOCELLULAR CARCINOMA LESS THAN 3 cm BY PERCUTANEOUS LIVER BIOPSY

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Objective: The present study prospectively evaluated the accuracy of noninvasive imaging criteria for small hepatocellular carcinoma (HCC) by percutaneous liver biopsy.

Methods: We consecutively enrolled 265 patients in high risk group for HCC who showed arterial hepervascularization and venous/delayed phase washout in at least one dynamic imaging study including contrast-enhanced dynamic computed tomogram (CT), gad-oxetic acid-enhanced magnetic resonance (MR) and contrast-enhanced ultrasonography (CEUS). After liver biopsy, patients were undergone for radiofrequency ablation for treatment of suspicious nodule.

Results: There were 41 patients with nodules less than 1 cm, 89 patients with nodules between 1 and 2 cm, and 126 patients less than 3 cm. A total of 241 patients (94.1%) were diagnosed as HCC by liver biopsy. Diagnosis of HCC were confirmed histologically at 85.3% (35/41) in nodules less 1 cm, 92.1% (82/89) in nodules between 1 and 2 cm, and 97.6% (241/256) in nodules over 2 cm. In histologically proven HCC patients, the positive predictive values among dynamic CT, MR, and CEUS was similar in nodules over 2 cm (97.6%, 99.2%, and 98.4%, respectively), and nodules between 1 and 2 cm (91.5%, 98.9%, and 96.3%). However, the positive predictive value by single imaging modality was not accurate in nodules less than 1 cm (77.1%, 94.3%, and 85.7%, respectively). When combining MR and CEUS together, the sensitivity, specificity, and positive predictive value were 85.3%, 100% and 97.2%.

Conclusion: The EASL-AASLD diagnostic criteria for HCC could establish diagnosis of HCC over 1 cm. However, multiple diagnostic imaging modalities or liver biopsy should be considered for accurate diagnosis of HCC in nodules less than 1 cm.

ARE SELECTIVE SEROTONIN RECEPTOR INHIBITORS (SSRI) ASSOCIATED WITH HEPATOCELLULAR CANCER IN PATIENTS WITH HEPATITIS C?

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Background and Aim: Antidepressants are commonly prescribed for patients with hepatitis C (HCV). Research suggests SSRIs may be involved in development of cancers, and serotonin could promote growth of hepatocellular cancer (HCC). A retrospective cohort study was conducted to test the hypothesis that exposure to SSRIs is associated with increased risk of HCC in patients with HCV.

Methods: Data on 109,736 patients in the Department of Veterans Affairs HCV registry were analyzed. During 8 years of follow-up after a one year baseline period without an SSRI or HCC, 36,192 filled at least one prescription for an SSRI. Cases of HCC were identified by inpatient and outpatient diagnosis codes (ICD-9-CM: 155.0). Multivariable Cox regression analyses were used to estimate adjusted HCC hazard ratios (HR) after subjects were exposed to an SSRI or HCC, 36,192 filled at least one prescription for an SSRI. Cases of HCC were identified by inpatient and outpatient diagnosis codes (ICD-9-CM: 155.0). Multivariable Cox regression analyses were used to estimate adjusted HCC hazard ratios (HR) after subjects were exposed to an SSRI or HCC.

Results: There were 41 patients with nodules less than 1 cm, 89 patients with nodules between 1 and 2 cm, and 126 patients less than 3 cm. A total of 241 patients (94.1%) were diagnosed as HCC by liver biopsy. Diagnosis of HCC were confirmed histologically at 85.3% (35/41) in nodules less 1 cm, 92.1% (82/89) in nodules between 1 and 2 cm, and 97.6% (241/256) in nodules over 2 cm. In histologically proven HCC patients, the positive predictive values among dynamic CT, MR, and CEUS was similar in nodules over 2 cm (97.6%, 99.2%, and 98.4%, respectively), and nodules between 1 and 2 cm (91.5%, 98.9%, and 96.3%). However, the positive predictive value by single imaging modality was not accurate in nodules less than 1 cm (77.1%, 94.3%, and 85.7%, respectively). When combining MR and CEUS together, the sensitivity, specificity, and positive predictive value were 85.3%, 100% and 97.2%.

Conclusion: The EASL-AASLD diagnostic criteria for HCC could establish diagnosis of HCC over 1 cm. However, multiple diagnostic imaging modalities or liver biopsy should be considered for accurate diagnosis of HCC in nodules less than 1 cm.
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VALIDATION OF THE MESIAH SCORE AS PREDICTOR OF SURVIVAL IN HIV-INFECTED PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)

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Background: Recently, the MESIAH score (Model to Estimate Survival In Ambulatory HCC Patients) has been proposed as predictor of survival in patients with HCC. It has not been validated in HCC patients with HIV infection, which is the objective of this study.

Methods: Overall, 206 HIV-infected patients with HCC were retrospectively identified from 1992–2012 in 38 centers in North and South America, Europe, and Australia. Predictability of survival using the MESIAH score was compared with 4 other published prediction models: BCLC (Barcelona-Clinic-Liver-Cancer), CLIP (Cancer-of-the-Liver-Italian-Program), JIS (Japan-Integrated-System) and Okuda. Univariate analysis was determined by Kaplan Meier analysis for univariate analysis of categorical variables and unadjusted Cox regression for continuous variables. Multivariate analysis was performed by Cox regression. The likelihood ratio and c-statistic determined the goodness of the fit of the Cox models.

Results: Among the 206 patients, there were 124 deaths with a median survival of 10.0 months (95% confidence interval, 6.5–13.5 months). The median MESIAH score was 3.960 (range, 2.09–7.39, interquartile range, 3.16–4.79) with 82% of patients having a performance status (PS) of 0–1. In univariate analysis, all 5 models significantly (all p < 0.0001) predicted survival. In multi-variable stepwise Cox regression analysis using the 5 models but no other variables, only MESIAH (hazard ratio, 1.63; p < 0.0001) and BCLC (HR, 1.35; p = 0.0001) independently predicted of survival, but not CLIP, JIS and Okuda. After controlling for age, CD4+ cell count, log HIV viral load and MELD score, both MESIAH (HR, 1.53; p = 0.0002) and BCLC (HR, 1.28; p = 0.0003) remained independent predictors of survival. MESIAH correlated well with BCLC stages (p < 0.05 for all comparisons of MESIAH scores between stages). The strength of the predictability of MESIAH (likelihood ratio, 1071; C statistic, 0.67) was similar to that of BCLC (likelihood ratio, 1075; C statistic, 0.71). Within BCLC stage A (but not stages B-D), MESIAH further differentiated survival using the median as cut-off (median survival, 13.5 months). The median MESIAH score was 3.960 (range, 2.09–7.39, interquartile range, 3.16–4.79) with 82% of patients having a median survival of 10.0 months (95% confidence interval, 6.5–13.5 months).

Conclusion: MESIAH and BCLC complement each other in predicting survival of HCC in HIV-infected patients. In BCLC stage A, MESIAH can further differentiate survival.

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SHOULD LIVER BIOPSIES BE REPORTED BY PATHOLOGISTS WITH A SUBSPECIALIST INTEREST IN HEPATOLOGY?

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Introduction: Histopathologists working in a district general hospital usually do not have a subspecialist interest in hepatology. Most district general hospitals have a gastroenterology service and local pathologists usually report liver biopsies. The Royal college of Pathologist (RCP) recommend that ‘as minimal acceptable practice’ a liver biopsy report should include the clinical diagnosis, biopsy size, overall architecture, degree of fibrosis, severity in chronic liver disease (staging/grading), a definitive diagnosis or discussion of the differential diagnosis. Appropriate negative findings (e.g. lack of iron overload or alpha-1-antitrypsin globules) should be documented in the report.

Aims and Methods: A single centre retrospective analysis of all the liver biopsies between January 2010 to February 2012 at two district general hospitals (Barnet and Chasefarm NHS trust) in North London was performed. Data was collected from medical records and electronic results. Our aim was to assess whether liver biopsies provided the clinician with adequate information about diagnosis.

Results: 107 liver biopsies were performed during this period under ultrasound guidance by a radiologist. Mean patient age was 62 (Range 19–90). The mean number of core biopsies per patient was 1.5 (range 1–6). 10.7% (10/107) of the report did not mention a clinical diagnosis. 30% (32/107) of the biopsy report did not have a definitive or a differential diagnosis about possible etiology of underlying liver disease. However 98% (47/48) of patients with cancer had a definite or a differential diagnosis. Only 53% (9/17) patients with chronic hepatitis had severity scoring (Ishak staging/grading).

Conclusion: About one third of liver biopsies did not have diagnosis or discussion about a differential diagnosis. This number goes up to 47.5% (28/59) if we exclude malignancies. The mortality associated with percutaneous liver biopsy ranges between 0.13 and 0.33%. From an audit from UK district general hospital. With the advent of fibroscan there is less need to perform liver biopsies except in diagnosing malignancies or in hepatitis of unknown/unclear etiology. We believe that non-cancer liver biopsies should be reported by pathologists with subspecialist interest in hepatology or the procedure should be performed in a tertiary hospital to give the clinician an accurate diagnosis to aid treatment.
77 (15.4%) infected with HBV, 414 (83%) with HCV and 8 (1.6%) with HDV.

Results: At inclusion, α-fetoprotein levels >400 ng/dl, presence of metastasis and esophageal varices were similar. Male gender was more frequent in group A: 52 (92.9%) vs 324 (64.9%) (p < 0.0001). Median age was 62.5 years (range 46–80) in group A and 72 (26–92) in group B (p < 0.0001). HCC was detected during US surveillance in 24 patients in group A (42.9%) and in 329 (66.1%) in group B (p < 0.0001). Single HCC was detected in 22 (39.3%) patients in group A and in 309 (61.9%) in group B (p = 0.001). The size of larger nodules was similar (34.9±19.2 in group A vs 32±18.5 mm in group B). Diffuse HCC was more frequent in group A than in group B: 10 (17.9%) vs 35 (7%) patients, p < 0.05.

Child–Pugh score and BCLC stage were higher in group A (p < 0.05 and p < 0.001, respectively). Twenty-five (44.6%) patients in group A and 322 (64.5%) in group B met the Milan criteria (p < 0.005). Performance Status grade 0 was similar in both groups. Potentially curative treatment was possible in 22/48 (45.8%) vs 227/475 (47.7%) patients in groups A and B, respectively.

Conclusions: The NASH-related HCC accounts for about 6% of all observed HCC cases. NASH-related HCC was significantly more frequent in male gender and was detected at a younger age. Multifocal and diffuse HCCs were more frequent in NASH-related HCCs, that were detected in patients with more advanced Child and BCLC stage.

672 TUMOR GROWTH AND LYMPH NODE METASTASIS OF INTRAHEPATIC CHOLANGIOCARCINOMA ARE ASSOCIATED WITH hsa-miR-126 AND hsa-miR-128 EXPRESSION

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Introduction: Angiopoietin-1 (Ang1) and vascular endothelial growth factor (VEGF) are potent (lymph-) angiogenic factors involved in tumor cell invasion and lymph node metastasis. Given the growing interest in microRNA (miR)-dependent modulation of (lymph-) angiogenic signalling, we here tested three miR targeting genes that encode Ang1, VEGF-C and VEGF-D to be of (lymph-) angiogenic signalling, we here tested three miR

Methods: Human tissue samples of ICC and matched adjacent noncancerous liver tissue (n=43) were collected after liver resection. Tissue was snap-frozen and relative expression of hsa-miR-126, hsa-miR-128, has-miR-107 was assessed by quantitative reverse transcriptase-PCR. miR expression patterns in human ICC relative to adjacent noncancerous liver tissue (ΔΔCt) were correlated with clinicopathological parameters.

Results: Expression of hsa-miR-126, hsa-miR-128, hsa-miR-107 was detected in all ICC. High relative miR-126 expression in ICC was associated with absent perineural sheet infiltration and no lymph node metastasis (both p < 0.05). High relative miR-128 expression in ICC was associated with large tumors (>5 cm; p < 0.05). No correlation was found between miR-107 expression in ICC and clinicopathological parameters.

Conclusions: Our study provides first evidence that miR targeting genes that encode potent (lymph-) angiogenic factors are differentially expressed by ICC and associate with clinicopathological parameters. miRNA might serve as potential biomarkers in ICC, whereas further studies are needed to elucidate their functional roles in development and spread of ICC.

673 PROGNOSTIC FACTORS IN PATIENTS WITH CHOLANGIOCELLULAR CARCINOMA – COMPREHENSIVE ANALYSIS OF 570 PATIENTS

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Background and Aims: Patients with cholangiocellular carcinoma (CCC) have very poor survival. Due to small patient numbers, only few randomized prospective studies exist. The aim of the study was to characterize patients’ characteristics, evaluate the treatments and find prognostic factors in patients with CCC.

Methods: We collected data from all patients with CCC treated in our institution from 2000 to 2010 and analyzed their clinicopathological characteristics.

Results: 570 patients with CCC were identified and classified into intrahepatic CCC, extrahepatic CCC, ampullary carcinoma (AC), gallbladder carcinoma or mixed CCC/HCC. 39% of all patients were diagnosed at UICC stage IV. 10% had primary sclerosing cholangitis (PSC), which was not associated with a difference in median overall survival (OS). 46% underwent resection. After tumour resection, OS was 25 months compared to 9.3 months in non-resected patients (p < 0.0001). The 1, 3 and 5 year survival was 77±3%, 35±3% and 25±3% in resected patients, and 43±3%, 10±2% and ±1% in non-resected patients. The best surgical results were achieved in patients with AC with a median survival of 5.2 years. Adjuvant chemotherapy with gemcitabine was not benificial. Patients with metastatic disease or non-resectable tumours had a significantly longer survival when treated with chemotherapy (15.8 vs. 1.8 months median OS, p < 0.0001 and 12.8 vs. 2.3 months median OS, p < 0.0001). Patients who received first line (N = 115), second line (N = 43) and third line (N = 39) chemotherapy had an OS of 8.4, 17.0 and 30 months, respectively. Incomplete tumour resection, lymph node involvement, metastatic disease and low histological differentiation were associated with a short OS. Cholestasis, anemia and the elevation of the tumour markers CA 19–9 or CEA were indicative for a poor survival.

Conclusions: Even after radical resection, prognosis of CCC is poor. Patients with AC have the best outcome of all CCC. There is no evidence for a benefit of adjuvant chemotherapy. We identified tumour-related characteristics and laboratory values which are associated with a poor survival.

674 INSULIN INCREASES THE RISK OF HEPATOCELLULAR CANCER IN PATIENTS WITH DIABETES MELLITUS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Diabetes mellitus (DM) increases the risk of hepatocellular cancer (HCC). Several preclinical and observational studies have shown that anti-diabetic agents may modify the risk of HCC. While metformin appears to be chemopreventive, sulfonylureas may be oncogenic. The cancer-modifying effects of insulin are unclear. We performed a systematic review and meta-analysis of existing studies evaluating the effect of insulin on the risk of HCC in patients with DM.

Methods: We conducted a systematic search of Medline, Embase and Web of Science up to August 2012, and manually reviewed meeting abstracts and reference lists of all articles. Studies were included if they

1. evaluated and clearly defined exposure to insulin,
2. reported HCC outcomes, and
3. reported relative risks (RR) or odds ratio (OR) or provided data for their estimation. Pooling OR estimates with 95% confidence intervals (CI) were calculated using the random-effects model. Statistical heterogeneity was assessed with the Cochran’s Q statistic and the I² statistic.

**Results:** Seven studies (240,220 patients with DM) reporting on 22,611 cases of HCC were included in the analysis. Meta-analysis of these studies revealed a significant 16% higher risk of HCC with insulin use (as compared to non-use) (adjusted OR, 2.61; 95% CI, 1.46–4.65), though there was considerable heterogeneity among studies (Cochran’s Q test p < 0.01; I² = 88%). This risk was significantly higher in the Asian population (n=2 studies; OR, 4.36; 95% CI, 4.16–4.58) as compared to the Western population (n=5 studies; OR, 2.01; 95% CI, 1.77–3.44). The results were stable across different study design (case-control and cohort) and setting (population-based and hospital-based). The oncogenic effect of insulin continued to be significant on restricting analysis to high-quality observational studies (n=6 studies; OR, 2.50; 95% CI, 1.30–4.80). There was no evidence of significant publication bias.

**Conclusion:** Based on this meta-analysis, insulin use may be associated with an increased risk of HCC in patients with DM in observational studies. Results should be interpreted with caution since the comparator group (no insulin use) is composed of other anti-diabetic medications which have an inherent cancer-modifying effect, as well as the potential of residual confounding, particularly confounding by indication.

### 675 HIGH RESOLUTION-MAGIC ANGLE SPINNING (HRMAS)-NMR-BASED METABOLIC FINGERPRINTS OF EARLY AND RECURRENT HEPATOCELLULAR CARCINOMA


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Recurrent hepatocellular carcinoma (HCC) is more aggressive than early HCC. The metabolite changes associated with such a progression are unclear. We compared the metabolite profiles obtained by 1D proton HRMAS NMR spectroscopy of 46 needle biopsies (17 primary HCC nodules, 13 recurrent HCC nodule, and 16 paired adjacent cirrhotic specimens). In all cases the diagnosis of HCC was based on the radiological features of two different imaging techniques and was confirmed by histology in 10 cases. Median diameter of primary and recurrent nodules was 20 mm (range 14–45) and 17 (10–35), respectively. Median AFP was 13.4 ng/mL (range 0.8–27) and 39.7 (2.2–3159). Median MELD was 12 in both groups. Spectroscopy was performed using a Bruker AVANCE II 600 spectrometer. Mono-dimensional proton spectra were acquired using water-suppressed (zgcppr) pulse and spin-echo CPMG sequences. Statistics was based on the SIMCA P (version 13) package. Well resolved spectra were obtained from 44 samples (28 from the neoplastic and 16 from the non-neoplastic tissue). A clear separation between tumor and cirrhosis related spectra was obtained applying the orthogonal projections to latent structures discriminant analysis (OPLS-DA) (cumulative Q2X = 0.776; predictive component = 0.0811). This difference was maintained when the analysis of paired samples from primary and recurrent nodules was split. The comparative analysis of spectra derived from primary and recurrent nodules also showed a significant difference (cumulative Q2X = 0.941; predictive component = 0.906). Signals differentiating tumor from non-neoplastic samples, as identified by the statistical total correlation spectroscopy (STOCSY) analysis, were at 3.22, 1.34, 0.86, 3.64, 3.54, 4.62 ppm. According to BBIOREFCODE and HMDB database these signals correspond to phosphocholine, lactate, fatty acids, valine, glutaminine, and glucose. When our analysis was focused on small metabolites differentiating primary from recurrent HCC, the signal log ratio from early vs recurrent HCC was 2.13 for phosphoethanolamine, 1.87 for acetate, 1.42 for alanine and 0.9 for lactate. These findings suggest that “de-novo” lipogenesis is a characteristic of early HCC, whereas recurrent HCC is associated with an enhanced Warburg effect. HRMAS-NMR spectroscopy provides a suitable fingerprint of small HCC with potential pathophysiological and clinical implications.

### 676 CLINICAL IMPACT OF VIRAL LOAD ON THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA AND LIVER-RELATED MORTALITY IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION

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**Background and Aims:** Serum hepatitis B virus (HBV) DNA level is well known as an independent risk factor affecting the disease progression to hepatocellular carcinoma (HCC), decompensated liver cirrhosis, and liver-related death in chronic HBV-infected patients. However, the influence of hepatitis C virus (HCV) on the liver disease progression remains to be elucidated. The present study was conducted to assess the clinical impact of viral load of hepatitis C on the development of HCC and liver-related death in patients with chronic HCV infection.

**Methods:** A total of 111 subjects with chronic HCV infection who were available in serum quantitation of HCV RNA at enrollment were recruited in this retrospective cohort. All recruited subjects were followed for at least 3 years, and mean follow-up period was 54±16 months. The development of HCC and liver-related mortality according to serum HCV RNA titer during follow-up were analyzed by Cox proportional hazard model.

**Results:** HCC newly developed in 14 patients during follow-up period. One-, two-, three-, and five-year cumulative incidence rates of HCC were 2.7%, 6.3%, 9.0%, and 12.4%, respectively. The cumulative risk of HCC development was higher in subjects with high titer of HCV RNA (log titer of HCV RNA > 6 IU/mL, [n = 52]) than subjects with low titer (log titer of HCV RNA ≤ 6 IU/mL, [n = 59]) (HR = 4.639, p = 0.032) with being 10,000 person-years of 47.1 and 111.5 in the incidence rate of HCC, respectively. Old age (HR = 1.113, p = 0.004), low platelet level (HR = 0.978, p = 0.010), and initial presence of cirrhosis (HR = 19.342, p = 0.007) were other independent risk factors for the development of HCC. Liver-related death occurred in 7 patients whose 1-, 2-, 3-, and 5-year cumulative occurrence rates were 0%, 0%, 3.2%, and 6.5%, respectively. The cumulative risk of liver-related death was not affected to be serum HCV RNA titer.

**Conclusions:** Serum HCV RNA titer may be considered to be an independent risk factor affecting HCC development but liver-related mortality.
PREDICTABILITY OF PREOPERATIVE [18F]FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY FOR POSTOPERATIVE HISTOPATHOLOGIC DIFFERENTIATION AND EARLY TUMOR RECURRENCE OF PRIMARY MALIGNANT INTRAHEPATIC TUMORS


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Purpose: To evaluate the correlation between [18F]fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET) scan and histopathologic differentiation, overall and early (time-to-recurrence < 6 months) tumor recurrences among patients underwent surgical operation of primary malignant intrahepatic tumors.

Methods: Among total patients, 115 cases with primary malignant intrahepatic tumors (94 for HCC, 21 for IHCC) fulfilled inclusion criteria. The mean pSUVtumor was 3.98, and the mean TNR was 1.42 in HCC, but 7.63, and 1.81 in IHCC respectively (p < 0.050).

Results: Considering to a postoperative histopathologic differentiation, there was a correlation of TNR with HCC, and TNR, with IHCC respectively. However, on multivariate analysis, only early recurrence was shown to be reflected with TNR in HCC, and pSUVtumor in IHCC respectively. Analysis of the area under the ROC curve showed various predictive values of PET results for tumor recurrences and differentiation (Figure).

Conclusions: These results suggest that preoperative [18F]-FDG PET scan might be considered a useful reference for overall and early tumor recurrences, and histopathologic differentiations in cases of HCC and IHCC.

<table>
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Data expressed as p-value.

Figure: ROC curves for prediction based on pSUVtumor & TNR.

678 [18F]-FLUORO-DEOXYGLUCOSE PET/CT PREDICTS TUMOR PROGRESSION IN INTERMEDIATE-STAGE HEPATOCELLULAR CARCINOMA TREATED WITH LOCOREGIONAL TREATMENT


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Purpose: [18F]-fluoro-deoxyglucose positron-emission tomography ([18F]-FDG PET) monitoring of FDG uptake may be an useful for assessment of the biological behavior of hepatocellular carcinoma (HCC). We evaluated the correlation between [18F]-FDG PET with clinical characteristics and prognosis.

Methods: In total, 58 HCC patients undergoing [18F]-FDG PET before transarterial chemoembolization (TACE) between May, 2007 and May, 2010 at Seoul St. Mary's hospital were evaluated retrospectively. The ratio of tumor maximal SUV to liver mean SUV (Tsuvmax/Lsuvmean) was tested as a predictive factor. Primary endpoints were the clinical characteristics and treatment response according to Tsuvmax/Lsuvmean. The secondary endpoint was time to progression.

Results: A high SUV ratio (cutoff value of 1.70) correlated significantly with tumor size (≥5 cm) and serum AFP level (≥400 ng/mL). Objective response rates were significantly different above (15.7%) and below (66.6%) the cutoff value (P = 0.023). Patients in the low SUV group had a median TTP of 16.8 months compared with 8.1 months in the high SUV group (P = 0.011). Overall survival in the high SUV ratio group was worse than that in the low SUV ratio group (median, 56.5 vs. 23.3 months), although the difference was not statistically significant in a multivariate analysis.

Conclusions: Tumor metabolic activity (Tsuvmax/Lsuvmean), assessed by PET/CT, is an independent predictor of treatment response with TACE in intermediate-stage HCC. The Tsuvmax/Lsuvmean can be used to predict tumor progression. Thus, [18F]-FDG PET can provide valuable information for prediction of the prognosis and aid in decisions regarding treatment strategy.
Aim: Non-alcoholic fatty liver disease (NAFLD) is rapidly increasing in developed countries and can progress to hepatocellular carcinoma (HCC). In contrast, a histologic subtype of HCC—steatohepatitic HCC (SH-HCC)—shows features resembling non-neoplastic SH, including large droplet steatosis, Mallory bodies, ballooning of malignant hepatocytes, and pericellular fibrosis. In this study, we aimed to compare the clinicopathological characteristics of HCC rising from NAFLD (NAFLD-HCC), including its relation with SH-HCC, with HCC rising from hepatitis C virus-related chronic liver disease (HCV-HCC) – the major cause of HCC in Japanese patients.

Methods: We investigated non-neoplastic and neoplastic tissue specimens from 34 NAFLD-HCC patients (22 men; median age, 71 years), diagnosed on the basis of American Gastroenterological Association Clinical Practice Guidelines; 30 patients were surgically treated, and 4 were treated with radio-frequency-ablation therapy (RFA). For comparison, we investigated 34 age- and sex-matched HCV-HCC cases (30; surgery; 4; RFA) during the same period.

Results: The findings in non-neoplastic tissues were as follows: cirrhosis NAFLD-HCC 53%/HCV-HCC 74% (p < 0.05), mild fibrosis, 24%/12%; over 33% of hepatocyte steatosis, 50%/15% (p < 0.05); Mallory bodies, 91%/35% (p < 0.05); and moderate-to-severe portal inflammation, 9%/32% (p < 0.05). The rate of solitary tumor were 79%/74%, and the median diameters of the main tumor were 40 (10–150) mm/20 (10–80) mm (p < 0.05). The histological differentiations of HCC were well differentiated in 21%/24%, moderately differentiated in 71/65% (nodule in nodule pattern with peripheral well differentiated areas in 6 cases/2 cases), and poorly differentiated in 9%/11%; histological patterns were trabecular in 71%/74%, pseudoglandular in 15%/15%, and compact in 3%/6%. The positivities of features as SH-HCC in neoplastic tissues were 70%/73%, including large droplet steatosis, Mallory bodies, ballooning of malignant hepatocytes, and pericellular fibrosis, 50%/15%, respectively. SH-HCC in neoplastic tissues were 71%/74%, pseudoglandular in 15%/15%, and compact in 3%/6%. On histological patterns were trabecular in 71%/74%, pseudoglandular in 15%/15%, and compact in 3%/6%.

Conclusion: The characteristic features of NAFLD-HCC compared to HCV-HCC were development of non-cirrhotic liver, solitary and well differentiated for tumor size, and high prevalence of features as SH-HCC.
Patients and Methods: Two hundred pairs of patients with cirrhosis and HCC and unrelated patients with cirrhosis alone were enrolled. Polymorphisms of TNFα −238, TNFα −308, and lymphotoxin (LTα) +252 were genotyped by the polymerase chain reaction with direct sequencing or restriction fragment length polymorphism. TNF haplotypes were also analyzed. From routine laboratory data, the following surrogate markers associated with hepatic fibrosis were measured: platelet count, aspartate aminotransferase/alanine aminotransferase ratio, aspartate aminotransferase/platelet ratio index, Pohl score, and cirrhosis discriminant score. These fibrosis surrogate markers were analyzed with polymorphisms and haplotypes of TNF locus.

Results: The frequencies of the variant genotypes and alleles of LTα +252 and TNFα −308 in patients with HCC were significantly higher than those in patients with cirrhosis alone. There was no such a difference in the TNFα −238 polymorphisms. Univariate analysis indicated that LTα +252 G/G genotype (odds ratio (OR) = 2.31) and TNFα −308 G/A genotype (OR = 2.31) were significantly associated with HCC. Multivariate analysis indicated that LTα +252 G/G (OR = 2.05, 95% confidence interval (CI), 1.20–3.51), TNFα −308 G/A (OR = 1.77, 95% CI, 1.02–3.07), and TNF haplotypes (AGA, OR = 5.69; GAG, OR = 7.10; GGA, OR = 2.07; 95% CI, 1.18–5.06) and thrombocytopenia (OR = 3.26; 95% CI, 1.14–9.30) were independent risk factors for HCC. Among patients with cirrhosis and HCC, significant hepatic fibrosis was found between 71.4% and 93.5% of patients with variant TNF genotypes and between 54.4% and 86.8% of patients harboring TNF haplotypes (AGA, GAG and GGG). Multivariate analysis showed that factors associated with variant genotypes included cirrhosis with Child–Pugh C (OR = 8.85; 95% CI, 1.18–3.63; and GGG, OR = 1.51, 95% CI, 1.11–2.04) were haplotypes (AGA, OR = 5.69; 95% CI, 1.90–17.10; GGA, OR = 2.07; 95% CI, 1.18–3.63; and GGG, OR = 1.51, 95% CI, 1.11–2.04) were independent risk factors for HCC. Among patients with cirrhosis and HCC, significant hepatic fibrosis was found between 71.4% and 93.5% of patients with variant TNF genotypes and between 54.4% and 86.8% of patients harboring TNF haplotypes (AGA, GAG and GGG). Multivariate analysis showed that factors associated with variant genotypes included cirrhosis with Child–Pugh C (OR = 8.85; 95% CI, 1.18–3.63; and thrombocytopenia (OR = 3.26; 95% CI, 1.14–9.30).

Conclusions: Patients with cirrhosis who carry the TNF genetic variants were correlated with advanced fibrosis and severe liver damage, which may contribute to a higher risk to HCC.

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APPARENT DIFFUSION COEFFICIENT DETERMINED TARGET RESPONSE AT DIFFUSION-WEIGHTED MRI IS AN INDEPENDENT PREDICTOR OF OUTCOME IN HEPATOCELLULAR CANCER PATIENTS TREATED WITH TRANSLARY CHEMOEMBOLIZATION

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Background and Aims: EASL and mRECIST improve response assessment of locoregional therapy for hepatocellular carcinoma (HCC) compared to RECIST 1.1, but may lack sufficient accuracy to predict non-response to one transarterial chemo-embolization (TACE). Early treatment assessment by diffusion-weighted (DWI) magnetic resonance imaging (MRI) has been shown to be an independent predictor of outcome. We compared DWI with RECIST 1.1, EASL and mRECIST at 1 month for predicting response to TACE of HCC.

Methods: Response 1 month after initial TACE was assessed at MRI by RECIST 1.1, EASL, mRECIST and apparent diffusion coefficient changes (ADCRatio) in 38 patients with HCC. Eight patients were transplanted after TACE allowing correlation of 1-month response with histopathological necrotic ratio. In 30 patients, Kaplan–Meier and log-rank tests were used to correlate the responses defined by each method to progression-free survival (PFS) and overall survival (OS). Comparative accuracy of 1 month response assessment was determined by receiver operator characteristic curves.

Results: In the 30 patients, median PFS and OS were respectively 5 and 17.5 months. Univariate analysis showed significant correlation of EASL and ADCRatio with PFS (p<0.05) and solely significant correlation between ADCRatio and PFS at multivariate analysis (p<0.001). ADCRatio correlated substantially with OS (p=0.07). For predicting 5 months PFS, sensitivity was 93.8% and specificity 85.7% for the ADCRatio, 37.5% and 71.4% for mRECIST and EASL and 87.5% and 42.9% for RECIST 1.1. In the 8 transplanted patients, the ADCRatio correlated significantly to the tumor histopathological percentage necrotic ratio (p=0.03).

Conclusions: One month ADCRatio after initial TACE for HCC is an independent predictor of PFS. Therefore DWI may show additional value in guiding early treatment decisions.

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TGF-β, CYFRA21-1, IGF2 AND IL_8 SERUM LEVELS CATEGORIZE HEPATOCELLULAR CARCINOMA (HCC) WITH FASTER GROWTH AND SHORTER SURVIVAL

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Background and Aims: HCC is a highly heterogeneous cancer whose extremely variable growth speed remarkably influences HCC’s course, therapy and prognosis (2012, LB Abstracts. Hepatology. doi: 10.1002/hep.26139). TGF-β has been demonstrated to play a key role in HCC progression in preclinical experimental models. Aim of this study was therefore to investigate the role of TGF-β1 and of other possibly related cytokines in dependence of HCC growth speed and survival.

Methods: HCC doubling time (DT) was prospectively calculated as fractional tumor growth (*log2(logTV1-logV0)) on 2 CTs performed 6 weeks apart (no therapy in between) in 78 patients with HCC at first diagnosis. Survival, disease-free survival after down-staging and transplant-free survival [Kaplan–Meier (KM)] were analyzed in relation to imaging and molecular data. Baseline Insulin, TGF-β1, CYFRA21-1, IL-1, IL-6, IL-8, IGF2, TNF-α, HFG and VEGF were measured in sera with commercial ELISA kits and compared by Mann–Whitney. (IRB10/08_CE_UniRer; NCT01657695).

Results: DT for 25th, 50th, 75th quartiles was 55.83 and 112 days respectively. Survival was significantly lower for DT <55 days vs. all and each of the other quartiles (median survival: DT <55 days:11 months; DT>55 <83 days: 41 months; DT>83 <112 days:42 months; DT>112 days:48 months; P<0.0001). TGF-β1 levels were significantly increased in patients with faster DT vs. other quartiles (P=0.002). No difference were observed regarding the other cytokines. TGF-b1, CYFRA21-1, IGF2, IL_8 levels were significantly higher at baseline in patients who died during follow-up. IL_6 and IL_8 were higher in alcohol-related HCC while IL_10 was higher in viral-related HCCs. VEGF levels were higher in patients with lesions<4cm at presentation and in patients with NASH/cryptogenic HCCs.

Conclusions: This study underscores the role of TGF-β1 in patients with HCC with aggressive growth behavior; TGF-β1 in combination with CYFRA21-1, IGF2, IL_8 is able to identify patients with worst survival. The detection of elevated levels of TGF-β1 in the serum of patients with a more aggressive HCC could select a cohort of patients suitable for receiving an anti-TGF-β inhibitor as systemic therapy.

Supported by Grant Progetto Regione Emilia-Romagna_Università 2007.
Background and Aims: Foxp3-expressing regulatory T cells impede effective immune surveillance against cancer and inhibit anti-tumor immune responses, thereby influencing the biological behavior of many, but not all tumors. This study aims to determine the microenvironment-specific influence of Foxp3 expression on tumor biology of hepatitis B-associated hepatocellular carcinoma (HBV-HCC).

Methods: This prospective study includes 90 HBV-HCC surgical resection patients enrolled between 2008–2012 at the Mount Sinai Medical Center in New York. Foxp3 mRNA in four regions of the resection specimens (center of the tumor, periphery of the tumor, non-neoplastic liver bordering tumor, non-neoplastic liver distant from tumor) was quantitated for each case using real-time PCR. Resection specimens were assessed by a single dedicated liver pathologist to evaluate tumor characteristics as well as liver fibrosis using the Ishak method.

Results: Foxp3 mRNA was detectable in all four regions of all the samples. While no differences were observed in median Foxp3 expression between tumor and non-neoplastic liver, Foxp3 expression in center of the tumor showed the greatest variance and skewed most towards the highest values (variance: 1603, 371, 66 and 110; and skewness: 6.8, 4.0, 3.4, 2.3, in center of the tumor, periphery of the tumor, non-neoplastic liver bordering tumor, non-neoplastic liver distant from tumor, respectively). This high variance in the tumor was due to the high variance in the highest quintile of Foxp3 expression, and was not observed in tumors that were associated with less fibrotic liver (Ishak stage 1–4), or in tumors that were well-differentiated. High Foxp3 expression in the tumor (highest quintile) was associated with mortality (6/17 (35%) vs. 5/64 (8%), p = 0.009). Likewise, mortality was associated with high Foxp3 expression (13.1 vs. 2.7, p = 0.015) and high variance (9325 vs. 524) in center of the tumor, but not in the other three compartments.

Conclusions: A small population of HBV-HCC patients (20%) had high Foxp3 expression within the tumor microenvironment that is highly variable and associated with mortality following surgical resection. Therefore, HCC predominate by a Foxp3-dependent immune-suppressive environment is associated with poor prognosis following surgical resection.

Reference(s)

686 PROGRESSION AND PATHOGENESIS ROLE OF RNA BINDING PROTEIN La-RELATED PROTEIN 1 IN HEPATOCELLULAR CARCINOMAS
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Background: La-related protein 1 (LARP1), was first described in Drosophila and shown to be required for embryogenesis, spermatogenesis, cell cycle progression and epithelial–mesenchymal transition (EMT). However, the functional significance of LARP1 in human cancer remains unknown.

Methods: We investigated LARP1 expression in 263 hepatocellular carcinomas (HCC) paraffin-embedded archival samples using immunohistochemistry. mRNA and protein levels of LARP1 expression in 15 HCC cell lines, and 6 paired HCC lesions and the adjacent noncancerous tissues were examined. Statistical analyses were applied to derive prognostic and diagnostic associations between LARP1 and clinical characters. The effect of endogenous LARP1 on cell invasion and anchorage-independent growth ability was examined in vitro. Western blotting and Immunofluorescence analyses were performed to identify the effects of LARP1-overexpression or -knockdown on expression of cell EMT markers.

Results: The expression of LARP1 was markedly up-regulated in HCC cell lines and surgical HCC specimens at both transcriptional and translational levels. Immunohistochemical analysis revealed that 160 of 263 (60.8%) HCC specimens exhibited high levels of LARP1 expression. Statistical analysis suggested the upregulation of LARP1 was significantly correlated with the clinical staging of the HCC patients (P = 0.001), TNM classification (P = 0.002), and tumor number (P = 0.045) and those patients with high LARP1 levels exhibited shorter survival time (P < 0.001). Importantly, this...
correlation remained significant in patients with early-stage HCC or with normal serum AFP level. Multivariate analysis indicated that LARP1 expression might be an independent prognostic indicator of the survival of patients with HCC. Furthermore, we found that ectopic expression of LARP1 induced, while silencing LARP1 inhibited, cell invasion and anchorage-independent growth ability. Moreover, we demonstrated that the upregulation of LARP1 could reduce the expression of E-cadherin and induce the expression of fibronectin.

Conclusions: Our findings suggest that the LARP1 protein is a valuable marker of HCC progression and may represent a novel prognostic biomarker and therapeutic target for the disease.

687 SERUM IP-10 LEVEL ASSOCIATED WITH HEPATOCELLULAR CARCINOMA DEVELOPMENT IN PATIENTS WITH CHRONIC HEPATITIS B


Background and Aims: Hepatocellular carcinoma (HCC) is considered a long-term sequela of interaction between virus, host, and environmental factors. Interferon-gamma-induced protein 10 (IP-10) is a member of the CXC chemokine family, which has been shown to be a chemoattractant and involved in a variety of immune and inflammatory reactions. This study aimed to investigate the serum level of IP-10 in association with HCC development in patients with chronic hepatitis B (CHB).

Methods: This study was based on the community-based REVEAL HBV cohort involving 3,448 CHB participants without HCV co-infection and free of cirrhosis at enrollment. The baseline serum IP-10 level was assayed using Millipore Human Cytokine/Chemokine Magnetic Bead kit. A total of 175 HCC cases were ascertained from enrollment to the end of 2008 by data linkage with the national Cancer Registry database. Multivariate Cox proportional hazards regression analysis was used to estimate the hazards ratio (HR) and 95% confidence interval (CI) of developing HCC for various IP-10 levels with adjustment for other documented risk predictors.

Results: Serum IP-10 level was positively associated with increasing age, hepatitis B e antigen (HBeAg) status, serum alanine aminotransferase (ALT), HBV DNA, and serum hepatitis B surface antigen (HBsAg) levels (all P < 0.0001). It was not associated with family history of HCC and alcohol consumption habit. The incidence rate of developing HCC per 100,000 person-year was 171.0, 345.0, 534.0, and 673.2, respectively, for IP-10 levels < 333 pg/mL (median), 333–<414 pg/mL (70% percentile), 414–<615 pg/mL (90% percentile), and ≥615 pg/mL. The corresponding HR (95% CI) was 1.0 (reference), 1.54 (1.00–2.38), 1.62 (1.08–2.45), and 1.89 (1.17–3.06), respectively, after adjustment for gender, age, HBeAg, serum ALT, HBV DNA, and HBsAg levels. The IP-10 level was statistically significant with a substantial effect size in subgroups of HBeAg-seronegative or HBV DNA <10^6 copies/mL; the corresponding multivariate-adjusted HR (95% CI) was 6.00 (2.64–13.64) and 5.95 (2.49–14.23), respectively, for per log10 increment in IP-10.

Conclusions: Serum chemokine IP-10 level was an independent host risk factor of HCC. The predictability of IP-10 level on HCC was prominent in HBeAg-seronegative patients with moderate to low viral load.
Results: Genotyping was successful in >99% of all samples. The control and case populations were both in HWE (all p > 0.05). At the allelic level, no significant difference between the cancer and control group was detected (20.1 vs. 23.0%; odds ratio (OR) 0.84, 95% confidence interval (CI) 0.63–1.12; p = 0.24). Similarly, Armitage’s trend test to assess genotypic differences yielded no significant results. Power analysis indicated sufficient statistical power (>95%) to exclude major SNP effects as per OR >1.95, thus limiting the chance of a type 2 error.

Conclusions: Our data do not support a significant modulation of biliary cancer risk by the common PNPLA3 variant unlike the situation for HCC. However, subgroup analyses and integration of metabolic co-factors, which might act in concert with variant adiponutrin to promote cholangiocarcinoma development, are pending [3].

Reference(s)

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04b. MOLECULAR AND CELLULAR BIOLOGY: BILIARY TRACT PATHOPHYSIOLOGY

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SERUM FGF19 LEVELS ARE INDEPENDENTLY RELATED TO BMI IN HEALTHY BLOOD DONORS: AN INTERIM ANALYSIS OF AN ONGOING STUDY

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Background and Aim: Fibroblast growth factor 19 (FGF19) is an enterokine playing key roles in enterohepatic signalling, bile acid (BA) synthesis, gallbladder motility and metabolic homeostasis. Aim of our study was to evaluate the correlation between serum fasting FGF19 and glucose, lipid metabolism and BMI in healthy subjects.

Materials and Methods: 285 blood donors were prospectively enrolled from our Transfusion Center, from January 2011 to March 2012. Exclusion criteria were: increased ALT, γGT or ALP, history of liver, Gl or gallstone disease, previous abdominal surgery and treatment with metabolic or Gl medications. All patients underwent lab-tests: fasting glucose and insulin, total cholesterol, HDL, LDL, triglycerides, ALT, γGT, ALP, fasting FGF19 serum level (ELISA assay), serum BA levels (HPLC-ESI-MS/MS in 250 subjects). Student t-test was used for the comparison of groups; multivariate analysis was used to identify variables independently related.

Results: 279 subjects (153M/126F; age 41.6±11.6 years) met the inclusion criteria. Mean BMI was 25.1±3.7 (male 25.6±3.1; female 23.9±3.9 P = 0.0001); mean fasting FGF19 was 124.8±84.2 pg/ml. Fasting serum FGF19 levels were significantly higher in subjects with BMI <25 (144.4±106.6) vs overweight subjects (102.9±76; P <0.001). FGF19 was inversely correlated with BMI (r = -0.245; P <0.0001). 52 subjects presented insulin-resistance (HOMA-R ≥2.5). Insulin resistance was not correlated with FGF19 level (131.7±102.9 no IR vs 112.6±89.1 IR subjects). Serum cholesterol and triglycerides did not correlate with FGF19 serum levels. On multivariate analysis, FGF19 was independently related to BMI (r = -0.778; P < 0.001).

Conclusions: Serum FGF19 was significantly lower in otherwise healthy overweight subjects and a linear inverse correlation
between BMI and serum FGF19 was observed. The mechanisms responsible for these findings are probably related with a different bile acid homeostasis, and deserve further investigation.

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NOS-3 REGULATION BY OXIDATIVE STRESS IN A CELLULAR MODEL OF CHOLESTATIC DAMAGE
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Background: During cholestatic liver disease, excessive accumulation of hydrophobic bile acids exerts a cytokotoxic effect leading to cell death and tissue damage. Oxidative stress plays a key role in this process by promoting the development of fibrosis, cirrhosis, portal hypertension and chronic liver failure. Furthermore, in cellular models of cholestatic damage it has been established a cytoprotective role for nitric oxide (NO). The aim of the study was to evaluate the regulation of endothelial nitric oxide synthase (NOS-3) in a cellular model of cytotoxicity by glycochenodeoxycholic acid (GCDCA) and its relationship with the oxidative stress and cell death.

Materials and Methods: A kinetic study was performed (0–24 hours) for induction of cell death by GCDCA (0.5 mM) in the human hepatocarcinoma cell line HepG2. The compound Mn (III) tetrakis (4-benzoic acid) porphyrinchloridin (MnTBAP, 1 mg/mL) was tested as an antioxidant molecule. The detection of reactive oxygen species and assessment of cell death was performed spectrophotometrically by using the probes 2,7-dichlorofluorescein diacetate and dihydroethidium, and by measuring caspase-3 activation and lactate dehydrogenase cellular release, respectively. NOS activity was determined by analyzing nitrite and nitrate accumulation in the extracellular medium. NOS-3 expression was measured by RT-qPCR and western-blot. The promoter activity of Nos-3 gene (1601 bp) was assessed using the luciferase activity assay. The identification of transcription factors (TFs) that could be involved in the NOS-3 regulation was performed using prediction programs. Chromatin immunoprecipitation assay and western-blot were used for further analysis and for the identification of the TFs binding sites in the Nos-3 promoter.

Results: GCDCA administration was associated to oxidative stress increase and Nos-3 promoter activity decrease, with a reduction in NOS-3 expression and cellular NO production. The expression and the binding of TFs cjun, cfos and SP1 to the Nos-3 promoter (identified positions), as well as the phosphorylation of protein kinases JNK and ERK1/2, were related to GCDCA-induced hepatocellular damage. MnTBAP treatment prevented the cellular effects of GCDCA.

Conclusions: GCDCA-induced cell death was associated to NOS-3 expression/activity decrease by oxidative-stress. This fact was related to JNK and ERK1/2 phosphorylation, and Nos-3 promoter binding increase of TFs cjun, cfos and SP1.

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PREVENTION OF CHOLESTEROL GALLSTONES FORMATION BY TWO EXTRACTS OF RAPHANUS SATIVUS L. var niger IN MICE
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Background: Cholesterol gallstones is a frequent disease in Western countries, as well as in Chile and Mexico. Furthermore cholesterol gallstones formation results from an imbalance in lipid components of bile through dysregulation of biliary transporters. On the other hand, glucosinolates are active metabolites of Raphanus sativus L. var niger (black radish), which therapeutic effects have shown to be antioxidant and hypolipidemic (J Biomed Biotechnol. 2012; 2012: 161205; Phytother Res, 2005; 19: 587).

Aim: To investigate the effects of two extracts of black radish in the prevention of cholesterol gallstones formation in mice.

Methods: Thirty-three male adult mice (C57BL/6Nhsd) were used in this study. Intragastric aqueous (H2O) or methanolic (MeOH) extract from black radish 10, 100, 1000 mg/kg plus lithogenic diet for 40 days was administered. As control groups, animals were fed with normal diet (ND) or lithogenic diet (DL) or ursodeoxycholic acid (UDCA) plus lithogenic diet. After experimental period, animals were sacrificed. Total cholesterol, bile salts, phospholipids and triglycerides were determined in serum and bile. Biliary transport protein expression from Abcb11, Abcb4, Abcg5, Abcg8 was evaluated by western blot. The presence of gallstones was determined by micro- and macroscopic analyses of the gallbladder.

Results: MeOH extract (10, 100, 1000 mg/kg) inhibited gallstones formation (Table 1). In those groups the expression of Abcg8 and Abcg5 was decreased, being dose dependent in the former one. On the other hand, mice that received MeOH extracts (10 and 100 mg/kg) showed a lower expression of Abcb11 as compared to lithogenic diet group. Abcb4 increased its expression with H2O extract (1000 mg/kg) and MeOH extract (10 and 100 mg/kg) as well, however, MeOH extract (1000 mg/kg) decreased its expression.

Conclusions: Raphanus sativus L. var niger may have important anti-lithogenic properties for prevention of cholesterol gallstones, regulating components in bile through a modulation in expression of biliary transporters.

Table 1. Effect of black radish extracts in gallstones formation and binary lipids

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Incidence of gallstones (%)</th>
<th>Bile salts</th>
<th>Phospholipids</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>0</td>
<td>148.7±1.2*</td>
<td>15.8±0.6</td>
<td>7.8±0.1*</td>
</tr>
<tr>
<td>LD</td>
<td>100</td>
<td>170.1±2.2</td>
<td>8.9±0.4</td>
<td>31.9±1.4</td>
</tr>
<tr>
<td>UDCA</td>
<td>0</td>
<td>167.6±4.0</td>
<td>15.1±0.7</td>
<td>20.6±0.7</td>
</tr>
<tr>
<td>H2O 1000</td>
<td>42.9</td>
<td>154.6±2.9</td>
<td>12.1±0.4</td>
<td>24.0±1.1</td>
</tr>
<tr>
<td>MeOH 10</td>
<td>28.5</td>
<td>158.4±2.1</td>
<td>16.3±0.5*</td>
<td>20.0±0.6*</td>
</tr>
<tr>
<td>MeOH 100</td>
<td>0</td>
<td>166.6±3.5*</td>
<td>34.2±1.7*</td>
<td>21.3±0.5</td>
</tr>
<tr>
<td>MeOH 1000</td>
<td>0</td>
<td>174.7±2.92</td>
<td>27.38±1.38*</td>
<td>8.41±0.24*</td>
</tr>
</tbody>
</table>

*Values indicate significant differences (p<0.05) between groups versus LD group. One-Way ANOVA with Tukey post-hoc.

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LYSYL OXIDASE-LIKE 2 (LOXL2) PROTEIN IS INDUCED IN HUMAN CHOLESTATIC LIVER DISEASE AND IN ANIMAL MODELS OF CHOLANGIOPATHIES
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Background and Aims: In a pathological setting, the extracellular matrix cross-linking enzyme LOXL2 creates an excess of highly cross-linked collagen which increases tissue stiffness and results in fibrotic scarring and activation of local disease-mediating cells. Since fibrosis is a feature of human cholestatic liver disease, we
sought to investigate LOXL2 expression in human samples of primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC), as well as in mouse models of cholestatic liver disease.

**Methods:** Liver explants from patients with PSC or PBC, or biopsies from non-diseased liver tissue, were evaluated by immunohistochemistry (IHC) and immunofluorescence (IF). IHC and IF as well as qRT-PCR were performed on liver tissue from Mdr2−/− and bile duct-ligated (BDL) mice, at various stages of disease progression. LOXL2 protein levels were measured by immunoassay in plasma drawn from an independent set of PSC patients (n=14) and healthy controls (n=12).

**Results:** LOXL2 protein was abundantly expressed in diseased regions of human PSC (Fig. 1A) and PBC (1B) livers, and LOXL2 protein and mRNA were induced in both the Mdr2−/− (1C & 1E) and BDL (1D & 1F) models relative to non-diseased controls. LOXL2 was present in regions of fibrosis, co-localizing with tracts of fibrillar collagen deposition in clear association with onion skin-type fibrosis and fibrotic septa. LOXL2 expression was also observed in non-fibrotic regions of ductular proliferation as well as in endothelial cells of portal vessels. Plasma LOXL2 protein was detected in 86% of PSC patients versus 8% of healthy donors (p=0.0002, Fisher’s exact test). The plasma LOXL2 levels among PSC patients positively correlated with their Mayo Risk Scores (r=0.64, p=0.002, Spearman correlation).

**Conclusions:** LOXL2 expression is elevated in PSC and PBC liver explants and is significantly increased in plasma from PSC patients, implicating this enzyme in pathogenesis of these cholestatic diseases. A similar elevation and pattern of expression were observed for LOXL2 in livers of Mdr2−/− and BDL mice, suggesting these models may be used to study the potential of anti-LOXL2 therapeutics in PSC and PBC.

**Background:** Occlusive diseases of the extrahepatic bile duct result in a dilatation of the intrahepatic bile ducts. Furthermore it is supposed that cholestasis promotes as well an increase of the smaller intrahepatic bile ducts. The congestion of bile fluid causes a periductular and periporal inflammation, which in turn acts as a proliferative stimulus for the cholangiocytes and bile ducts. The technique of bile duct ligation (BDL) is a proofed model for extra- and intrahepatic cholestasis.

The Aim of our investigation was the three-dimensional reconstruction of the intrahepatic architecture of the biliary tree in the time course of cholestasis following BDL.

**Materials and Methods:** We performed the bile duct ligation in a small rodent model (mouse). Afterwards the animals were sacrificed at several time points. As a control group we used Sham operated animals. The time points were days 3, 5, 7, 14 and 28 after BDL, respectively. To envision the biliary architecture we injected a rubber-silicone mixture (Microfil MV-122; Flow-Tech Inc., USA) into the biliary tract. After polymerization we harvested the entire liver of the animal. The specimens were scanned using a micro-CT (1 voxel, side with 20 μm). The 3D-reconstruction was performed using a non-commercial software.

**Results:** The 3D-reconstruction showed a prominent dilatation of the main intrahepatic bile ducts after cholestasis even after a short-term of up to 5 days. At the later time points a marked torquise-twisted elongation of main and smaller bile duct were found. We could not found a clear evidence for an increased branching of the bile ducts after long-term cholestasis.

**Conclusion:** For the first time the kinetics of the biliary architecture in the time course of cholestasis could be reconstructed in a 3D-modus. We could show that an increase in bile ducts seems not to result from an increased branching, rather it seems to originate from a twisted elongation of the bile ducts.

**Background and Aims:** We have previously reported that administration to rats of L-NAME (NO synthesis inhibitor) abrogates the ability of cholic acid (CA) to block bile salt (BS) synthesis leading to increased BS pool. The aim of this work was to analyze the role of CA-induced NO production in the regulation of BS synthesis.

**Methods:** Isolated rat hepatocytes were incubated with 100 μM CA in the presence or absence of 1 mM L-NAME. Chip assay was used to analyze the interaction of SHP (inhibitor of Cyp7a1 transcription) with the Cyp7a1 promoter. Nitrosylation of GAPDH (protein which transnytrosilates nuclear proteins after nuclear translocation), HDAC2 and 6 (proteins of the SHP binding complex), and Sirt1 (deacetylsase which regulates SHP activity) was studied with biotin switch assay.
Results: Treatment of hepatocytes with CA induced SHP upregulation and inhibited Cyp7a1 expression. When L-NAME was present in the incubation medium, CA increased SHP mRNA but was not able to inhibit Cyp7a1 expression. ChIP analysis showed that L-NAME significantly blocked the binding of SHP to the Cyp7a1 promoter upon CA administration. Interestingly, CA induced GAPDH nitrosylation and its nuclear translocation. Likewise, CA promoted nitrosylation of both Sirt1 and HDAC2, proteins which assemble with SHP to form the Cyp7a1 repressor complex. Nitrosylation of GAPDH, Sirt1 and HDAC2 were prevented by treatment of hepatocytes with L-NAME.

Conclusions: BS induce NO production in hepatocytes leading to nitrosylation and nuclear translocation of GAPDH and nitrosylation of proteins that integrate the Cyp7a1 repressor complex. Blockade of NO synthesis prevented the binding of SHP to Cyp7a1 promoter impairing the ability of BS to control their own synthesis.

697 HEPATOBILIARY EXCRETION MEASURED BY PET/CT USING THE CONJUGATED BILE ACID TRACER 11C-CSAR: FIRST EXPERIENCES IN PATIENTS WITH INTRAHEPATIC CHOLESTASIS

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Background and Aims: We recently developed the conjugated bile acid [N-methyl-11C]cholylsarcosine (11C-CSar) for PET/CT studies of hepatobiliary excretion (1), a physiological function which hitherto has been impossible to investigate. The method was recently validated in pigs using various experimental interventions (2). Here we present the experiences from the first human applications of 11C-CSar PET/CT.

Methods: Dynamic liver 11C-CSar PET/CT was performed in patients with intrahepatic cholestatic disorders of different etiology. During the PET/CT studies, blood was sampled from a radial artery and liver vein for radioactivity concentration time courses. We analyzed data by applying a kinetic model with 11C-CSar entering hepatocytes from blood (K_m, ml blood/min/ml liver tissue), backflux from hepatocytes-to-blood (k_2, 1/min), transport from hepatocytes-to-bile canaliculi (k_3, 1/min) and biliary flow (Fb, ml bile/min; rate constant k_4, 1/min).

Results: Severe intrahepatic cholestasis caused a significant backflux of 11C-CSar from hepatocytes-to-blood as indicated by a dramatic decrease in the extraction fraction of 11C-CSar. The uptake of 11C-CSar from blood-to-hepatocytes could be separated from the subsequent excretion from hepatocytes-to-bile canaliculi; the latter step was dramatically impaired during cholestasis.

Conclusions: Dynamic 11C-CSar PET/CT makes it possible to investigate hepatobiliary excretory function in patients with intrahepatic cholestasis. These first experiences are promising for routine clinical use of 11C-CSar PET/CT because the method enables noninvasive investigation of this key function of the liver.

Reference(s)


698 FN14 IS EXPRESSED ON NEOPLASTIC CHOLANGIOCYTES IN INTRA-HEPATIC CHOLANGIOCARCINOMA AND PROMOTES NECROSIS AFTER INTERACTION WITH TWEAK

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Background and Aims: Cholangiocarcinoma is is the second most common primary hepatic malignancy worldwide. The incidence of intra-hepatic cholangiocarcinoma in the UK has steadily increased over the last 40 years. The main treatment is chemotherapy whilst the 5 year survival after radical surgery is only 25%. The carcinogenic mechanisms involved in cholangiocarcinoma remain elusive. Tumour necrosis factor-like weak inducer of apoptosis (TWEAK) and its cognate receptor fibroblast growth factor-inducible molecule 14 (Fn14) have been shown to be of importance in cellular proliferation and tumour angiogenesis in hepatocellular carcinoma. The aim was to demonstrate the expression of Fn14 and TWEAK in cholangiocarcinoma and to determine the functional significance of Fn14/TWEAK interaction on neoplastic cholangiocytes in vitro.

Methods: Human liver samples were obtained with ethical approval and patient consent from the liver transplant programme at the Queen Elizabeth Hospital, Birmingham, UK. Sections were stained for Fn14 using immunohistochemistry. Expression of Fn14 on a cholangiocarcinoma cell line (CC-LP-1) stimulated with TNF-α, IFN-γ and FGF-basic was established quantitatively by flow cytometry. Cytokine stimulated CC-LP-1 cholangiocytes were exposed to different concentrations of TWEAK for 24 hours. Apoptosis, necrosis, autophagy and reactive oxygen species production at this time point were determined by flow cytometry using annexin, 7-aminoactinomycin D, dansylcadaverine and dichlorofluorescin assays respectively. Proliferation was determined using Ki67 nuclear staining.

Results: Immunohistochemistry reveals Fn14 on the intra-hepatic malignant small bile ducts in cholangiocarcinoma. The CC-LP-1 cholangiocarcinoma cell line expresses cell surface and intracellular Fn14 in vitro. Exposure of cytokine stimulated CC-LP-1 cholangiocytes to TWEAK for 24hrs induces necrosis and reduces apoptosis in FGF-activated neoplastic cholangiocytes in a dose-dependent manner.

Conclusion: Fn14 is expressed on neoplastic cholangiocytes in intra-hepatic cholangiocarcinoma. Activation of the Fn14/TWEAK receptor-ligand system induces necrosis. The role of Fn14/TWEAK in cholangiocarcinoma requires further investigation to ascertain the signalling mechanisms involved and outcome on overall tumour viability.

699 PROFOUND SELECTIVE REPRESSION OF Abcg5 LEADS TO MINOR CHANGES IN HEPATIC CHOLESTEROL SECRETION RATES

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Introduction: Gallstone disease is one of the most common gastroenterological diseases. Most gallstones consist of cholesterol, indicating that supersaturation of bile with cholesterol in relation to its solubilizers phosphatidylcholine and bile salts is the key pathobiological defect. Cholesterol is secreted into bile by the canalicular cholesterol hemitransporters ABCG5/G8. To date, disruption of biliary homeostasis was studied primarily in double-knockout and overexpression mouse models. Our aim now was to assess hepatobiliary phenotypes in a mouse model with more physiological changes of cholesterol efflux rates. In particular, we
analyzed cholesterol contents and metabolism in a new model with repressed Abcg5 expression in liver and intestine.

**Methods:** Using BAC transgenesis, we established homozygous Abcg5-knockdown mice that only weakly express Abcg5 in liver and intestine. We collected gallbladder and hepatic bile from knockdown mice and wild-type controls. Hepatic bile was obtained after cannulation of the bile duct for up to 8 hrs, and bile flows and hepatic secretion rates of biliary lipids were measured in the first hour of the acute bile fistula. Biliary lipids were measured using standard enzymatic assays. Expression of alternative hepatic cholesterol transporter systems was performed using qRT-PCR.

**Results:** Abcg5 expression was profoundly repressed in the transgenic mice (liver: −90%, small intestine −55 to −95%), whereas Abcg8 expression was less affected. Cholesterol concentrations in gallbladder bile were significantly lower in Abcg5 mice as compared to wildtype mice, while bile acid and phosphatidylcholine levels were not altered. Cholesterol secretion rates were selectively reduced (4.24±2.48 vs. 7.02±2.17 nmol/l/kg bw; p = 0.015), but phosphatidylcholine and bile salt outputs remained constant. In spite of nearly absent Abcg5 expression biliary coupling of cholesterol to lecithin decreased only by 24%, resetting the

2.17
±
±

to wildtype mice, while bile acid and phosphatidylcholine levels in gallbladder bile were significantly lower in Abcg5 mice as compared to wildtype mice, while bile acid and phosphatidylcholine levels were not altered. Cholesterol secretion rates were selectively reduced (4.24±2.48 vs. 7.02±2.17 nmol/l/kg bw; p = 0.015), but phosphatidylcholine and bile salt outputs remained constant. In spite of nearly absent Abcg5 expression biliary coupling of cholesterol to lecithin decreased only by 24%, resetting the biophysical conditions underlying cholesterol phase separation in bile. Of note, serum cholesterol levels did not differ between wildtype and Abcg5 deficient mice. The expression of potential alternative cholesterol transporters (Abca1, Acat2) showed no difference between transgenic and wildtype mice.

**Conclusion:** Our data show that selective knockdown of Abcg5 decouples biliary phosphatidylcholine and cholesterol secretion rates, but the ability to secrete cholesterol into bile is maintained without apparent induction of alternative transporters.

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**EFFECT OF HEPATIC CYP7A1 AND CYP3A11 MODULATION ONATHEROSCLEROSIS IN cyp27A1/apoE DOUBLE KNOCKOUT MICE**


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**Background:** The enzyme CYP27A1 accounts for bile acid and cholesterol metabolism. To explore the impact of CYP27A1 deficiency on atherosclerosis, cyp27A1+/− mice were crossed with apoE−/− mice. The resulting cyp27A1+/−/apoE−/− mice failed to develop atherosclerosis, even when fed a Western diet (WD). We hypothesize that the up-regulation of hepatic CYP7A1 and CYP3A11 mRNA protects cyp27A1+/−/apoE−/− mice from atherosclerosis development.

The aim of study was to analyze the effect of cholic acid (CA) and rifampicin (RIF) on hepatic mRNA levels of CYP7A1 and CYP3A11, lipid profile and atherosclerosis in cyp27A1+/−/apoE−/−, cyp27A1+/−/apoE−/− and cyp27A1+/−/apoE−/− mice.

**Study design and Methods:** Cyp27A1+/−/apoE−/− mice were divided into two groups (n=6); one group was fed for 2 months with WD, the other with a WD containing 0.1% CA. Cyp27A1+/−/apoE−/− and cyp27A1+/−/apoE−/− mice fed with WD were also divided in 2 groups (n=6). At the age of 6 weeks, a treatment consisting of RIF at a dose of 10 mg/kg ip daily, or vehicle only, was initiated for a month.

Lipids were analyzed in plasma, atherosclerosis quantified in the aortic valve and mRNA expression of CYP3A11, CYP7A1 and CYP27A1 measured in liver tissues.

**Results:** CA suppressed up-regulation of CYP7A1 and CYP3A11 mRNA levels in liver homogenates of cyp27A1+/−/apoE−/− mice. It increased total cholesterol (TC) ~3-fold, decreased the HDL/LDL ratio ~4-fold and unchanged triglycerides (TG). Atherosclerosis increased more than 30-fold (from 0.003 mm2 to 0.11 mm2) in aortic valve. RIF increased CYP3A11 mRNA levels in liver homogenates in both cyp27A1+/−/apoE−/− and cyp27A1+/−/apoE−/− mice without affecting CYP7A1 mRNA levels. In cyp27A1+/−/apoE−/− mice, TC decreased ~2-fold, the HDL/LDL ratio increased ~2-fold and TG increased 1.5-fold. Atherosclerotic plaque was decreased ~3-fold. In cyp27A1+/−/apoE−/− mice, RIF had no effect on lipids and atherosclerosis. Compared to cyp27A1+/−/apoE−/−, cyp27A1+/−/apoE−/− mice had ~2-fold increase of TC, ~2-fold decrease of HDL/LDL and TG. Atherosclerosis increased from 0.005 mm2 to 0.015 mm2.

Increased IL8 in bile and increased MUC5AC and CFTR expression in the gallbladder of patients with primary sclerosing cholangitis

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Introduction: Primary sclerosing cholangitis (PSC) is characterized by inflammation and fibrosis of extrahepatic and segmental branches of the intrahepatic bile ducts. We recently observed that gallbladder bile – but not ERCP (duetal) bile – of PSC patients has decreased concentrations of bile salts, phospholipids, cholesterol and FGF19, suggestive of defective concentration of bile in PSC. This concentration defect may be caused by factors from the inflamed biliary tree. This study was undertaken to elucidate the underlying mechanism.

Methods: Gallbladder bile and tissue of PSC (n = 12–13) and non-PSC liver disease patients (n = 13–14) were collected at the time of liver transplantation (LTx). IL8 levels in bile were measured by ELISA. Additional cytokines and chemokines in bile were studied by Luminex multiplex bead array. Transcript levels of genes involved in mucus formation and chloride/bicarbonate secretion were measured by RT-qPCR using mucosal epithelium-specific cytokeratins 7 and 19 as reference genes. Cultured primary human gallbladder epithelial cells (HGBECs) were incubated with a pro-inflammatory cytokine mix (TNF-α, IL-1β and IL-6) for 3 hours before gene expression analysis.

Results: IL-8, but not TNF-α, IL-1β and IL-6, was strongly elevated in PSC gallbladder bile (7.6, [2.1–16.5] vs. 2.3, [0.6–3.4] ng/mL in non-PSC patients; p < 0.02) and ERCP bile (4.5, [1.4–19.7] vs. 0.2, [0.005–0.6]; p < 0.0001). Expression of MUC5AC and CFTR was upregulated in gallbladder of PSC patients (3.9-fold, p < 0.007 and 1.8-fold, p < 0.03, resp.). In addition, Trefoil factors (TFFs) 1, 2 and 3, known to stabilize the mucus layer, were also upregulated (4.0, 17.1 and 4.3-fold, resp.; p < 0.001). HGBECs incubated with cytokine mix showed upregulation of MUC5AC, CFTR and TFF1 (5.6, 3.4 and 1.7-fold, resp.; p < 0.02).

Conclusions: Elevated gallbladder expression of MUC5AC, TFFs and CFTR may underlie dilution of gallbladder bile in PSC patients by increased secretion of mucus and chloride/bicarbonate and accompanying flow of water. The strongly increased IL8 levels in PSC bile – even at the time of liver transplantation – are suggestive of a persistent and ongoing inflammatory process in the biliary tree that may affect mucus and chloride/bicarbonate secretion by MUC5AC and CFTR in the gallbladder. The persistent inflammatory stimulus in PSC may originate from the compromised gut.

Quantitative Magnetic Resonance Imaging (MRI) in the Evaluation of the Degree of Steatosis, Iron Accumulation and Fibrosis in Chronic Liver Disease (MRker Study)

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Background: Half of all the liver biopsies performed are to assess the severity of pathology including grading of fat, iron accumulation as well as fibrosis. Liver biopsies are invasive tests associated with sampling errors; the coefficient of variation for fibrosis measurement is 45%.

Aim: To develop and validate non-contrast, non-breath-holding, quantitative MRI methodology to estimate the amount of fibrosis, fat and iron accumulation within the whole liver.

Methods: MRI relaxation time data (T1, T2 and T2*) were acquired (over 15–20 minutes) using a novel Echo Planar Imaging technique with a respiratory-triggered (r.t.) acquisition method. 1H MR spectra were acquired (r.t.) using a multiple echo PRESS acquisition which allowed for individual T2 correction to the spectrum for accurate quantification of the fat fraction in a 30x30x30 mm3 voxel.

Results: 115 patients (67 Training; 48 Validation cohort) with suspected chronic liver disease aged 19 to 72 years [alcoholic (13%), non-alcoholic (56%) fatty liver disease, chronic viral hepatitis (21%) and haemochromatosis (3%)] who had a liver biopsy ≥25mm were included in the study. The diagnostic accuracy of the T1 parameter in the detection of different histological stages of fibrosis, using receiver operator curves and areas under the curve (AUC), in the training and validation cohort are summarised in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Fibrosis Stage</th>
<th>AUC Training</th>
<th>AUC Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis (stage 4 vs. 0–3)</td>
<td>0.91</td>
<td>0.83</td>
</tr>
<tr>
<td>Advanced fibrosis (stage 3/4 vs. 0/1/2)</td>
<td>0.81</td>
<td>0.78</td>
</tr>
<tr>
<td>Mild fibrosis (stage 2/3/4 vs. 0/1)</td>
<td>0.67</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Figure 1.
There were significant correlations between MR measures of fat fraction and staging of steatosis (Figure 1), T2* with hepatic iron staging (Figure 2), as well as T1 with the percentage of fibrosis (on morphometry) assessed by histology (Figure 3), within the entire study population.

Conclusions: Across a range of chronic liver diseases, MR measures of fat fraction, hepatic iron content and fibrosis of the whole liver correlate well with related histological measures.

704 LIVER STIFFNESS CHANGES DETECTED BY ACOUSTIC RADIATION FORCE IMPULSE IMAGING (ARFI) IN PATIENTS WITH INTRAHEPATIC CHOLESTASIS

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Background: Acoustic radiation force impulse (ARFI) elastography is increasingly used to assess liver fibrosis. However, ARFI is limited in patients with high hepatic necroinflamatory activity. So far it is unclear whether intrahepatic cholestasis interferes with ARFI fibrosis assessment. The aim of this study was to prospectively evaluate liver stiffness changes in patients with biliary obstruction and cholangitis after bile duct drainage.

Patients and Methods: A total of 42 patients (31 male, mean age 60.2±15.6 years) with intrahepatic cholestasis due to inflammatory or neoplastic obstruction of the common bile duct were consecutively evaluated in this study. Twenty-eight patients (67%) had additional pre-ERCP cholangitis, indicated by a wall thickening of the biliary tree of at least 1.5 mm. Papillotomy was performed in 11 patients (26%), while 38/42 (91%) patients underwent biliary drainage by biliary stenting. Liver stiffness was measured by ARFI elastography in all patients immediately before and 1–2 days after ERCP. In 25/42 (60%) patients a second ARFI follow-up was performed 1–12 weeks after endoscopic intervention. Clinical and laboratory parameters were recorded before and after ERCP.

Results: Mean liver stiffness was significantly declining immediately after bile duct drainage was performed (from 1.97 m/s to 1.66 m/s, p < 0.0001, respectively). During further follow-up liver stiffness was not significantly changing any more (from 1.68 m/s to 1.67 m/s, p = 0.502, respectively). Overall, ARFI values decreased in 37 patients (88%) after biliary drainage with a mean decrease of 20% at first follow-up. ARFI values did not significantly change in 5 patients (12%). Mean ARFI values were not significantly higher in patients with ultrasonographic signs of cholangitis than in those without (1.82 m/s vs. 1.74 m/s, p = 0.28). There was no correlation between ARFI baseline values and serum tests such as CRP, alkaline phosphatase, gamma-GT and bilirubin.

Conclusion: Liver stiffness is significantly decreasing after biliary drainage. Thus, assessment of liver fibrosis by ARFI elastography is limited in case of intrahepatic cholestasis and should be ruled out before ARFI elastography of the liver is performed.
706 COMPARISON OF LIVER BIOPSY, ELASTOMETRY AND SERUM MARKERS FOR LIVER FIBROSIS ASSESSMENT IN GENOTYPE 4 HCV-INFECTED PATIENTS IN EGYPT. RESULTS OF THE ANRS12184 STUDY
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Background and Aims: The lack of alternative techniques to liver biopsy (LB) may be the cause of undertreatment and insufficient monitoring of liver fibrosis progression. In this study, we compared elastometry (FS©) and serum markers (SM) with LB in Egypt, a country with 6 million individuals chronically infected with genotype 4 (G4) HCV.

Methods: All enrolled patients had a LB, a FS© and SM (APRI, Fib4, and Fibrotest©) within the same month. Only LBs at least 15 mm long and including 10 portal tracts, were kept in the final analysis. The F0/F1, F2, F3, and F4 stages were defined as unreliable in the following situations: fewer than 10 valid shots; success rate (SR) <60% and/or interquartile range interval (IQR) ≥30%.

Results: 474 patients were initially enrolled, of which 162 (34.2%) were excluded for inadequate biopsies. Patients were 39±10, 70%M/30%F, with a body mass index of 28±4 kg/m². The liver fibrosis distribution was F0/F1: 36%, F2: 33%, F3: 15%, and F4: 16%. Only 1.5% of the LBs had schistosomiasis-linked granulomatosis despite 37.3% of positive serology. Thresholds differentiating F0/F1 vs F2/F3/F4 were 4.43, 1, 0.37, and 7.8 kPa for APRI, Fib4, FT© and FS©, respectively, and the AUROC were 0.71, 0.77, 0.73 and 0.72, respectively. Thresholds differentiating F0/F1 vs F2/F3/F4 were 8.37, 1.27, 0.81 and 10.4 kPa, respectively, and AUROC were 0.74, 0.80, 0.75 and 0.87, respectively. Fib4 alone would satisfactorily categorize nearly 70% of patients as F0/F1 and F4, thus avoiding LB, and with the addition of FS, 80% would be properly categorised.

Conclusions: These data show that Fib4 and FS performed well among G4 infected Egyptian patients, and that “easy to use” algorithms including these two techniques may avoid the majority of LBs.

707 FACTORS ASSOCIATED WITH IMPOSSIBILITY TO OBTAIN RELIABLE LIVER STIFFNESS MEASUREMENTS BY MEANS OF ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY (ARFI)
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Background and Aim: ARFI elastography is a non-invasive method for liver fibrosis evaluation increasingly used in the last years. Our aim was to identify factors associated with impossibility to obtain reliable liver stiffness (LS) measurements by means of ARFI.

Material and Methods: Our study included 994 subjects, with or without hepatopathy, in which LS was evaluated by means of ARFI. Ten valid ARFI measurements were performed in each subject and median values were calculated, expressed in meters/second (m/s). Failure of TE measurements was defined as no valid measurement obtained after at least 10 shots, and a measurement was considered as unreliable in the following situations: fewer than 10 valid shots; success rate (SR) <60% and/or interquartile range interval (IQR) ≥30%.

Results: Our subjects were: healthy volunteers – 207 (20.8%); patients with chronic hepatitis B (67 – 6.8%) and chronic hepatitis C (73 – 7.3%), with various stages of fibrosis on liver biopsy; patients with liver cirrhosis diagnosed by means of clinical, biochemical, ultrasonad and/or endoscopic criteria – 616 (61.9%). Failure of LS measurements by means of ARFI was observed in 4 subjects (0.4%), unreliable measurements in 59 subjects (5.9%), so reliable measurements were obtained in 931 subjects (93.7%). The factors associated with failed and unreliable measurements are presented in the table.

Conclusions: Valid ARFI measurements were obtained in 93.7% of the subjects. Older age and higher BMI were associated with impossibility to obtain reliable ARFI measurements.

708 FACTORS INFLUENCING FAILURE OF LIVER STIFFNESS MEASUREMENTS USING TRANSIENT ELASTOGRAPHY – MONOCENTRIC EXPERIENCE
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Background and Aim: Liver stiffness measurement (LSM) using transient elastography (FibroScan® device) is a non-invasive technique that evaluates liver fibrosis. In some cases, however, reliable elasticity measurements are not obtained. The aim of this study was to assess the prevalence and factors associated with failed and unreliable LSMS in patients with chronic liver disease.

Material and Methods: Our study included 8218 patients with chronic liver diseases of diverse etiologies. In each patient 10 LSMS...
were performed with a FibroScan device (M probe – Echosens, France); a median value was calculated and the results were expressed in kilopascals (kPa). Failure of TE measurements was defined if no valid measurement was obtained after at least 10 shots; and unreliable if: fewer than 10 valid shots obtained; success rate (SR) <60% and/or interquartile range interval (IQR) ≥30%. We analyzed the factors associated with failed and unreliable measurements.

**Results:** Failed and unreliable LSMs were observed in 29.2% (2404/8218) cases. In univariate analysis, the following risk factors were associated with failed and unreliable measurements: age ≥50 years (OR 2.04; 95%CI 1.84–2.26, p < 0.0001); female gender (OR 1.32; 95%CI 1.20–1.45, p = 0.0001); BMI ≥27.7kg/m² (OR 2.89, 95%CI 2.62–3.19, p < 0.0001); weight >77kg (OR 2.17; 95%CI 1.97–2.40, p < 0.0001); and height <162cm (OR 1.26; 95%CI 1.14–1.40, p < 0.0001). In multivariate analysis all factors mentioned above were independently associated with the risk of failed and unreliable measurements.

If all positive factors were present (men <50 years, BMI ≤27.7kg/m², lighter than 77kg and taller than 162cm) the rate of failed and unreliable measurements was 58.5% (185/316). In obese patients (BMI >30kg/m²), the rate of failed and unreliable measurements reached almost 50% (969/1956 – 49.5%). If all positive factors were present (woman ≥50 years, BMI ≥27.7kg/m², heavier than 77kg and shorter than 162cm) the rate of failed and unreliable measurements was 58.5% (185/316). In obese patients (BMI >30kg/m²), the rate of failed and unreliable measurements reached almost 50% (969/1956 – 49.5%).

**Conclusion:** Failed and unreliable LSMs were observed in 29.1% of the patients. Female gender, older age, higher BMI, higher weight and lower height were significantly associated with failed and unreliable LSMs. This study underlines the need to use the XL probe in overweight patients to increase the rate of valid measurements in this type of patients.

**Table 1. Transient Elastography**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-discordant patients (%)</th>
<th>Discordant patients (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 (19–77)</td>
<td>52 (19–78)</td>
<td>0.97</td>
</tr>
<tr>
<td>Gender: female</td>
<td>n = 150 (47.4%)</td>
<td>n = 69 (54.4%)</td>
<td>0.21</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 ± 3.8</td>
<td>25.2 ± 3.8</td>
<td>0.47</td>
</tr>
<tr>
<td>European patients</td>
<td>n = 280 (80.7%)</td>
<td>n = 90 (70.8%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Asian patients</td>
<td>n = 94 (19.3%)</td>
<td>n = 37 (29.2%)</td>
<td>0.02</td>
</tr>
<tr>
<td>ALT (×ULN)</td>
<td>1.3 (0.2–22)</td>
<td>1.3 (0.3–26)</td>
<td>0.41</td>
</tr>
<tr>
<td>Fibrosis: F0–2</td>
<td>n = 182 (52.4%)</td>
<td>n = 94 (74.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Length of LB specimen (cm)</td>
<td>3 (0.8–6)</td>
<td>2.5 (0.7–5)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Results:** In 92.5% of patients reliable measurements were obtained both by elastographic methods. Discordance with biopsy of at least 2 stages of fibrosis was observed in a similar number of cases in TE and ARFI: 26.8% vs. 25.6%, p = 0.78. The factors associated with discordances are presented in tables.

**Conclusions:** Mild stages of fibrosis were associated with discordance in both TE and ARFI elastography. For TE the Asian race and for ARFI female gender were also associated with discordances.

**710 TIMP-1 IN PATIENTS WITH CIRRHOSIS: RELATION TO LIVER DYSFUNCTION, PORTAL HYPERTENSION, AND HAEMODYNAMIC CHANGES**

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**Background:** Patients with cirrhosis and portal hypertension often develop complications relating to haemodynamic changes. The diagnosis and consequences hereof are often based on invasive procedures, such as liver biopsy and measurements of the hepatic venous pressure gradient. Thus, there is obviously a need to identify non-invasive markers of the severity of liver cirrhosis. Matrix metalloproteinases and their specific inhibitors, tissue inhibitors of metalloproteinases (TIMPs) play a pivotal role in hepatic fibrogenesis and fibrolysis.

**Aim:** To investigate plasma TIMP-1 in liver cirrhosis patients undergoing catheterisation and relate the findings to the degree of liver dysfunction, portal hypertension and haemodynamic changes.

**Methods:** We included 96 patients with verified cirrhosis (Child A/B/C: 31/33/23) and 15 matched controls without liver disease. All individuals underwent a liver vein catheterisation with haemodynamic assessment. TIMP-1 was determined in arterial and hepatic venous plasma using the in-house MAC-15 TIMP-1 ELISA, which measures total (complexed and uncomplexed TIMP-1) with high analytical performance.
Results: There was no significant difference between arterial and hepatic venous concentrations of TIMP-1 in either patients or controls. Hepatic venous concentrations of TIMP-1 was significantly increased in patients with liver cirrhosis 317 (193) ng/ml versus 153 (104) (median/IQ range) (p < 0.001) with a progressive increase throughout the Child classes with the highest levels in Child class C patients (p < 0.001). Circulating TIMP-1 correlated significantly with ICG-clearance (r = −0.62, p < 0.0001), CEC (r = −0.40, p < 0.0001), the hepatic venous pressure gradient: (r = 0.59, p < 0.0001), mean arterial pressure (r = −0.40, p < 0.0001), and systemic vascular resistance (r = −0.36, p < 0.0001).

Conclusions: TIMP-1 is significantly increased in patients with liver cirrhosis. TIMP-1 correlates significantly with the severity of the liver disease, degree of portal hypertension, and the vasodilatory state. TIMP-1 may represent a promising non-invasive marker to predict development of haemodynamic-related complications in patients with cirrhosis.

711 CIRRHOSIS DIAGNOSIS IN CHRONIC HEPATITIS C: ELASTOMETRY VS. A SPECIFIC BLOOD TEST
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Background and Aim: Cirrhosis is an important diagnosis in chronic hepatitis C for treatment regimen and complication screening. Our aim was twice: to compare performance of elastometry vs a specific blood test, and classical binary diagnosis vs new classification for cirrhosis diagnosis.

Methods: 679 patients with chronic hepatitis C had liver biopsy (Metavir staging and morphometry), liver elastometry by Fibroscan and CirrhoMeter, a specific blood test for cirrhosis. Cirrhosis prevalence was 15%. Diagnostic indices of tests were compared according to classical binary diagnosis (single cut-off determined by maximum Youden index or accuracy) or multinomial fibrosis classification owing to percentile method.

Results: Binary diagnosis: Youden cut-off provided good accuracy (Fibroscan: 85.4%, CirrhoMeter: 79.2%, p < 0.001) and maximized sensitivity (Fibroscan: 83.3%, CirrhoMeter: 82.3%, p = 1) and negative predictive value (Fibroscan: 96.9%, CirrhoMeter: 96.4%, p = 0.68). Accuracy cut-off provided excellent accuracy (Fibroscan: 91.2%, CirrhoMeter: 89.8%, p = 0.28) and maximized specificity (Fibroscan: 97.3%, CirrhoMeter: 97.4%, p = 1) and positive predictive value (Fibroscan: 72.9%, CirrhoMeter: 77.3%, p = 0.57). The patient rate having at least 95% predictive values for cirrhosis was: Fibroscan: 83.4% vs CirrhoMeter: 75.6% (p < 0.001). Fibrosis classification: the same 6 fibrosis classes were obtained with both tests: F0/F1, F2/1, F2/1, F3/1, F4 and F4. These classifications were validated by a good correlation (Spearman rho, all p < 0.001) with the area of portal fibrosis: Fibroscan: 0.535, CirrhoMeter: 0.453. Classification accuracy was excellent: Fibroscan: 88.2% vs CirrhoMeter: 88.8% (p = 0.77). Sensitivity of classes including F4 was: Fibroscan: 83.3% vs CirrhoMeter: 82.3% (p = 1). Positive predictive values were in F4 class: Fibroscan: 78.8% vs CirrhoMeter: 82.4% (p = 0.94), in F3/4 class: Fibroscan: 72.2% vs CirrhoMeter: 67.6% (p = 0.68) and in F3/1class: Fibroscan: 27.7% vs CirrhoMeter: 27.5% (p = 0.96).

Conclusion: We provided the first detailed fibrosis classification of Fibroscan. Compared to classical binary diagnosis, this classification provides several advantages: increased accuracy, precision, sensitivity and positive predictive value. The limit is still a small grey zone but the cirrhosis probability is clearly depicted. A specific blood test markedly attenuates the known difference in accuracy between usual blood tests (constructed for significant fibrosis) and Fibroscan. Therefore, a blood test designed for cirrhosis could be an alternative to Fibroscan.

712 EVALUATION OF CIRRHOSIS MISCLASSIFICATION RATE BY NON-INVASIVE TESTS IN HEPATITIS C TRIALS
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Background and Aim: Several trials, especially in chronic hepatitis C, relies cirrhosis diagnosis (inclusion or exclusion) on a single cut-off of non-invasive test(s). False positives are generally thought to be fibrosis stage close to cirrhosis. Yet, these statements are based on any recommendation. Therefore, we evaluated predictive values for cirrhosis of available tests including a detailed fibrosis classification.

Methods: All patients had chronic hepatitis C and liver biopsy with Metavir fibrosis (F) staging. We evaluated negative (NPV) and positive (PPV) predictive values of tests considering either the F4 class or all the classes including F4 (e.g. F3/4) called Fx/4. The highest value of NPV and PPV determined the choice of fibrosis class and test. In population #1 including 1056 patients, we compared blood tests: Fibrotest, FibroMeter and CirrhoMeter. In population #2 including 679 patients, we compared blood tests, liver stiffness (Fibroscan) and their combination (CombiMeter). Other characteristics were evaluated: F distribution, morphometry, markers of liver function or portal hypertension (details not shown).

Results: Population #1: considering a cirrhosis trial, the optimal choice relies on the cut-off of CirrhoMeter F4 class since its NPV provides a high inclusion rate of cirrhosis (88%) whereas this rate is only 37% with Fibrotest, but at the expense of a higher number of patients to screen. Considering trials excluding cirrhosis, the optimal choice relies on the cut-off of CirrhoMeter Fx/4 classes since its NPV provides a low inclusion rate of cirrhosis (1%) whereas this rate is 4% with Fibrotest, but at the expense of a higher number of patients to screen. Population #2: results validated the best PPV of CirrhoMeter F4 class (89%). They also validated an excellent NPV of Fx/4 classes in all single tests (NPV>97%) with, nevertheless, a small advantage for the test combination (NPV: 98%).

Table: Patient rate with predictive values for cirrhosis

Population: classes F | PPV/NPV
---|---
Fibrotest | 77.7/89.7, 81.6/90.3, 84.3/90.9, 88.8/91.0, 90.2/91.5
FibroMeter | 72.7/89.2, 75.1/89.6, 77.0/90.0, 81.7/90.5, 88.8/91.0
CirrhoMeter | 71.7/89.2, 75.1/90.0, 76.3/90.5, 81.2/90.8, 87.7/91.1
Fibroscan | 72.7/89.2, 75.1/90.0, 76.3/90.5, 81.2/90.8, 87.7/91.1
CombiMeter | 75.1/90.0, 76.3/90.5, 81.2/90.8, 87.7/91.1, 92.2/91.5

Conclusion: A blood test designed for cirrhosis can affirm (88–89% prediction) or exclude (97–99% prediction) cirrhosis by using different cut-offs (F4 and Fx/4, respectively) of a detailed fibrosis classification. By contrast, certain inclusion criteria in cirrhosis trials are misleading inducing the inappropriate inclusion of around 2/3 of patients without cirrhosis, 35% being F2 or F1.

713 IMPROVEMENT OF TRANSIENT ELASTOGRAPHY APPLICABILITY TO EVALUATE LIVER STIFFNESS BY TRAINED OPERATORS AND USING XL PROBE
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Background: Transient elastography (TE) is the elastographic reference method to identify liver stiffness. However, TE fails to
obtain a liver stiffness measurement (LSM) in 3.1% of cases and the results are considered unreliable (ratio <60% or interquartile range/stiffness (IQR/LSM) >30%) in 15.8% (Castera et al. Hepatology 2010).

Aims: To show the applicability of TE in chronic liver diseases (CLD) patients with inadequate LSM, after a second evaluation by trained operators.

Methods: The inadequate LSM evaluated from March 2011 to March 2012 were included. LSM were categorized as inadequate (no values or ratio <60% and/or IQR/LSM >30%) or adequate. A second exploration (M probe) and a third register (XL probe) in patients with body mass index (BMI) >28 were performed by trained operators (>500 LSM) in those with a previous inadequate LSM.

Results: 895 LSM were performed in 840 patients with CLD (52.4% with chronic hepatitis C). Inadequate LSM were obtained in 164 (18.3%) patients. The IQR/LSM was >30% in 90 (54.9%), there were no values in 59 (36%) and the ratio was <60% in 15 (9.1%). A second LSM (M probe, n=119) by trained operators (JAC and MP) achieved valid LSM in 68.9% (n=82) from those previously considered as inadequate. No values were obtained in 8 (6.7%) patients and 5 (4.2%) had an IQR/LSM >30%. Patients with BMI <24 (n=17), from 24 to 28 (n=33) and >28 (n=69) showed inadequate LSM in 0%, 18.2% and 44.9% of the cases, respectively (p<0.001). A third LSM was performed with the XL probe in patients with a BMI >28 (n=31). The XL probe achieved an adequate LSM in 27 (87.1%) patients in whom the M probe performed by trained operators had not obtained adequate values.

Conclusions: The operators’ experience increases the applicability of TE particularly in patients with a BMI <28. For patients with a BMI >28 the use of XL probe would be recommendable.

714 LIVER STIFFNESS VALUES IN HEALTHY SUBJECTS WITHOUT EVIDENCE OF LIVER DISEASE PERFORMED IN A LARGE COMMUNITY-BASED POPULATION

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Background and Aims: Chronic HCV patients with advanced fibrosis or cirrhosis are difficult to treat and cure. The aims of this study were to determine if hepatic function measured with Cholate Testing could predict sustained virological response (SVR) to peginterferon/ribavirin (PEG/RBV) and to measure the improvement in hepatic function relative to baseline function in those achieving SVR.

Methods: Chronic HCV patients (Ishak F2–6) enrolled in the HALT-C Trial, characterized by advanced fibrosis and failure of prior treatment with interferon-based treatment, were tested at baseline and then retreated with PEG/RBV. Patients achieving sustained virological response (SVR) and nonresponders (NR) were retested at 2 years. Oral cholate-2,4,4-d4 targets the portal circulation, and its clearance defined Portal Hepatic Filtration Rate (HFR). IV cholate-24,14C clearance measured Systemic HFR. The ratio of Systemic to Portal HFR defined SHUNT. The disease severity index (DSI) was calculated from log Portal HFR and SHUNT. Labeled cholates were measured in serum by LCMS methods validated to FDA guidelines.

Results: Of 230 patients tested at baseline, 32 achieved SVR. SVR could not be achieved in patients with SHUNT >64% (n=13), Portal HFR <6.7 mL/min/kg (n=30), or DSI >29 (n=49). Five of the 32 patients achieving SVR had cirrhosis (Ishak F5 or F6). Retesting at 2 years showed declining function in NR (n=80) and improving function with SVR (n=23). Portal HFR declined 1.3±0.6 mL/min/kg (mean ± SEM) in NR and improved 3.8±1.0 mL/min/kg with SVR (p=0.00003). SHUNT increased 5.5±1.9% in NR and declined 6.1±2.0% with SVR (p=0.003). DSI increased 2.5±0.8 in NR and decreased 3.4±0.8 with SVR (p=0.0002). The improvements in function with SVR correlated with the degree of dysfunction at baseline (Graphs 1–3, boxes are healthy control values).

Figure 1. Portal HFR.
Conclusions: Non-invasive Cholate Testing predicts which patients are unable to achieve SVR when retreated with PEG/RBV and quantifies significant improvements in hepatic function after SVR. Improvement after SVR is greatest in those with the most severe baseline dysfunction.

716 THE EFFICACY OF A NEW QUALITATIVE SCORING SYSTEM FOR ADVANCED LIVER FIBROSIS DIAGNOSIS USING GADOXETATE DISODIUM-ENHANCED MRI IN PATIENTS WITH CHRONIC DIFFUSE LIVER DISEASES

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Purpose: To assess if gadoxetate disodium-enhanced (Gd-EOB-DTPA) contrast-enhanced MRI (CE-MRI) using quality scores is able to predict advanced liver fibrosis (LF) in patients with chronic liver diseases (CLD).

Materials and Methods: We analyzed retrospectively 95 consecutive patients (mean age 54.18 year) with histologically proven LF, who underwent dynamic 3 Tesla CE-MRI with Gd-EOB-DTPA. A single expert pathologist assessed LF according to Metavir scoring system: F0 and F2 (n=45, 47.4%) was considered no-to-moderate LF and F3 and F4 (n=50, 52.6%) as advanced LF. The CE-MRI images were obtained blinded to histology before contrast injection and in hepatocyte-phase (20 min) and we assessed three scores as LF predictors: inhomogeneous enhancement (IE), enhancement quality score (EnQS) and excretion quality score (ExQS). The accuracy of the three quality scores for the assessment of the stages of fibrosis was evaluated by calculating sensitivity (Se%), specificity (Sp%), positive and negative predictive values (PPV, NPV), and ROC curves (receiver operating characteristic curve).

Results: LF correlated strongly with EnQ score (r = −0.70, p < 0.0001) and moderately with ExQ score (r = −0.41, p < 0.0001) and IE (r = 0.35, p = 0.0008). Independently, advanced LF can be predicted by a EnQS ≤3 with AUROC=0.90 [Se=93.8%, Sp=73.3%, PPV=79.6%, NPV=91.5%], by ExQS ≤2 with AUROC=0.72 [Se=36.73%, Sp=97.78%, PPV=94.8%, NPV=58.2%] and by IE with AUROC=0.67 [Se=40.82%, Sp=93.33%, PPV=87.2%, NPV=58.7%]. The three quality scores together are able to accurately assess the severity of LF, with a specificity of 93.33% and a positive predictive value of 92.6%. The inter-observer agreement and reliability was very good for all three scores: EnQS (k=0.84, ICC=0.91), ExQs (k=0.91, ICC=0.91), IE (k=0.80, ICC=0.87).

Conclusion: In CE-MRI, the degree of liver parenchymal enhancement and the extent of biliary excretion of gadoxetate disodium-enhanced MR imaging are reliable predictors for evaluation of advanced LF in CLD patients.
Introduction: Measurement of hepatic venous pressure gradient (HVPG) is the gold standard method for the assessment of portal pressure and correlates with the occurrence of its complications. Transient elastography (TE) is a non-invasive procedure that assesses liver fibrosis through the measurement of liver stiffness. Aim: This study aimed to determine the correlation between TE and HVPG for the diagnosis of significant portal hypertension in chronic liver disease. And the relationship between the clinical and epidemiological characteristics and the presence of decompensation in follow-up for 42 months.

Patients and Methods: Liver stiffness was measured by TE in fifty-eight chronic liver disease patients who underwent a haemodynamic measurements. Patients were thereafter followed-up for a mean of 15.8±1.015 months until they have a complication of liver disease or death.

Results: In 51 patients, 87% have clinically significant portal hypertension (HVPG>10mmHg). The relationship between TE and HVPG has a Pearson correlation r = 0.71, p < 0.0001, and the AUROC 0.96±0.03, p < 0.0001. This curve gives us the choice of cut points for the diagnosis of clinically significant portal hypertension, 17.15kPa (≥90%, Sp 86%, PPV 86.5% and NPV 89.5%). 37 patients (63.8%) had at least one clinical decompensation during follow up, 12.1% encephalopathy, 8.6% variceal bleeding, 22.4% ascites, 12.1% hepatocarcinoma and 8.6% death. In the relationship between patients characteristics by presence of clinical decompensation, in the univariate were significantly male sex, the presence of esophageal varices, MELD ≥10, platelets ≥120 10^3/l, splenomegaly, HVPG ≥10mmHg and TE ≥17.15kPa. In the multivariate analysis only the liver elastography is significant (p < 0.05).

Conclusion: TE is correlated with HVPG predicts the presence of significant portal hypertension and in following up clinical decompensation in patients with chronic liver disease.

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COMPARISON OF THE NAFLD FIBROSIS SCORE VERSUS 7 OTHER BLOOD TESTS FOR LIVER FIBROSIS IN NAFLD
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Background and Aims: According to last AASLD practice guidelines, a blood test, the NAFLD Fibrosis Score (NFS), may be used for the diagnosis of advanced fibrosis in NAFLD. We aimed to compare the NFS with 7 other blood fibrosis tests, specific or not to NAFLD.

Methods: 371 patients with NAFLD, liver biopsy, and blood samples were included. 8 blood fibrosis tests were calculated: BARD, NFS, FibroMeter Steatosis (FMS) or Virus (FMV), APRI, FIB4, Fibrotest, Hepascore. The reference for liver fibrosis was the NASH-CRN F staging. Diagnostic accuracy was evaluated using Obuchowski index (adjusted AUROC), the reference of patients included in the grey zone between the thresholds of ≥90% negative (NPV) and positive (PPV) predictive values, and the rate of well classified patients by fibrosis classification that estimates the pathological fibrosis stage(s) from the blood test result, without any biopsy required.

Results: 40% of patients had NFS <1.455 (NFS for F≥3: 88.2%), 16.4% had NFS >0.676 (PPV: 76.3%), and 43.6% were in the grey zone between these 2 cut-offs. FMS and FMV Obuchowski indexes were not significantly different (0.820±0.021 vs 0.820±0.020, p=0.901) but were significantly higher than those of NFS (0.784±0.024), FIB4 (0.778±0.023), BARD (0.765±0.023), Hepascore (0.755±0.027), Fibrotest (0.716±0.027), APRI (0.713±0.024); excepted FMV vs NFS (p=0.347) or vs FIB4 (p=0.127), FMS vs Hepascore (p=0.166). NFS included 74.4% of patients in the grey zone between the thresholds of ≥90% predictive values for F12, 59.4% for F≥3, and 38.3% for F4 (specific VPN95%). Among the 8 blood tests, FMV allowed for the lowest rate of patients in the grey zone: 57.4% for F2 (p=0.038 vs other tests), 47.3% for F3 (p=0.006 vs other tests), and 28.9% for F4 (p=0.043 vs other tests, except vs 30.5% for Hepascore; p=0.504). NFS classification (5 stages) had 74.5% diagnostic accuracy, FMV (7 stages): 72.6% (p=0.731 vs FMS), and Fibrotest (8 stages): 36.0% (p<0.001 vs FMS and FMV).

Conclusion: NFS is an accurate blood test for liver fibrosis in NAFLD. Other blood fibrosis tests are at least as accurate, but the biopsy requirement is significantly lower with FM tests. Further step will be comparison with elastometry.
721 SOLUBLE LIGANDS FOR ACTIVATING NATURAL KILLER RECEPTOR (NKG2D) AS SCREENING TOOL TO PREDICT SEVERITY OF ALLOGRAFT FIBROSIS AFTER LIVER TRANSPLANTATION

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Introduction: The role of HLA mismatches and soluble ligands for activating natural killer group 2 (NKG2D) such as major MHC class I-related chain A and B [sMICA/sMICB] and UL16 binding protein 2 [sULBP2] in fibrosis progression has not yet been investigated in the liver transplant (LT) setting.

Aim: To investigate clinical, laboratory and immunological risk factors for progression of hepatic fibrosis following LT.

Methods: A total of 174 LT recipients were enrolled in the study. Cytokines were assessed by flow cytometry with the “BD Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Cytokine Kit”. Assessment of sMICA, sMICB and sULBP2 was realized by enzyme linked immunosorbent assay. Screening for anti-HLA class I, class II or MICA antibodies (AB) was performed using Luminex technology. Chi-square or Fischer’s exact test was used for comparing categorical data and Student’s t-test or Mann–Whitney U test, when appropriate, was used for comparing continuous variables. Multivariate logistic regression analysis was performed to identify independent risk factors for development of cirrhosis after LT.

Results: In the univariate analysis, donor age >50 years (p=0.001), presence of anastomosis stenosis (p=0.009), high serum levels of alanine aminotransferase (p=0.0001), alkaline phosphatase (p=0.0001) and creatinine (p=0.0001) were associated with progression to cirrhosis. Immunological risk factors included high serum levels of inflammatory cytokines [Tumor necrosis factor-alpha (TNF-α) (p=0.001), interleukin (IL)-10 (p=0.004), IL-6 (p=0.0001)], presence of donor specific anti-HLA class II AB (p=0.01), high serum levels of sMICA (p=0.04), sMICB (p=0.004) and ULPB2 (p=0.0001). In multivariate analysis, high serum levels of total bilirubin, sMICB and sULBP2 were independent predictors for cirrhosis after LT.

Conclusion: sMICB and ULPB2 molecules were associated with cirrhosis in LT patients. Early screening of these biomarkers in LT patients may be useful for identifying patients at high risk of hepatic fibrosis progression after LT.

722 LIVER STIFFNESS AND HEPATOCELLULAR CARCINOMA: IS IT REALLY USEFUL?

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Background: Hepatocellular carcinoma (HCC) is the leading cause of death among cirrhotic patients and the degree of liver fibrosis is known as the strongest risk factor for liver carcinogenesis. Liver stiffness measurement (LSM) by means of transient elastography (TE) has been suggested as a useful indicator of the risk of HCC development in patients with chronic hepatitis (CH). We aimed at assessing the relationship between LSM and HCC presence in CH-patients.

Methods: From January 2010 to January 2012, 97 consecutive CH-patients (64 M, median age 64, 55% cirrhosis) who underwent fine-needle biopsy to investigate hepatic focal lesions detected on ultrasound examination, were consecutively recruited to be concomitantly evaluated by FibroScan® (Echosens, Paris, France). The TE findings were compared with those of 156 CH-patients (101 M, 55 F, median age 50, 16.6% cirrhosis) free from hepatic nodules, who performed concomitant TE and liver biopsy to stage CH.

Results: Nine patients were excluded due to LSM success rate <65%. In the 88 patients with nodules enrolled (median age 63 yr, 69% male) median TE value was 14.7 kPa and was significantly higher than in CH-patients (8.8 kPa, p<0.0001), paralleling serum AFP levels (r=0.29, p=0.011), AST levels (r=0.38, p=0.0003), alkaline phosphatase (r=0.4, p=0.0002), PLT count (r=−0.64, p<0.0001). Among the 88 patients with nodules, histology revealed HCC in 31 patients, metastases from other neoplasms in six patients, non-neoplastic nodules in 51. Overall diagnostic accuracy of TE in diagnosing HCC was 55.4% (CI: 0.440 to 0.664). TE cut-off value with the best accuracy was 16.8 kPa. When considering only cirrhotic patients, TE values did not differ between patients with or without hepatic lesions. No differences were observed according to different etiology or number of lesions.

Conclusions: In patients with chronic liver disease LSM values are significantly higher in patients with hepatic nodules, as compared to those of patients without, thus demonstrating a possible role in identifying a subgroup of patients at high risk of HCC. However the exact role of TE as an indicator in predicting HCC has to be elucidated.

723 RISK ASSESSMENT OF LIVER-RELATED EVENTS DEVELOPMENT USING TRANSIENT ELASTOGRAPHY IN PATIENTS WITH CHRONIC HEPATITIS B RECEIVING ENTECAVIR

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Background and Aims: Liver stiffness (LS) values using transient elastography provides accurate assessment of liver fibrosis in patients with chronic liver disease. We investigated whether LS values can predict liver-related events (LREs) development in patients with chronic hepatitis B (CHB).

Methods: Between June 2007 and May 2010, a total of 162 patients with CHB who completed 2-year ETV treatment were evaluated. The primary end-point was LRE development (hepatic decompensation,
Results: The median age of the patients (99 men, 63 women) was 51 years, and the median LS value was 14.8 kPa. During the 2-year ETV treatment, 15 (9.3%) patients experienced LREs. On univariate analysis, age, the proportion of patients with liver cirrhosis, platelet counts, and baseline LS values were significantly associated with LRE development (all \( P < 0.05 \)). Together with age, multivariate analysis identified baseline LS values as an independent predictor of LRE development (\( P = 0.046 \); hazard ratio, 1.040; 95% confidence interval, 1.101–1.084). The cutoff LS value maximizing the sum of sensitivity and specificity was 12.0 kPa (area under the receiver operating characteristics curve, 0.736; \( P = 0.003 \); sensitivity, 93.3%; specificity, 42.2%). In addition, the changes in LS values between baseline and 1-year ETV treatment showed significant correlations with LRE development (\( P = 0.030 \)).

Conclusions: Our data suggest that LS values are predictive of LRE development during 2-year ETV treatment in patients with CHB. The potential role of LS value as a monitoring tool for predicting dynamic changes in the risk of LRE development during long-term ETV treatment should be investigated further.

LIVER FIBROSIS AMONG DRUG USERS ATTENDING HARM REDUCTION FACILITIES IN PARIS’ URBAN AREA

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Background and Aims: 59 to 70% actual or former drug users have positive HCV serology in France. Even though access to treatment for drug users is a public health priority that reduces HCV transmission risk, it also faces several individual perception barriers, such as the fear of treatment, heavy medical procedures or the underestimation of the disease’s seriousness by the patient. Contact with drug users attending harm reduction facilities are a good opportunity for screening, counseling and healthcare refering.

Methods: From June 2009 to July 2012, 1018 attendees of 25 Harm reduction facilities in Paris’ urban area had a medical consultation and their liver stiffness measured with a Fibroscan® mobile device. Socioeconomic and drugs consumption data were collected, a medical file was also filled when biological data was available.

Results: The average fibrosis score was 7.8 Kpa. 70.8% had no fibrosis (below 7 Kpa), 19.7% had a moderate to severe fibrosis (7–12 Kpa) and 9.5% had a cirrhosis (over 12 Kpa). 182 people had a confirmed HCV positive status. Serum was obtained from 92 people, out of which HCV-RNA was positive in 85%. The most frequent genotypes were 1 (39%) and 2 (29%). Hepatitis C and B prevalence are significantly higher among drug users who have already injected than among non-injectors (73.1% vs. 20.4% and 13.6% vs. 5.2% respectively; \( P < 0.001 \)). Adjusted multivariate analysis showed that positive HCV serology is correlated with an age ≥35 years, being or having been injecting, a daily consumption of alcohol and having a score of Fibroscan ≥7Kpa. In multivariate analysis adjusted to age and sex, factors significantly associated with a significant liver fibrosis (from 7 Kpa) are being or having been injector, being on substitution therapy and daily alcohol consumption (\( P < 0.001 \)).

Conclusions: This is the largest prospective study to assess liver fibrosis among drug users in France, showing significant fibrosis in 29.2%, including 9.5% cirrhosis. Providing drug users with an estimate of the liver stiffness created an opportunity to pass on prevention messages about liver protection and made HCV related liver disease concrete to those with significant fibrosis.
Results: Hepatic VQT SWV demonstrated a significant correlation with histological stage (Spearman’s $p=0.847$; $P<0.001$). The optimal cut-off values for predicting liver fibrosis by APRI were 1.29 m/s for F2 ($AUC=0.93$), 1.37 m/s for F3 ($AUC=0.97$) and 1.62 m/s for F4 ($AUC=0.97$). The optimal cut-off values for predicting liver fibrosis by APRI were 0.34 m/s for F2 ($AUC=0.85$), 0.49 m/s for F3 ($AUC=0.87$) and 0.45 m/s for F4 ($AUC=0.89$).

Conclusions: The hepatic VQT SWV correlates well with the histological liver fibrosis stages. This novel technology enables simultaneously noninvasive assessment of liver fibrosis in patients suffering from chronic Hepatitis B with high diagnostic accuracy and convenience.

727 FIBROTEST AND LIVER STIFFNESS MEASUREMENT (LSM) BY FIBROSCAN FIBROSIS DIAGNOSIS IN HIV–HCV CO-INFECTED PATIENTS FROM THE FRENCH HepaVIH COHORT. HIV–TREATMENTS IMPACT ON NON-INVADE BIOMARKERS


Background: In HIV–HCV coinfected patients (French HepaVIH cohort): (1) To estimate the impact of HIV–treatments on Fibrotest and (2) To compare the performance of Fibrotest and LSM by Fibroscan. Patients with non-discordance had a higher discordance rate (p=0.001; odds ratio 5.95). After adjusting fibrosis stages, neither advanced fibrosis (AF) and cirrhosis and by the prevalences of Non-App Fibrotest according to treatments. Non-App related to Fibrotest was compared to LSM (success rate <60% and IQR/median ratio=30%).

Results: 248-patients with LB and Fibrotest were pre-included. Non-App Fibrotest and LSM were 5/248 (2%) and 5/153 (3.3%), respectively. 243-patients had App-Fibrotest: 69.5% males, age=44 (18–70 years), 59.3% AF, 14% cirrhosis according to LB. Fibrotest AUROC (SE) were: AF 0.65 (0.02) and cirrhosis 0.76 (0.05). Fibrotest AUROC versus App-LSM (N=147) were: AF 0.59 (0.05) vs 0.63 (0.04), $p=0.38$ and cirrhosis 0.78 (0.04) vs 0.78 (0.06), $p=0.98$, respectively. In patients with >6-months delay with the LB, Fibrotest (N=162) AUROC were: AF 0.70 (0.04) and cirrhosis 0.77 (0.04) and versus concomitant App-LSM (N=62-patients) AF 0.63 (0.07) vs 0.77 (0.06, $p=0.09$) and cirrhosis 0.81 (0.07) vs 0.89 (0.04), $p=0.16$. Despite higher median bilirubin levels in patients treated with atazanavir (ATZ, N=42) compared to non-ATZ (N=133) (29 vs 9 $\mu$mol/L, $p<0.0001$), there was no impact on Fibrotest AUROC for AF 0.70 vs 0.71 ($p=0.91$) and cirrhosis 0.73 vs 0.76 ($p=0.76$). There was no difference in prevalences of non-App Fibrotest according to ATZ-treatment: 2.3% (1/43) vs 2.9% (4/137), $p=0.84$. 25-patients had efavirenz (EFV) and 11 nevirapine (NVP) treatments. Median GGT were higher in both EFV and NVP-treated patients compared to not-treated: 133 vs 74 IU/L ($p<0.01$) and 264 vs 811 IU/L ($p=0.06$), respectively, without impact on Fibrotest AUROC for AF for both p=NS). While prevalences of non-App Fibrotest were similar in EFV vs non-EFV groups: 0/25 vs 3.2% (5/155), $p=0.48$, there was a significant higher prevalence of non-App Fibrotest in NVP-treated 21% (3/14) vs non-NVP 1% (2/166), $p<0.01$. No significant impact on the diagnosis of fibrosis as per Fibrotest was observed for the other treatments.

Conclusion: Fibrotest performances were not changed by atazanavir, efavirenz and nevirapine treatments in HIV–HCV co-infected, despite changes in bilirubin or GGT. Nevirapine was associated with a higher rate of non-applicable Fibrotest. Fibrotest and LSM by Fibroscan had similar diagnostic values.
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RESULTS: We performed 895 LSM in 840 patients with CLD: chronic hepatitis by HCV (52.4%), HBV (18.8%), HIV–HCV coinfection (11.5%), non-alcoholic steatohepatitis (4.4%), alcoholic liver disease (3.7%), autoimmune (2.8%) and cholestatic diseases (1.9%). Inadequate LSM were obtained in 164 (18.3%) patients: IQR/LSM >30% in 90 (54.9%), no values in 59 (36%) and ratio <60% in 15 (9.1%). Patients with inadequate LSM were older and had higher weight, body mass index (BMI), waist circumference and levels of glucose and lower levels of albumin than patients with adequate measurements (p<0.01 in all cases). Multivariate analysis (odds ratio, OR; confidence interval 95%, CI95, p) identified BMI (OR: 0.91; CI95: 0.86–0.97; p<0.01), waist circumference (OR: 0.98; CI95: 0.96–0.99; p=0.02), glucose (OR: 0.99; CI95: 0.98–1.0; p=0.04), albumin (OR: 1.6; CI95: 1.01–2.6; p=0.04) and age (OR: 0.98; CI95: 0.97–1.0; p=0.07) as independent predictors to obtain an inadequate LSM. Patients with a BMI (kg/m2) <20 (n=56), from 20 to 28 (n=581) and >28 (n=249) showed an inadequate LSM in 19.6%, 11.7% and 34.1%, respectively (p<0.001).

Conclusions: BMI is the main variable to obtain inadequate LSM. The rate of inadequate examinations is higher in patients with obesity or overweight probably due to anatomical reasons.

730 INVESTIGATION OF CELLULAR IMMUNITY IN DIFFERENT STAGES OF LIVER FIBROSIS

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Background: Immune mechanisms – cellular and humoral, play a major role in the pathogenesis of degenerative diseases of the liver. Therefore, the aim of this study was to determine the dependence of the stage of liver fibrosis on the functional state of the immune system, in particular, an imbalance of cellular immunity.

Methods: The study included 146 people: 118 patients with chronic liver diseases (60 patients with chronic hepatitis C (CHC) and 58 patients with alcoholic hepatitis (AHI)), 28 healthy individuals were the comparison group. Liver elastometry (FibroScan) has been used to evaluate fibrosis stages according to METAVIR classification. Functional activity of phagocytic cells (monocytes, neutrophils) was investigated (phagocytic ability, chemotactic function analysis of neutrophils and monocytes; expression of cell adhesion molecules (ι2-integrins: LFA-1, CR3), the cAMP/cGMP ratio and the level of intracellular Ca2+ were explored.

Results: In patients with fibrosis, oxygen-dependent bactericidal performance of neutrophils and monocytes in the nitroblue tetrazolium test (NBT-test) were unidirectional, regardless of etiology, significantly higher than the similar characteristics of leukocytes from healthy donors. Early stages of fibrosis were characterized by increased number of neutrophils expressing cell adhesion molecules which determine transendothelial migration of leukocytes, while in the final stages of the disease, regardless of etiology, levels of monocytes which express b2-integrins (LFA-1 and CR3) increased. Unidirectional increase in the ratio of cAMP and cGMP levels by the significant increase of cAMP and decrease of cGMP were recorded in neutrophils and monocytes in patients with liver fibrosis at different stages of the disease.

Conclusions: Analysis of the data allows us to conclude that a significant amplification of microbial mechanisms of leukocytes in degenerative diseases of the liver can lead to the realization of cytotoxic effects on host cells. An increased migration of leukocytes into the parenchyma of the liver causes damage of the liver cells by phagocytes, due to increased secretion of bioactive molecules with damaging and profibrotic effect (ROS, chemokines, profibrotic cytokines), which may contribute to further progress of the disease. Explored changes in the system of secondary messengers showed increased activation of cells of non-specific resistance in liver fibrosis.

731 LIVER STIFFNESS EVALUATION BY MEANS OF TRANSIENT ELASTOGRAPHY IN TYPE 2 DIABETES PATIENTS

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Aim: To assess the feasibility of liver stiffness (LS) evaluation by means of Transient Elastography (TE) in type 2 diabetes patients. Also, we investigated the presence of NAFLD and the infection with hepatitis B and C virus.

Material and Method: We enrolled 262 patients with type 2 diabetes mellitus. In each patient we performed: abdominal ultrasound, liver stiffness measurements by means of TE, and biological samples were collected (HBsAg, antiHCV-Ab, amino-transferrases). TE measurements were considered reliable if 10 valid measurements could be acquired with at least 60% success rate and less than 30% IQR. We used the Wong criteria in TE for NAFLD1 to divide the patients according to fibrosis severity TE <7.9 kPa (F2±3 excluded); TE 7.9–9.6 kPa (“gray zone”); TE >9.6 kPa (F3±3).

Results: According to the body mass index (BMI), patients were classified as: 16.8%-normal weight, 30.2%-overweight and 53%-obese patients. Reliable LS measurements by TE were obtained in 72.1% of cases. In obese patients, the rate of reliable measurements was significantly lower as compared with normal weight and overweight patients: 61.8% vs. 82.2% (p=0.007) and 61.8% vs. 86.3% (p=0.006), respectively. The rate of reliable measurements was similar in normal weight and overweight patients: 86.3% vs. 82.2% (p=0.73).

Out of the entire cohort, 8 patients (3%) were found with positive HBsAg, 6 patients (2.2%) with positive antiHCV-Ab, and one patient (0.3%) with both viruses. We also found NAFLD (moderate and severe liver steatosis at ultrasonography and normal aminotransferases) in 143 patients (54.5%) and NASH (liver steatosis at ultrasonography and elevated aminotransferases) in 53 patients (20.2%).

In the cohort of 189 NAFLD and NASH patients with reliable LS measurements, using the Wong criteria, 72.4% had no severe fibrosis, 13.8% were in the ‘gray zone’ in which biopsy is recommended and 18.5% had severe fibrosis.

Conclusions: TE was feasible in 72.1% of diabetes patients, the rate of reliable LS measurements decreasing with the increase of BMI. We found infections with hepatitis B or C viruses in 5.5% of cases, and NASH in 20% cases, with quite severe liver involvement in 18.5% of NAFLD patients.

Reference(s)
732 ENHANCED LIVER FIBROSIS (ELF) SCORE IS RELATED TO LIVER DYSFUNCTION AND PREDICTS MORTALITY IN CIRRHOSIS

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Background: The Enhanced liver fibrosis (ELF) test is a noninvasive fibrosis panel composed of hyaluronic acid (HA), procollagen-3 N-terminal peptide (P3NP), and tissue inhibitor of metalloproteinase-1 (TIMP1). Previous studies have established high diagnostic accuracy of the ELF score to assess hepatic fibrosis and to predict liver-related clinical outcomes in chronic viral hepatitis and fatty liver disease. Aim of the present study was to relate baseline ELF scores to mortality in a cohort of cirrhotic patients.

Methods: We enrolled 63 consecutive cirrhotic patients admitted to our outpatient liver clinic (29% females; age: 58±10 years; etiology alcohol in 64%). Serum samples obtained at baseline were used to perform ELF test (Siemens Health Care, Vienna, Austria). The prognostic value of ELF score, Child-Pugh (CP) score and model for end-stage liver disease (MELD) obtained at baseline was assessed by receiver operating characteristic (ROC) analysis. Logistic regression analysis with backward elimination of variables was used to determine independent prognostic variables.

Results: Baseline characteristics of our study cohort were: CP-A 38%, CP-B 43%, CP-C 19%, CPS 7 (6.9), MELD 13 (10, 16). ELF score increased progressively with the stage of cirrhosis as estimated by CP stage (CP-A: 10.8±1.0; CP-B: 12.2±1.0; CP-C: 13.2±1.3). During a median follow-up of 43 months, 27 patients developed hepatic decompensation, 18 patients died and 5 patients underwent liver transplantation (LT). ROC analysis of baseline variables revealed superior diagnostic accuracy of ELF score compared to CP score and MELD for prediction of a combined endpoint death/LT at 1 year (AUC: ELF 0.80, CP score 0.71, MELD 0.71). On logistic regression analysis, ELF score remained the only significant prognostic variable for the combined endpoint death/LT at 1 year.

Conclusion: Our results suggest that ELF score, a noninvasive marker of hepatic fibrosis, is related to severity of liver dysfunction and to mortality in cirrhosis and may represent a novel prognostic marker in chronic liver failure.

733 NEOEPITOPE DERIVED FROM EXTRACELLULAR MATRIX DEGRADATION REFLECT LIVER FUNCTION, FIBROSIS AND HVPG IN HIV-PATIENTS

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Background: HIV-patients, especially with HCV-co-infection, show accelerated fibrogenesis and increased hepatic-venous pressure gradient (HVPG). Liver injury includes fibrogenesis, sinusoidal remodelling and deposition of extracellular matrix-proteins (ECM). As we have recently shown (AASLD2012), matrix-metalloproteinases (MMP) degrade these structural proteins, which present specific epitopes at the degradation site. Highly-active-antiretroviral therapy (HAART) might improve fibrosis, but also induce liver injury. Here, we extended the analysis of the Protein Fingerprint by assessment of circulating levels of these neoepitopes of ECM-proteins in HIV- and HIV/HCV-co-infected patients with regard to liver injury and HVPG.

Methods: We analyzed serum from 403 serum samples of HIV patients (100 HIV/HCV co-infected). The circulating neo-epitopes of ECM-proteins were determined using specific ELISA assays. As markers for ECM-remodelling we used biglycan degraded by MMP9 (BGM), collagen type I, type 3 and type 4 degraded by MMP2/9/13 (respectively C1M, C3M, C4M) and elastin degraded by MMP12 (ELM). Collagen IV 7S domain (P4NP7S) and pro-collagen III (P3NP) were used as markers of collagen synthesis. The results were correlated with clinical data, fibroscan (n=110) and HVPG (n=58).

Results: Liver size measured by ultrasound correlated with levels of BGM, C1M, C3M, VICM and P4NP7S (n=0.3; p<0.01). HCV-co-infection and HCV-viral load correlated significantly with ECM-remodelling as reflected by higher levels of C4M (p=0.041) and lower levels of C1M (p=0.002). Alcohol abuse was associated with increased collagen-type-VI synthesis (P4NP7S). Serum albumin levels correlated inversely with markers of ECM-remodelling (BGM, C4M, C3M), while INR correlated positively with C4M and P4NP7S. Moreover, P3NP correlated with severity of fibrosis as assessed by fibroscan (R=0.275), ALT (R=0.300) and HVPG (R=0.354; p=0.02). Interestingly, HCV-viral load significantly correlated with ECM-degradation and synthesis, shown by levels of BGM, C3M, C4M, ELM and P4NP7S (R=0.12–0.34). Similarly, the administration and the duration of HAART correlated inversely with circulating levels of C3M, C4M, ELM and P4NP7S (Rs = −0.17 to −0.3).

Conclusion: These protein fingerprint markers correlate with liver injury, liver synthesis and HVPG in HIV patients. Moreover, HIV-viremia is associated with ECM-degradation and HAART appears to attenuate these processes in HIV-patients. Therefore, these ECM-neo-epitopes might have prognostic values in the management of HIV patients.

734 LIVER STIFFNESS BY SHEAR WAVE ELASTOGRAPHY IS INFLUENCED BY MEAL AND MEAL-RELATED HAEMODYNAMIC MODIFICATIONS

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Background: Shear Wave Elastography has an emerging role in the non-invasive evaluation of liver fibrosis. Physiological factors can influence liver stiffness (LS) and should be considered when performing LS measurement.

Aim: To evaluate the effect of meal and meal-related haemodynamic variations on LS measurement by Shear Wave Elastography.

Methods: 10 healthy volunteers were enrolled in the study. LS, echo color Doppler analysis of portal vein and hepatic artery, as well as liver and spleen dimensions were evaluated before a meal (T0) and 30, 60, 120′ (T30), 60 (T60) and 120′ (T120) postprandially.

Results: LS values increased 30′ postprandially and decreased from 60′ postprandially, returning to baseline values after 2 hours (T0: 5.2±1.06 kPa, T30: 6.3±0.8 kPa, T60: 6±1.3 kPa, T120: 5±1 kPa; p: 0.02). Portal flow showed comparable kinetics (T0 0.52±0.22 L/min, T30 1.08±0.32 L/min, T60 0.77±0.52 L/min, T120 0.48±0.21 L/min; p: 0.02). Portal mean velocity was significantly increased at T30 (T0 11.3±2.5 cm/s, T30 16.1±4.7 cm/s; p: 0.02), and returned to values comparable to baseline from T60 on (T60 14.2±3.6 cm/s, T120 13.5±3.4 cm/s; p: >0.05). Hepatic artery maximal (Vmax) and mean (Vmean) velocity,
after a decrease at T30, progressively increased up to T120, where they reached values higher than baseline (Vmax: T0 52.8±36.8 cm/s, T30 45.4±17.4 cm/s, T60 55.6±23.5 cm/s, T120 73.7±44.2 cm/s; p: 0.04; Vmean: T0 36.4±27 cm/s, T30 28.3±11.7 cm/s, T60 35.6±16 cm/s, T120 48.1±29 cm/s; p: 0.04). The Resistivity Index (RI) of the hepatic artery increased from T30 after meal (T0 0.62±0.07 cm/s, T30 0.74±0.08 cm/s; p: 0.02, T60 0.73±0.05 cm/s; p: 0.02) and decreased at T120 (T120 0.69±0.08 cm/s; p: >0.05). Liver and spleen diameters did not show variations.

**Conclusions:** LS transiently increases postprandially in relation to the meal-induced haemodynamic variations in portal and hepatic arterial flow, and returns to baseline 120′ after the meal. LS evaluation should hence be performed fasting or at least 120′ after a meal.

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**07c. VIRAL HEPATITIS B & D: CLINICAL (THERAPY, NEW COMPOUNDS, RESISTANCE)**

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**735**

**PEGINTERFERON ADDED TO LONG-TERM NUCLEOS(T)IDE ANALOGUES ENHANCES THE DECLINE OF SERUM HBeAg AND HBsAg LEVELS IN CHRONIC HEPATITIS B**

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**Background:** Although treatment with nucleos(t)ide analogues (NA) for chronic hepatitis B (CHB) is effective and safe, response will probably not sustain off treatment and most patients therefore require long-term treatment. This pilot study investigated the effect of Peginterferon (PEG-IFN) add-on therapy on serum HBeAg and HBsAg levels in HBeAg positive CHB patients who are on long-term NA-treatment.

**Methods:** Eleven patients stably suppressed with an HBVDNA <200IU/mL during NA treatment (5ETV/6TDF±LAM/FTC) were studied. Five patients were randomized to receive 24 weeks of PEG-IFN α-2b add-on therapy and were compared with 6 patients who continued NA-monotherapy. Follow-up (FU) was 48 weeks. Quantitative HBeAg and HBeAg was measured at week 0, 12, 24 & 48 using the Cobas E411 (Roche).

**Results:** Patients were predominantly male (82%) and of Asian (55%) or Caucasian (45%) origin. Mean age was 34 years. Seven patients (64%) had previously been treated with IFN. Patients harboured HBV genotypes B/C/D/other in 36/28/27/18%. At baseline mean HBeAg and HBsAg were 1.0logIU/mL and 3.8logIU/mL, respectively, and did not differ between groups (p-values>0.2). HBVDNA remained <200IU/mL in all patients. Patients who received PEG-IFN add-on therapy showed more HBeAg decline at end of treatment (0.84 vs. 0.14IU/mL, and end of FU (0.93 vs. 0.29IU/mL) than those who remained on NA monotherapy. One patient (PEG-IFN arm) achieved HBeAg seroconversion. HBSAg decline was more profound in patients who received PEG-IFN add-on at end of treatment (0.35 vs. 0.12IU/mL), and end of FU (0.26 vs. 0.18IU/mL). HBsAg loss was not observed.

**Conclusion:** Twenty-four weeks of PEG-IFN added to long-term NA treatment may improve HBeAg and HBsAg decline in HBeAg positive CHB. PEG-IFN add-on treatment should therefore be further investigated as an option to finalize long-term NA therapy in CHB.

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**736**

**NOVEL EFFECTOR OF HBV CAPSID ASSEMBLY REDUCES THE ACTIVITY OF cccDNA AND INTERACTS SYNERGISTICALLY WITH NUCLEOSIDE ANALOGS IN THE HUMAN HEPATOCYTES**

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**Background:** HBV reverse transcriptase (RT) is an essential enzyme for viral replication and has multiple inhibitor binding domains making it a major target for antiviral intervention. Given that this enzyme lacks proofreading activity, the emergence of viral resistance to antiviral agents frequently develops. New drugs targeting different steps of nucleocapsid assembly have the potential to interfere with cccDNA formation and in combination with HBV RT inhibitors could effectively circumvent and control the emergence of drug-resistant variants and eliminate cccDNA from chronically infected individuals. Hence, we evaluated dual and triple combinations of the novel nucleocapsid assembly effector heteroarylidihydropyrimidine (HAP12) with lamivudine (3TC), emtricitabine (FTC), tenofovir disoproxil fumarate (TDF) and entecavir (ETV). Additionally, the effect of the levels of cccDNA formation was determined.

**Methods:** HepAD38 cells replicating wild type HBV were used for single and drug combination studies. After drug treatment for 5-days, total DNA was purified and HBVDNA was amplified by real-time-PCR. Drug interaction analyses were performed using CalcuSyn program. HBV e antigen was measured as a cccDNA-dependent marker. Cellular pharmacology was performed in HepG2 cells and extracts were analyzed by LC-MS-MS.

**Results:** Triple combinations of HAP12+FTC+TDF or HAP12+ETV with either FTC or TDF or 3TC resulted in a strong synergistic antiviral effect, with weighted average combination index value (CIwt) of ≤0.5. In addition, dual combinations of HAP12 with either 3TC, FTC or ETV also synergistically inhibited HBV-DNA replication,
whereas an additive antiviral effect (CI=1.3) was observed for HAP12+TDF. The levels of the active metabolites FTC-TP were on average 1.4-fold higher for FTC+HAP12 than the levels achieved with FTC alone. Reduced levels of secreted HBeAg were demonstrated when cells were exposed to HAP12 (10 μM) for 7-days, with a fold-change of ≤0.2 when compared to untreated control, while 3TC (50 μM) showed a fold-change between 0.8–1.2.

**Conclusion:** These promising in vitro data warrant further investigation of HAP12 together with RT-inhibitors in animal models and toxicological studies in order to implement more effective treatments for HBV-infection. This synergy should translate into strong viral suppression, thus preventing drug-resistant variants as well as cccDNA formation and ultimately contributing to HBV eradication.

**737**

**POLYMORPHISMS NEAR THE IL28B GENE ARE NOT ASSOCIATED WITH RESPONSE TO PEGINTERFERON IN HBeAg-NEGATIVE CHRONIC HEPATITIS B PATIENTS**

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**Background and Aims:** Peginterferon therapy is effective in only a subset of HBeAg negative chronic hepatitis B patients. Polymorphisms near IL28B were recently associated with serologic response in HBeAg positive patients. Our aim was to investigate the association of IL28B genotypes with response to peginterferon in HBeAg negative patients.

**Methods:** We studied 133 HBeAg negative chronic hepatitis B patients treated in a randomized controlled trial with peginterferon alfa-2a (± ribavirin) for 48 weeks at 25 European centers. IL28B genotypes rs12980275, rs12979860 and rs8099917 were tested for the association with response defined as the combined presence of an HBV DNA level below 1,714 IU/ml (10,000 copies/ml) and normalization of ALT at 24 weeks post-treatment. IL28B single-nucleotide polymorphism variants were determined using competitive allele-specific PCR (KASP™, KBioscience Hoddesdon, UK).

**Results:** Ninety-five percent of the patients were Caucasian and 74% male. In majority patients were infected with genotype D (81%). At 24 weeks post-treatment, twenty-four patients had a combined response (18%) of whom 23 patients were infected with genotype D (96%). At baseline, response was not influenced by age (p = 0.16), sex (p = 0.63), HBV DNA levels (p = 0.20) or serum ALT (p = 0.31). The distributions of rs12980275 AA/AG/GG, rs12979860 CC/CT/TT and rs8099917 TT/TT/GG were 55/36/9, 55/33/12 and 68/22/10 percent, respectively. The single-nucleotide polymorphisms at rs12980275 and rs12979860 were in linkage disequilibrium for 75%. When performing Chi-square analysis, none of the IL28B genotypes were associated with response to peginterferon (p = 0.73, p = 0.96 and p = 0.31 for rs12980275, rs12979860 and rs8099917, respectively). The response rates were 19% vs. 23% for AA and non-AA genotypes (p = 0.62), 18% vs. 20% for CC and non-CC (p = 0.83) and 17% vs. 28% for TT and non-TT (p = 0.20). For all IL28B genotypes, decline of HBVDNA and HBsAg levels on week 12, 24, 48 and 72 did not differ significantly between homozygosity for the major allele versus heterozygosity and homozygosity for the minor allele.

**Conclusions:** Polymorphisms near the IL28B gene were not associated with the kinetics of HBV DNA and HBsAg levels during treatment and follow-up, nor with 24 week post-treatment response to peginterferon therapy in HBeAg negative chronic hepatitis B patients.

**738**

**SWITCH FROM LONG-TERM DE-NOVO LAMIVUDINE + ADEFOVIR THERAPY TO TENOFOVIR OR/AND ENTECAVIR IMPROVES VIRAL RESPONSE AND RENAL/BONE SAFETY**

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De-novo combination therapy with lamivudine (LAM) 100 mg/d + adefovir (ADV) 10 mg/d represented good therapeutic approach in chronic hepatitis B (CHB) between 2007–2008 prior to registration of 2nd generation nucleos(t)ide analogues (NA), tenofovir (TDF) 245 mg/d or entecavir (ETV) 0.5 mg/d, which are highly efficacious antivirals with high resistance barrier and provide alternative to LAM+ADV. Switch of long-term LAM+ADV patients to TDF or/and ETV might be associated with concerns for renal/bone safety for TDF patients, but improvement of virological responses.

**Aims:** To investigate serological and virological responses and renal/bone safety parameters after switch from LAM+ADV to other antivirals.

**Patients:** 192 NA naive CHB patients (35%HBeAg+, 34%cirrhosis) were treated with de-novo LAM+ADV (median 36 months). 178 patients from this cohort were switched to other antivirals: 72% TDF monotherapy, 16% TDF+ETV, 8% ETV and 4% other antivirals (median 30 months). Reasons for switch were: 19% suboptimal response, 8% ADV-related renal impairment, 3% pregnancy, 1 liver transplantation and 60% were switched for non-clinical reasons.

**Methods:** Proportion of patients achieving HBeAg/HBsAg seroconversion and virological response (VR=HBVDNA <12IU/ml) was assessed at switch baseline and every 6 months (M) post switch. Differences between serum levels of HBVDNA [log10 IU/ml], creatinine and phosphate [mmol/l] and estimated glomerular filtration rates (eGFR) [ml/min] were tested at each time-point and compared between LAM+ADV and switch baselines. Results are presented as medians.

**Results:** During LAM+ADV therapy HBeAg/HBsAg seroconversion and virological response were achieved in 21% and 2% patients and in additional 8% and 1% patient after switch. VR improved significantly post-switch than on LAM+ADV (M0: 75% vs. 0% M6: 91% vs. 60%, M12: 93% vs. 72%, M18: 94% vs. 74%, M24: 95% vs 77% and M30: 94% vs. 84% patients). Creatinine levels, eGFR at LAM+ADV and switch baselines were comparable (eGFR: 80.5 vs. 78) and proportion of patients with eGFR <60 was similar 6% vs. 5%, but phosphate levels were lower at time of switch (1.02 vs. 0.95, p = 0.04). Post switch eGFR gradually increased and this difference was significant from M18 (M6: 79 vs. 80; M12: 81 vs. 77; M18: 85 vs. 77, p = 0.02; M24: 84 vs. 75, p = 0.05 and M30: 85 vs. 74, p = 0.03). The proportion of patients with eGFR <60 remained unchanged post-switch. Phosphate levels increased steadily after switch and difference was significant from M12 (M6: 0.98 vs. 0.99; M12: 1.01 vs. 0.95, p = 0.03; M18: 1.03 vs. 0.92, p = 0.02; M24: 1.01 vs. 0.5, p = 0.02, M30: 1.01 vs. 0.88, p = 0.001).

**Conclusions:** Switch to other antivirals from long-term de-novo LAM+ADV therapy was efficient in viral control and was associated with improvement of renal and bone parameters.
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ALT FLARES DURING TREATMENT WITH PEGINTERFERON LAMBDA OR PEGINTERFERON alfa IN PATIENTS WITH HBeAg-POSITIVE CHRONIC HEPATITIS B INFECTION (CHB)

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Background: Treatment-associated ALT flares are common during peginterferon alpha (alfa) therapy, reported in 25–40% of treated CHB patients. Peginterferon lambda-1a (Lambda) is a type III interferon with more restricted receptor distribution than alfa. Interim analysis of an ongoing comparison of Lambda vs alfa in HBeAg+ CHB showed greater early reductions of HBV-DNA and quantitative HBsAg with Lambda, and improved safety/tolerability. We present a detailed analysis of on-treatment ALT flares through Week 24 from this dataset.

Methods: Adult HBeAg+, interferon-naïve patients with HBV-DNA >105 IU/mL and elevated ALT received 180 μg Lambda or Alfa-2a SC weekly for 48 weeks. The primary efficacy endpoint is HBeAg seroconversion at Week 72. Two flare definitions were employed: ALT >10×ULN and >twice baseline (NIH-adopted criteria); ALT >5×ULN and >twice baseline (minimum accepted criteria). ALT/DNA plots were employed to characterize flare onset and mechanism.

Results: Frequency of flares was higher with Lambda vs alfa irrespective of flare definition (Table). Patients with flares had mean baseline HBV-DNA=7.99 log10 IU/mL, ALT=136 U/L, and qHBsAg=4.26 IU/mL; HBV genotypes B (28%) and C (58%) predominated. More Lambda vs Alfa flares occurred early (Weeks 4–16), associated with HBV-DNA decline, suggesting host-mediated pathogenesis (25/27 [93%] vs 8/13 [62%, respectively). For 3/5 Lambda vs 0/7 alfa recipients with HBeAg seroconversion, ALT flare (NIH criteria) preceded the event. Five flares (1 Lambda, 4 alfa) were preceded by HBV-DNA increase/lack of response, suggesting viral-mediated pathogenesis. The majority of Lambda flares were asymptomatic and required no intervention; none was associated with decompensation.

Conclusions: Lambda was associated with more frequent on-treatment ALT flares than alfa. Over 90% of Lambda flares were consistent with host-mediated pathogenesis; 60% of Lambda-associated HBeAg seroconversions were preceded by host-mediated flares. Further characterization of predictive factors, flare-associated biomarker changes, and correlative outcomes is ongoing.

Table 1

<table>
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<th>Event</th>
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<th>Alfa (N=83)</th>
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<td>6/83 (7)</td>
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<tr>
<td>ALT flares (ALT &gt;5×ULN and &gt;2× baseline)</td>
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<td>Managed with dose reduction</td>
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<td>Resulted in treatment discontinuation</td>
<td>2/27 (9)</td>
<td>2/13 (15)</td>
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THE FEASIBILITY STUDY OF ALLOGENEIC BONE MARROW MESCENGYMAL STEM CELLS TRANSPLANTATION IN ACUTE-ON-CHRONIC LIVER FAILURE PATIENTS CAUSED BY HEPATITIS B

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Background and Aims: Acute-on-chronic liver failure (ACLF) caused by hepatitis B virus (HBV) is a severe disease with high mortality. Our early study revealed that autologous bone marrow mesenchymal stem cells (BM-MSCs) transplantation for HBV induced liver failure patients made some improvements in liver functions, but without any survival benefit. The insufficiency of BM-MSCs transfused may be the main reason. Our study aimed to assess the safety and efficacy of allogeneic BM-MSCs transplantation via peripheral veins for HBV induced ACLF patients by increasing the doses of the BM-MSCs and the frequency of transplantation.

Methods: 28 HBV induced ACLF inpatients were divided into 3 groups randomly. The first group (Group SMT) received the standard medicine treatment. The second (Group MSC-1) and the third group (Group MSC-2) received 4 doses of allogeneic BM-MSCs via peripheral veins once a week, in addition to the standard medicine treatment. The doses of BM-MSCs infused were 1×10^6/kg for Group MSC-1 and 1×10^6/kg for Group MSC-2 respectively. All the groups were followed up for 24 weeks.

Results: No serious adverse reactions were observed from 1–24 weeks. Comparing with Group SMT, Group MSC (Group MSC was defined as the combine of Group MSC-1 and MSC-2) achieved higher 24-week cumulative survival rate (P=0.015). Besides, Group MSC acquired markedly improvements of liver functions from 1 to 4 weeks. Comparing with Group MSC-1, Group MSC-2 achieved better improvement in the level of alanine aminotransferase (ALT) at week 1 (P=0.041) and 2 (P=0.016). However, Group MSC-1 got dramatic improvements in the level of International Normalized Ratio for blood clotting time (INR) at 2 (P=0.035) and 4 (P=0.024) weeks, and in the level of Model for End-Stage Liver Disease (MELD) score at 3 (P=0.024), 4 (P=0.018) and 8 (P=0.045) weeks. There were no significant differences in the 24-week cumulative survival rates between Group MSC-1 and Group MSC-2.

Conclusions: Allogeneic BM-MSCs transplantation via peripheral veins is safe for HBV induced ACLF patients. It can improve the 24-week survival rate and the liver functions. The efficacy of Group MSC-2 with higher injected doses of BM-MSCs is not superior to that of Group MSC-1.

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OPTIMIZED STRATEGY: SEQUENTIAL PEGYLATED INTERFERON a-2a THERAPY IN ENTECAVIR-TREATED PATIENTS CHB WITHOUT SATISFACTORY END-POINT LED TO HBeAg SEROCONVERSION

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Background and Aim: To investigate the efficacy and safety of pegylated interferon a-2a (PEG-IFN a-2a) therapy in chronic hepatitis B (CHB) patients who failed to achieve satisfactory end point with entecavir (ETV) treatment.

Method: This randomized, open-labeled, double-controlled clinical study enrolled 57 HBeAg positive CHB patients who had received ETV for at least 96 weeks and achieved HBV DNA <500 copies/mL and HBeAg <50 PEIU/mL, but without HBsAg seroconversion. All patients were randomly assigned to receive ETV for 48 weeks (n=30) or PEG-IFN a-2a for 48 weeks (with ETV overlapped in the first 12 weeks) (n=27).

Result: There were no significant differences between PEG-IFN a-2a group and ETV group at baseline ALT level (37.7 U/L vs 36.5 U/L), qHBsAg level (28.19 PEIU/mL vs 28.63 PEIU/mL) and qHBsAg level (4613.7 HI/mL vs 4560.4 HI/mL). The HBeAg clearance and...
seroconversion rate at 48 weeks were 40.7% and 37% in PEG-IFN a-2a group, respectively, which are both higher than those in ETV group (16.7% and 13.3%, P all <0.05). At 24 weeks, 3.7% of patients in PEG-IFN a-2a group cleared HBeAg, and at 48 weeks, the percentage reached 7.4%. Contrarily, none in ETV group achieved that response (P >0.05). The mean qHBsAg level in PEG-IFN a-2a group declined over time during treatment. The qHBsAg level at 48 weeks was significantly lower than that in ETV group (2866.0 ± 2580.4 vs. 4335.8 ± 2650.0 IU/mL, P = 0.027). A greater HBsAg decline was observed in HBeAg seroconverters compared with non-seroconverters. The decline from baseline was significantly different between seroconverters and non-seroconverters especially at 36 weeks (1763.4 ± 3161.2 vs. 1333.5 ± 2483.4 IU/mL, P = 0.036), and 48 weeks (1979.6 ± 2897.1 vs. 1631.8 ± 2395.8 IU/mL, P = 0.01) as well. Although 11.1% of participants in PEG-IFN a-2a group developed HBV DNA relapse (>500 copies/mL) at 48 weeks, which did not occur in ETV group, there was no statistical difference between the two groups at virological relapse rate.

Conclusion: For HBeAg-positive CHB patients who fail to achieve satisfactory endpoint with ETV therapy, sequential PEG-IFN a-2a treatment is an appropriate treatment option to achieve sustained immune control.

742 EFFICACY AND SAFETY OF ENTECAVIR (ETV) PROPHYLAXIS IN INACTIVE HBV CARRIERS WHO UNDERWENT CHEMOTHERAPY FOR SOLID OR HAEMATOLOGICAL CANCER: INTERIM ANALYSIS OF A COHORT STUDY

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Background and Aim: Hepatitis B virus (HBV) carriers who receive chemotherapy for cancer are at high risk of HBV reactivation and a prophylaxis with antiviral drugs is recommended in such patients. The aim of the study was to assess the efficacy and safety of a definite prophylaxis with Entecavir (ETV) in inactive HBV carriers who underwent chemotherapy for solid or haematological cancer.

Patients and Methods: In this open cohort study were included inactive HBV carriers (normal ALT, serum HBV-DNA <2,000 IU/mL, absence of liver disease) who received the first line of chemotherapy because of cancer. All patients received 0.5 mg daily of Entecavir from the start to six months after the discontinuation of chemotherapy. Serum HBV-DNA and ALT levels were measured every 12 weeks and an increase of serum HBV-DNA above 2,000 IU/ml was considered as HBV reactivation.

Results: From June 2009 to October 2012, we included 36 patients (24 males, median age 60 years, range 21–82). Eighteen patients had a solid cancer (9 gastrointestinal, 6 lung and 3 breast tumors) and 18 patients had haematological cancer (3 leukemia, 10 non Hodgkin lymphoma, 2 Hodgkin lymphoma, 3 myeloma). Twenty-nine patients (80%) received corticosteroids and 12 out of 18 patients with haematological cancer received Rituxamab. At baseline, the median values of ALT were 32 IU/mL and the median value of serum HBV-DNA level was 345 IU/mL. During the observation (median 12 months, range 2–21) were not observed reactivations of HBV, and therapy with ETV was not interrupted in any patient because of adverse events.

Conclusion: Our study confirm that prophylaxis with Entecavir is effectiveness in patients receiving anti-cancer chemotherapy, even in patients who have profound immunosuppression because receive Rituxamab in their therapeutic schedule.

743 A RANDOMIZED TRIAL OF GRANULOCYTE-COLONY STIMULATING FACTOR THERAPY IN PATIENTS WITH HBV-ASSOCIATED ACUTE-ON-CHRONIC LIVER FAILURE

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Objective: The aim of our study was to evaluate safety and efficacy of granulocyte-colony stimulating factor (G-CSF) therapy in patients with hepatitis B virus (HBV)-associated acute-on-chronic liver failure.

Methods: Fifty-five patients with HBV-associated acute-on-chronic liver failure were randomized into two groups: the treatment group and the control group. 27 patients in treatment group received G-CSF (5 μg/kg/day, six doses) treatment plus standard therapy, and 28 patients in control group received only standard therapy. The level of peripheral CD34+ cells was consecutively measured by flow cytometry. Circulating white blood count, biochemical parameters and other clinical data of these patients were recorded and analyzed. All patients were followed up for at least 3 months for evaluation of changes in liver function and survival rate.

Results: The peripheral neutrophil and CD34+ cell count in G-CSF group increased on day 3 of therapy, continued to rise on day 7 and remained on day 15 compared to those of the control group; Child-Turcotte-Pugh (CTP) score of patients in the treatment group was improved on day 30 after G-CSF therapy, compared to that in controls (P = 0.041); Model for end stage of liver disease (MELD) score of patients in the treatment group was improved on day 7 (P = 0.004) and remained high until day 30 after G-CSF therapy (P < 0.001). Safety, including bone marrow suppression (<500 copies/mL), as well as other adverse events were not observed. The survival rate of the treatment group (13/27) was significantly higher than that in the control group (6/28) (P = 0.0181).

744 TENOFOVR DF (TDF) IS SAFE AND WELL TOLERATED IN CHRONIC HEPATITIS B (CHB) PATIENTS WITH PRE-EXISTING MILD RENAL IMPAIRMENT

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Background and Aim: Tenofovir DF (TDF) is approved for the treatment of CHB patients with normal renal function (CrCl ≥80 mL/min). However, data are limited in CHB patients with mild renal impairment (MRI) as they are excluded from most trials. MRI patients (CrCL 50–80 mL/min by Cockroft-Gault) were included in a prospective, randomized, double-blind trial of TDF vs. FTC/TDF in lamivudine-resistant patients (Study 121) wherein no differences were observed in efficacy or safety between treatments (Fung S. AASLD 2012, #20).

Methods: Post-hoc analysis of Study 121 which compared MRI patients (74/280; 26%) and normal renal function (NRF; CrCl ≥80 mL/min) patients (206/280; 74%). Safety, including bone mineral density (BMD) monitoring by DXA, pharmacokinetics (PK; MRI patients only), and efficacy were assessed over 96 weeks.

Results: At baseline (BL), mean (SD) CrCl was 67 (9) mL/min for the MRI group and 104 (18) mL/min for the NRF group. Both groups (MRI vs. NRF) were well matched except: mean age 58 vs. 43 yrs (p <0.001), males 59% vs. 81% (p <0.001), prior IFN 18%
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vs. 32% (p = 0.015), and prior ADV 14% vs. 25% (p = 0.044). Tenofovir (TFV) PK parameters (AUC, Cmax, Tmax) in MRI were comparable to historical data in NRF, and there was no correlation between steady-state TFV exposures (AUC,) and BL CrCL. TDF was well tolerated overall; 3 patients (1.1%) discontinued the study early for an AE (2-MRI and 1-NRF). No patients had a confirmed increase in serum creatinine of ≥0.5 mg/dL, and 1% (2-NRF) had transient PO4 <2 mg/dL. Nine MRI patients had Clcr <50 mL/min (pre-treatment range: 49–61 mL/min) that stabilized with dose adjustment. No differences were observed in % change in spine or hip BMD over 96 weeks, and no clinically relevant bone loss was noted in either group. At Week 96 there was no significant difference (missing=failure) in % with HBV DNA <400 copies/mL, or rates of ALT normalization or HBeAg loss/seroconversion.

Conclusions: The safety, PK, and efficacy of patients with MRI receiving TDF were similar to NRF patients; in MRI patients there was no evidence of increased risk for renal- or bone-related complications.

745 eGFR IMPROVEMENT IN CHRONIC HEPATITIS B PATIENTS WITH TELBIVUDINE TREATMENT IS INDEPENDENT OF ANTIVIRAL ACTIVITY

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1Auckland City Hospital, Auckland, New Zealand; 2Chang Gung University College of Medicine, Chang Gung Memorial Hospital, Taipei, Taiwan R.O.C.; 3Southwest Hospital, Third Military Medical University, Chongqing, China; 4University Department of Medicine, Queen Mary Hospital, Hong Kong, Hong Kong S.A.R.; 5Albert-Ludwigs Universit¨ at Freiburg, Freiburg im Breisgau, Germany; 6/26 patients (23%) demonstrated evidence of viral breakthrough (shift from 60–90 to <60 ml/min/1.73m2) eGFR) at 2 years.

Results: At 2 years eGFR (1Smean) increased from baseline in telbivudine-treated patients by 5.5% compared to -0.52% in lamivudine-treated patients in the overall ITT population (p < 0.001). In the subgroup of patients >50 years, eGFR increased 11.4% in telbivudine compared to -2.4% decrease in lamivudine-treated patients. Patients with baseline eGFR 60–90 ml/min/1.73m2, eGFR increased by 16.8% in telbivudine versus 3.9% in lamivudine-treated group. eGFR improvements at 2 years were independent of baseline HBeAg status, markers of virologic response, including HBV DNA undetectable (<300 copies/ml), HBV DNA suppression (<5 logs) or virologic breakthrough/genotypic resistance at 2 years. Patients who had not achieved on-treatment HBeAg seroconversion (17.6%) or HBeAg loss (18.7%) had higher eGFR increase versus patients with HBeAg seroconversion (7.3%, p = 0.0223) or HBeAg loss (6.2%, p = 0.0035). In the stepwise regression model, no differences were observed for: baseline HBeAg status, baseline ALT (<2× ULN vs >2× ULN), baseline HBV DNA (<9 vs <9 log for HBeAg+ and <7 vs >7 log for HBeAg− patients), Genotype C and undetectable HBV DNA at Week24. Telbivudine treatment (Odds ratio (OR) 2.509 (95% CI: 1.663, 3.784) p < 0.0001) and age (continuous variable) OR 0.940 (0.923, 0.958), p < 0.0001 were the strongest significant predictors of eGFR increase. Other predictors were Caucasian race (vs Asian), OR 0.338 (0.175, 0.652), p = 0.0012 and Other vs Asian, OR 0.429 (0.183, 1.005), p = 0.0514.

Conclusion: In this subanalysis of the GLOBE study, the strongest predictors of improvement in renal function during antiviral therapy in patients with compensated CHB were age and telbivudine treatment, the latter effect independent of on-treatment antiviral response.

746 INDUCTION MAINTENANCE THERAPY IN CHRONIC HEPATITIS B; STEP-DOWN FROM COMBINATION LAMIVUDINE AND TENOFIVIR TO LAMIVUDINE MONOTHERAPY – A POTENTIAL NEW TREATMENT STRATEGY


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Background: Tenofovir is a potent and effective oral antiviral (OAV) for Chronic Hepatitis B (CHB) treatment. Recent data suggest that OAV therapy may be required for up to 40 years prior to HBsAg loss in HBeAg-negative disease. The cost implications and potential long-term toxicities of indefinite OAV use mandates further consideration for optimising CHB management. An induction-maintenance treatment strategy ‘stepping-down’ from potent drug combinations to monotherapy has been effective in HIV. In CHB, initiation with combination Lamivudine and Tenofovir followed by Lamivudine maintenance may be cost-effective. We report the first data with this approach.

 Patients and Methods: Patients receiving combination Lamivudine and Tenofovir for a minimum of 18 months were considered for the study. Selection criteria included HBeAg-negative disease, Ishak fibrosis stage <4/6, undetectable HBV-DNA and normal ALT for a minimum of 12 consecutive months. Patients meeting these criteria were invited to discontinue Tenofovir and step-down to maintenance Lamivudine monotherapy. Patients were followed monthly to determine whether viral suppression and ALT normalisation were maintained in the absence of Tenofovir.

Results: 26 patients (17 male), median age 49, (range 32–65) discontinued Tenofovir and were followed for up to 1 year. Median follow-up was 48 weeks (range 12–52), 20/26 patients (77%) sustained viral suppression during follow-up. In this group, pre-discontinuation of Tenofovir, the median ALT was 27U/L (range 15–40), and during follow-up on Lamivudine was 24U/L (range 15–40), (p=n.s). Median HBsAg level pre-discontinuation of Tenofovir was 3.54 logU/ml (range 1.21–4.48) and 3.45 logU/ml (range -0.35–4.35); (p=n.s) on Lamivudine monotherapy. 6/26 patients (23%) demonstrated evidence of viral breakthrough on Lamivudine monotherapy. Median time for HBV-DNA detection was 16 weeks (range 12–36). On sub-group analysis there was no significant difference in ALT (p = 0.74) and HBsAg (p = 0.83) levels pre-discontinuation of Tenofovir, in those with evidence of viral breakthrough compared to those with continuous viral control.

Conclusions: We demonstrate that in the majority of patients a step-down strategy to Lamivudine monotherapy is safe. These data suggest that an induction-maintenance strategy may be pursued in selected patients to avoid long-term exposure to Tenofovir; consequently limiting potential drug toxicity and reducing the burden on healthcare budgets.
Background and Aims: Entecavir has a higher potent antiviral efficacy and a lower drug resistance rate than Lamivudine in nucleoside-naïve chronic hepatitis B (CHB) patients. The switch from Lamivudine to Entecavir in patients who have undetectable hepatitis B virus DNA (HBVDNA <60 IU/mL) may lead to more prolonged viral suppression to undetectable level by PCR method, compared to patients with continuous Lamivudine treatment. This prospective, 96 week study investigated the antiviral efficacy, safety and tolerability of switching to Entecavir versus maintaining Lamivudine in CHB patients with virologic response to Lamivudine.

Patients and Methods: A total of 73 HBeAg-positive patients, with serum HBVDNA <60 IU/mL after at least 6 months Lamivudine monotherapy were randomized 1:1 into either switching to Entecavir 0.5 mg/day, or continuing with Lamivudine 100 mg/day.

Results: Mean duration of prior Lamivudine treatment (n = 35) was 25.7 months in the Lamivudine-maintained, and 27.4 months in the Entecavir-switch patients. At 96 weeks of follow-up, 20/35 (57.1%) patients in the Lamivudine arm had persistently undetectable HBVDNA, compared with 37/38 (97.4%) patients in the Entecavir arm (P <0.001). Out of total 16 patients with HBVDNA rebound, 8/15 in the Lamivudine arm had HBVDNA of more than 1000 IU/mL during rebound, while none in Entecavir arm. Genotypic resistance to assigned intervention emerged in 28.6% (10/35) of Lamivudine-maintained patients, and in 0% (0/38) of Entecavir-switched patients during 96 weeks (P <0.001). Seventeen Entecavir-switched (45.9%) and seven Lamivudine-maintained (21.2%) patients achieved HBeAg loss (P =0.043), and nine Entecavir (24.3%) and five Lamivudine (15.2%) patients achieved HBeAg seroconversion.

Conclusion: Switching to Entecavir in patients with undetectable HBVDNA to Lamivudine resulted in maintained virologic response without antiviral resistance at 96 week compared with maintaining Lamivudine.

748 VIROLOGICAL RESPONSE AND TOLERANCE OF TENOFOVIR DF (TDF) IN THE ELDERLY IS SIMILAR TO YOUNGER PATIENTS IN REAL LIFE PRACTICE

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Background and Aims: Phase III trials have reported high virological response (VR) and favorable safety profile of TDF up to 6 years. Unfortunately, patients with older age and severe comorbidities are usually excluded from clinical trials. Thus, data from real life cohorts are needed. The aim of this study was to analyze the 2-year data on the efficacy and tolerance of TDF treatment in a real life cohort, especially in elderly patients.

Methods: In a French multicentre prospective cohort (Vireal study), 441 HBV patients treated with TDF were included. Clinical, serological and virological data were collected at baseline and every 6 months. Preliminary analyses after 2-years of treatment were performed in the overall population and a subgroup of elderly patients (>65 years). VR was defined as HBV-DNA <69 IU/mL.

Results: Baseline characteristics (n = 441) were: mean age 45±14 years, 71% male, 67% abnormal ALT, 74% HBeAg-negative and 33% advanced fibrosis (F3/F4 Metavir). 58% were NUC-experienced or resistant. Overall VR was 94% at 2-years. Naïve patients had similar VR to treatment-experienced patients after 2-years (96% vs 93%, p = 0.42). HBsAg-loss was observed in 11 patients at 2 years (3 elderly, 8 HBeAg-). The rate of adverse events (AE) was 14%. No major safety issues were reported. Liver-related complications were observed in 3 patients (2 HCC, 1 SBP). Forty-eight elderly patients were subsequently analyzed: mean age 71±6 years, 73% male, 87% HBeAg-negative, 58% advanced fibrosis and 79% treatment-experienced. VR did not differ between elderly and younger patients (92% vs 94%, p =0.64) at 2 years. Four elderly patients under immunosuppressive therapy had received TDF and no cases of HBV reactivation were reported. AE rate in elderly was 15%. The most common AE was nausea (n = 3). Although 82% of elderly had prior GFR <90 mL/min (estimated by CKD-EPI formula), GFR remained stable or improved in 91%. The mean GFR was 73, 69 and 70 mL/min at baseline, 1 and 2 years.

Conclusions: In real life practice, TDF treatment was associated with a high virological response and an increasing number of HBsAg-loss (2.5%) at 2 years. TDF efficacy, safety and tolerance were similar in elderly and younger patients.
Methods: 119 treatment-naïve CHB ACLF patients were prospectively enrolled from a single center in this intention-to-treat analysis. Patients included were aged between 18 and 65 years, had pro-thrombin activity (PTA) ≤40%, total bilirubin ≥171 μmol/L (or increase ≥171 μmol/L), hepatitis B virus (HBV) DNA ≥10^6 copies/mL, alanine transaminase (ALT) > 5 × upper limit of normal and absence of hepatocellular carcinoma. Patients received either entecavir 0.5 mg/day (n = 65) or lamivudine 100 mg/day (n = 54). The primary efficacy endpoint was survival among non-liver transplant patients at Week 48. Secondary efficacy endpoints included mean change in HBV DNA and ALT levels, mean change in PTA and improvement in Child-Turcotte-Pugh (CTP) and model of end-stage liver disease (MELD) score at Day 60. Safety was analysed cumulatively in patients as-treated.

Results: Baseline characteristics were not statistically different between groups. All patients were followed-up through 48 weeks. The 48-weeks survival rate was 64.6% (42/65) in entecavir patients and 50.0% (27/54) in lamivudine patients (P = 0.108). The mean survival time was longer in entecavir patients than lamivudine patients (288.7 versus 231.0 days; P = 0.031). The mean reduction from baseline in HBV DNA and ALT levels and mean PTA were higher in entecavir than lamivudine patients at Day 60 (P < 0.01, P > 0.05 and P < 0.05, respectively). Improvement in CTP and MELD score was greater in entecavir than lamivudine patients at Day 60 (P < 0.05 and P < 0.01). No patient experienced serious adverse events, and there were no cases of severe lactic acidosis attributed to entecavir therapy. No patients required dose modifications or early discontinuation.

Conclusions: 48 week survival rates were not statistically different between groups; however, mean survival time was greater in entecavir than lamivudine ACLF patients. Entecavir was superior to lamivudine in terms of reducing HBV DNA and ALT levels, PTA, CTP and MELD scores at Day 60.

751 IS HBV-RELATED RENAL TUBULOPATHY BEFORE ANY ANTIVIRAL TREATMENT A REALITY?

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Background and Aims: Tenofovir (TDF) is a nucleotide analogue recommended since 2008 in HBV monoinfection. Its proximal renal tubular toxicity identified in HIV therapy is still debated in HBV monoinfection and the potential role of the virus itself on the tubule is unknown. Clinical studies have used little sensitive and specific late tools to diagnose tubular dysfunction (creatinine, urine test strip). The purpose of this work was to prospectively screen potential ‘infra-clinical’ changes of the proximal tubular function in HBV monoinfected and treatment-naïve patients.

Methods: Prospective consecutive study of 81 HBV monoinfected treatment-naïve patients with no treatment indication (74% inactive carriers, 26% chronic hepatitis patients with 18% HBeAg+ patients, 9% HBeAg+ patients of which 4% immunotolerant). According to the literature, an infra-clinical proximal tubulopathy was defined by the association of two early markers among the following; – non-diabetic glycosuria on the urine test strip, – abnormality of the phosphorus proximal tubular reabsorption (TmPi/GFR below 0.8 according to the Bijvoet’s diagram), – increase of the uric acid fractional excretion (UAFe) beyond 10%, – ratio of beta-2-microglobulinuria/creatininuria over 200 μg/g, – ratio of UCyS/creatininuria over 14 μg/mmol.

Results: No patient presented an infra-clinical proximal tubulopathy defined by at least two early criteria out of five. 95% of patients presented a 25-OH-Vitamin D3 insufficiency and 27% a deficiency defined by a concentration below 30 ng/mL and 10 ng/mL respectively. An isolated abnormality of the proximal tubular function was observed in 13 patients after the correction of the 25-OH-Vitamin D3 level: 12 TmPi/GFR below 0.8, 4 UAFEs over 10%, and one increase of the beta-2-microglobulin/creatininuria ratio. No difference was found between the 68 patients without any abnormality and the 13 patients with an early marker of tubular dysfunction in terms of age, race, gender, blood pressure, GFR, BMI, HBV viral load, ALAT, bilirubin level, PT, elastometry score and virological status.

Conclusion: This study is, to our knowledge, the first prospective study to show the low probability of infra-clinical proximal tubular renal dysfunction, pre-existing to any antiviral treatment and thus potentially related to HBV, using several early markers of proximal tubular dysfunction.

752 THERAPEUTIC VACCINATION FOR CHRONIC HEPATITIS B (CHB) USING HETEROLOGOUS PROTEIN PRIME/VECTO BOOST VACCINATION SCHEME

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Background and Aims: Infection with Hepatitis B virus (HBV) remains a major global health threat. Approximately 350 million individuals have chronic infection and are at risk of premature death from cirrhosis with liver failure or hepatocellular carcinoma. These patients lack HBV-specific T cell responses and neutralizing antibodies. Current therapies control but rarely eliminate HBV requiring alternative treatment approaches. Therapeutic vaccination provides an attractive strategy to mount a broad and strong virus-specific T cell response in CHB patients and additionally induce specific neutralizing antibodies for viral control and clearance.

Methods: We used recombinant S ayw/adw and/or C protein in combination with adjuvants polyphosphazene (PCEP) and/or CpG for vaccination. In addition, we constructed recombinant Modified Vaccinia Ankara (MVA) virus expressing the small envelope (S) with the HBV subtype ayw or adw or core (C). To investigate the ability of the vaccine to induce HBV-specific T cell and antibody responses, we tested it in HBV transgenic (HBVtg) mice that replicate HBV ayw in their livers and are immunologically tolerant to HBV.

Results: Protein priming followed by a boost vaccination with MVA-S ayw and MVA-C induced high frequencies of S- and C-specific CD8+ T cells, respectively, as well as neutralizing antibodies in C57Bl/6 wild type mice. In HBVtg mice, this heterologous prime-boost vaccination induced multifunctional S-specific CD8+ T cell responses in spleen and liver as well as anti-C and neutralizing anti-S antibodies. Vaccination, however, was only able to break tolerance in low-viremic, but not in high viremic mice unless CpG and PCEP were combined as adjuvants. MVA-S adw induced stronger T cell responses than MVA-S ayw and higher S- but similar C-specific antibody titers in sera of high viremic HBV transgenic mice. S-specific antibodies induced by S-protein/MVA-S adw were also found to neutralize viral particles of subtype ayw and lead to a significant reduction of HBsAg in the transgenic mouse model.

Conclusion: Heterologous protein prime/MVA vector boost vaccination is able to break tolerance in a mouse model of CHB even in high replicating animals if appropriate adjuvants are used. It therefore provides a promising scheme for therapeutic vaccination in future clinical trials.

753 BASELINE HBsAg PREDICTS HBsAg LOSS AFTER 2 YEARS OF TREATMENT-FREE FOLLOW-UP IN CHRONIC HEPATITIS B PATIENTS TREATED WITH PEGINTERFERON alfa-2a AND ADEFOVIR

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Introduction and Aim: Considering the low efficacy and significant side effects of peg-IFN based therapy, there is a need to establish predictors of response to allow selection of patients likely to benefit from treatment. Therefore we determined response and predictors of response in chronic hepatitis B patients treated with peg-IFN and adefovir combination therapy.

Patients and Methods: We treated 92 chronic hepatitis B patients (44 HBeAg-positive and 48 HBeAg-negative) with HBV-DNA >100,000 copies/mL (>17,182 IU/mL) with peg-IFN and adefovir for 48 weeks, and followed them up for 2 years. Markers for HBsAg loss, combined response (HBV-DNA levels ≤2,000 IU/mL and ALT normalization), and HBsAg loss were evaluated. Serum HBsAg quantitation was performed by the Abbott Architect. Predictors of response were examined by logistic regression analysis.

Results: After 2 years of treatment free follow up, rates of HBsAg loss and HBsAg loss in HBeAg-positive patients were 18/44 (41%) and 5/44 (11%), respectively. Four of them developed anti-HBs. In HBeAg-negative patients, a low baseline HBsAg was the only independent predictor of HBsAg loss (OR 0.02, p=0.01). All but one HBeAg-negative patient with HBsAg loss had developed anti-HBs at 2 years of follow up. In HBeAg-positive patients no baseline markers predicted HBsAg loss. HBeAg-negative patients with HBsAg loss had significantly lower baseline HBsAg levels than those without HBsAg loss (mean HBsAg 2.35 versus 3.55 log IU/mL, p <0.001). They also had lower HBV-DNA levels and were more often (peg-) interferon interfered. Baseline HBsAg was the only independent predictor of HBsAg loss (OR 0.02, p=0.01).

Conclusions: With combination therapy of peg-IFN and adefovir for 48 weeks, a high rate of HBsAg loss was observed in both HBeAg-positive (11%) and HBeAg-negative (17%) patients two years after end of treatment. In HBeAg-negative patients, a low baseline HBsAg level was a strong predictor for HBsAg loss.
New combination therapy of Pegylated interferon (PegIFN) with tenofovir (TDF) was assessed in order to improve the rate of HBsAg loss. The aim of this study is to investigate the S-gene variability of patients treated with combination of PegIFN and TDF.

Methods: Patients received 180 μg of Peg-IFN/week plus 300 mg of TDF/day during 48 weeks. Patients were seen every 3 months. Sustained virologic response (SVR) was defined as HBV-DNA <2000 UI/mL, 48 weeks after end of therapy. Non-SVR response (N-SVR) was defined as HBV-DNA >2000 UI/mL 48 weeks after end of therapy. HBsAg-encoding gene from each patient’s serum at baseline was PCR-amplified and cloned. At least 15 clones per patient were analysed.

Results: Thirty-two patients were included in this analysis. Baseline characteristics were: median age 44 years (range=27–67 years), 81% male, mean of HBV-DNA 4.9 log IU/mL (range 2.9–8.8 log IU/mL) and mean of serum ALT 102 IU/L (range 24–287 IU/L). After the 48 weeks of follow-up, 7/26 patients (27%) achieved a SVR and 5/26 patients (16%) had a loss of HBsAg. Comparison of variability along the S protein indicated a marked difference between patients with N-SVR vs SVR. When considering the full-length S coding region, the number of residues substitutions was 1.32 times more frequent in N-SVR vs SVR. When considering the full-length S coding region, the number of residues substitutions was 1.32 times more frequent in N-SVR vs SVR.

Conclusion: In patients receiving Peg-IFN plus tenofovir, a SVR was observed in 22% and an HBsAg loss in 16%. N-SVR patients showed more variability along the S protein. The Accumulation of residue substitutions frequency of the HBsAg “a” determinant region was more variability along the S protein. The Accumulation of residue substitutions frequency of the HBsAg “a” determinant region was more variability along the S protein.

Sustained virologic response (SVR) was defined as HBVDNA ≤400 UI/mL one month after end of therapy. HBsAg-encoding gene from each patient’s serum at baseline was PCR-amplified and cloned. At least 15 clones per patient were analysed.

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12 and throughout the study. No safety issues were reported. HCC developed in 5 non cirrhotics (2.4%) between month 19 and 46 of treatment, with an yearly rate of 0.5%. The 155 compensated cirrhotics remained clinically stable, yet 17 developed a HCC to give a 5-year cumulative rate of 14% and an yearly rate of 2.8%, despite full suppression of viral replication in most cases. Five cirrhotics died for non-liver related causes, 2 for HCC and 4 were transplanted for HCC: the overall 5-year cumulative survival and liver-related survival were 91% and 95%, respectively.

**Conclusion:** Entecavir monotherapy efficiently suppressed HBV in naïve patients with CHB, fully preventing clinical decompensation but not HCC.

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**SERUM HBsAg DECLINE IS FASTER THAN PREVIOUSLY ESTIMATED IN LONG-TERM NUC RESPONDERS WITH LOW TREATMENT-INDUCED HBsAg LEVELS**

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Serum HBsAg loss is the recommended stopping rule in NUC responders, yet this event occurs rarely. A 50% decline of HBsAg titer every 5 to 6 years has been described among HBeAg-negative NUC responders, leading to an estimated duration of treatment exceeding 30–50 years in most cases. Whether such a sloppy HBsAg kinetics applies also to patients with treatment-induced low HBsAg levels, is unknown.

**Methods:** 39 consecutive CHB patients displaying HBsAg titers between 100 and 10 IU/ml following long-term exposure to NUC, were recruited in a single center. HBeAg positive patients with a recent HBeAg seroconversion, immunocompromised patients, acute protracted cases and recently IFN treated patients were excluded. At baseline, age was 60 years, 87% males, 82% HBeAg-negative, 65% genotype D, 52% IL28B CC genotype, 59% cirrhotics, 97% with undetectable HBV-DNA, 95% with normal ALT, 64% on combo (LAM+ADV in 13, LAM+TDF in 12). Liver function tests, HBV-DNA and HBsAg levels (Abbott assay) were assessed every 3–4 months.

**Results:** During 39 (11–78) months of study, the median HBsAg titers progressively declined from 95 (range 16–270) IU/ml at baseline to 46 (0.02–139) at year 1, to 23 (0.11–87) at year 2, to 7 (0.07–68) at year 3. The median decline was 50% every year, with more than 1 log decline over 3 years of continuous NUC therapy. The proportion of patients achieving >1 log decline increased over time, from 22% at year 1 and 2, to 37% at year 3 and 54% at last follow-up visit. Serum HBV-DNA remained undetectable in all patients, HBeAg seroconversion occurred in 3 patients between 5 and 10 months. Eleven patients (28%), 9 HBeAg negative, lost HBsAg between 11 and 73 months (median 27) and 10 of them successfully discontinued NUC.

**Conclusion:** HBsAg kinetics is faster than previously estimated in long-term NUC suppressed CHB patients achieving low HBsAg levels, a finding that might help in the design of cost effective algorithms of immunomodulatory add-on or switch therapies in NUC suppressed patients.

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**ADDING PEGINTERFERON alfa-2a ON NUCLEOS(T)IDE ANALOGUES THERAPY IMPROVES HBeAg SEROCONVERSION AND qHBsAg DECLINE IN HBeAg-POSITIVE CHRONIC HEPATITIS B PATIENTS WHO HAVE ACHIEVED VIROLOGICAL RESPONSES**

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**Background and Aims:** Most HBeAg positive chronic hepatitis B (CHB) cannot achieve serological response with nucleos(t)ide analogues (NUCs). The aim of this study was to investigate whether adding peginterferon alfa-2a (PEG-IFN) on NUC therapy can improve serological response and thereby, shorten the course of treatment.

**Methods:** HBeAg-positive CHB who had achieved undetectable HBV DNA with at least one year of NUC therapy but remained HBeAg positive were enrolled. All subjects were non-randomly classified into two arms. Those in the combination therapy group were added Peg-IFNα-2a 180 μg sc/week on concurrent NUC therapy while those in the monotherapy group remained on NUC therapy. Serological and virological tests were performed at an interval of 12 weeks. Adverse events (AEs) were recorded.

**Results:** A total of 75 eligible subjects were recruited, among whom 19 (9 ETV, 8 ADV, 1 ETV+ADV, 1 LAM) were in the combination therapy group and 56 (35 ETV, 19 ADV, 2 LAM) in the monotherapy group. There was no difference at baseline characteristics between the two groups, including sex, age, ALT level, qHBsAg level, qHBeAg level and prior NUC treatment durations. The HBeAg seroconversion rates at 12, 24, 36 and 48 weeks were all significantly higher in the combination therapy group than in the monotherapy group.

**Conclusion:** PEG-IFNα-2a can be considered as an effective agent to enhance the seroconversion rate and HBsAg elimination in CHB patients who have achieved virological response on NUCs.
(Figure 1A). The HBeAg loss rate and mean HBeAg decline value from baseline were both significantly higher in the combination therapy group than in the NUC group at 48 weeks (42.1% vs. 10.7%, *P* = 0.002; 0.42 vs. 0.22 log_{10} s/co, *P* = 0.004). The mean HBeAg decline value from baseline was also higher in the combination therapy group than in the monotherapy group at 48 weeks (1.06 vs. −0.09 log_{10} IU/mL, *P* < 0.001; Figure 1B). Two subjects in the combination therapy group achieved HBeAg seroconversion. There was no difference in rate of AEs between the two groups.

**Conclusions:** Adding Peg-IFNα-2a on NUC therapy is well-tolerated and can improve HBeAg seroconversion and qHBeAg decline in HBeAg-positive CHB patients who have achieved virological response with NUC therapy, which made it possible to shorten the treatment duration in these population.

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ENTECAVIR PLUS PEGINTERFERON alfa-2a vs. ENTECAVIR ALONE IN THE TREATMENT OF HEPATITIS B e ANTIGEN-POSITIVE CHRONIC HEPATITIS B: AN INTERIM REPORT

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**Background and Aims:** Peginterferon alfa or nucleos(t)ide analogue monotherapy is the current standard of care for antiviral treatment of hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB). We aimed to assess the efficacy and safety of peginterferon alfa-2a plus entecavir (ETV) versus ETV alone for compensated HBeAg-positive CHB.

**Methods:** An open-label, multicenter randomized controlled trial was performed at 10 outpatient hepatology clinics in Taiwan. HBeAg-positive CHB patients were randomized at baseline to receive peginterferon alfa-2a 180mcg/week plus ETV 0.5 mg/day for 24 weeks followed by ETV monotherapy for additional 120 weeks (Group A) or ETV alone for 144 weeks (Group B). Post-treatment follow-up was 48 weeks. The primary endpoint was the rate of HBeAg seroconversion at 144 weeks after the start of treatment.

**Results:** A total of 168 patients (63% male and mean age 39 ± 9 years) were enrolled: 84 in each arm. Mean ALT level was 192 ± 120 U/L and HBV DNA level was 8.6 ± 9 Log_{10} IU/mL. At week 24, the rate of serum HBV DNA <1000 copies/mL, ALT normalization, and HBeAg seroconversion in Group A (n = 70) versus Group B (n = 68) was 80% versus 60%, 47% versus 71%, and 14% versus 7%, respectively. At week 48, the rate of HBeAg seroconversion in Group A (n = 65) versus Group B (n = 62) was 28% versus 15%. At end of 144-week treatment, the rate of HBV DNA <1000 copies/mL, ALT normalization, and HBeAg seroconversion in Group A (n = 25) versus Group B (n = 28) was 92% versus 89%, 92% versus 82%, and 40% versus 39%, respectively. Only 1 patient in Group B dropped out because of intolerable itching sensation. None of the patients in Group A developed peripheral neuropathy or other significant adverse effects.

**Conclusions:** In HBeAg-positive CHB, 24-week combined peginterferon alfa-2a plus ETV followed by 120-week ETV monotherapy was safe, but resulted in only transient greater efficacy than 144-week ETV monotherapy. No difference in end-of-treatment virologic and serologic response rates was observed between the two treatment arms.

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IMPACT OF ENTECAVIR VERSUS LAMIVUDINE ON HEPATIC COVALENTLY CLOSED-CIRCULAR DNA AND TOTAL HEPATIC HBV DNA IN NUCLEOSIDE-NAIVE HBEAG POSITIVE CHRONIC HEPATITIS B PATIENTS

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**Background:** Worldwide, chronic hepatitis B virus (HBV) infection is the leading cause of cirrhosis, decompensated liver disease, and hepatocellular carcinoma (HCC). The chronic nature of HBV infection is due to a pool of stable, covalently closed-circular HBV DNA (cccDNA) inside the nuclei of infected hepatocytes. Hepatic cccDNA and chromosomal HBV integration, together with liver inflammation resulting from the immunological reaction to the infection, are believed to contribute to HCC development. Limited data are available on the effect of nucleos(t)ide analogues on hepatic cccDNA and total hepatic HBV DNA levels. These results describe the effect of entecavir (ETV) on hepatic cccDNA and total hepatic HBV DNA levels compared with lamivudine (LVD) in biopsies from patients enrolled in the phase III study ETV-022.

**Methods:** Patients with evaluable hepatic cccDNA and total hepatic HBV DNA pairs (i.e. both baseline and Week 48 measurements from biopsies) were included. Differences (ETV vs LVD) in mean log_{10} changes in hepatic cccDNA and total hepatic HBV DNA were estimated using linear regression adjusted for baseline levels. Total hepatic HBV DNA was extracted from frozen liver samples using the Epicenter Masterpure kit. Hepatic cccDNA and total hepatic HBV DNA were quantified by real-time PCR (Roche LightCycler), and copy numbers per human genome equivalent (HGEq) were determined by normalizing samples to the cellular beta-globin gene (limit of detection for both hepatic cccDNA and total hepatic HBV DNA; 0.002 copies/HGEq).

**Results:** Overall, 305 patients had evaluable pairs (ETV: 159; LVD: 146). Baseline demographics and disease characteristics were comparable between the two arms. Compared with LVD, ETV demonstrated significantly greater reductions of hepatic cccDNA and total hepatic DNA levels at Week 48 from baseline.

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>ETV (N = 159)</th>
<th>LVD (N = 146)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Age, years, mean±SE (range)</td>
<td>35±11 (17–76)</td>
</tr>
<tr>
<td>Male, %</td>
<td>116 (73)</td>
<td>104 (71)</td>
</tr>
<tr>
<td>Race, %</td>
<td>Asian 92 (58)</td>
<td>87 (60)</td>
</tr>
<tr>
<td>White</td>
<td>64 (40)</td>
<td>53 (36)</td>
</tr>
<tr>
<td>Week 48</td>
<td>Total hepatic HBV DNA change from baseline, mean±SE log_{10} copies/HGEq</td>
<td>−2·1±0·07</td>
</tr>
<tr>
<td>Difference estimate ETV vs LVD (95%CI)</td>
<td>−0·5 (−0·6,−0·3), <em>P</em> &lt; 0·0001</td>
<td>−0·9±0·05</td>
</tr>
<tr>
<td>Hepatic cccDNA change from baseline, mean±SE log_{10} copies/HGEq</td>
<td><em>a</em></td>
<td>−0·2 (−0·3,−0·1), <em>P</em> = 0·0033</td>
</tr>
</tbody>
</table>

*a*Adjusted for baseline total hepatic HBV DNA level; **b** adjusted for baseline hepatic cccDNA level.
Conclusion: At Week 48, treatment with ETV is superior to LVD in reducing hepatic cccDNA and total hepatic HBV DNA from baseline.

760 A PHASE III CLINICAL TRIAL WITH A NASAL VACCINE CONTAINING BOTH HBsAg AND HBCAg IN PATIENTS WITH CHRONIC HEPATITIS B

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Background and Aims: The therapeutic efficacy of hepatitis B surface antigen (HBsAg)-based vaccine was not satisfactory even when this vaccine was used with antiviral drugs in patients with chronic hepatitis B. The phase III clinical trial described here was accomplished to develop a better therapeutic approach of immune therapy in CHB patients with modifications in nature of therapeutic vaccines and route of administration.

Methods: A phase III clinical trial was done with 151 patients with CHB with similar levels of HBVDNA and alanine aminotransferase (ALT). They were randomly assigned to receive either a therapeutic vaccine containing 100 microgram of both HBsAg and hepatitis B core antigen (HBcAg) or pegylated interferon (Peg-IFN). Seventy-five CHB patients (ALT). They were randomly assigned to receive either a therapeutic vaccine containing 100 microgram of both HBsAg and hepatitis B core antigen (HBcAg) (Center for Genetic Engineering and Biotechnology, Havana, Cuba), once in every two weeks, for 5 times through nasal route administered by a newly developed nasal vaccine device. Seventy-six patients with HBsAg/HBcAg therapeutic vaccine and then declined to receiving HBsAg/HBcAg-based vaccine by nasal route is almost comparable with 48-weeks treatment with Peg-IFN. Follow up study of this phase III clinical trial would yield important information about the role of nasally-administered HBsAg/HBcAg-based therapeutic vaccine in CHB.

Results: Notable adverse effects or acute flare of hepatitis was not detected in any patient of either group. All patients with CHB were expressing HBVDNA in the sera before study commencement; however 37 of 75 patients receiving HBsAg/HBcAg vaccine became negative for HBVDNA after receiving 5 vaccinations through nasal route. The levels ALT showed an early elevation in 90% patients receiving HBsAg/HBcAg therapeutic vaccine and then declined to below upper limit of normal value (<42U/L) in 46 of 75 patients. Out of 76 patients, 48 patients with CHB received Peg IFN became negative for HBVDNA and 36 express normal levels of ALT after the end of IFN therapy (48 weeks after therapy commencement).

Conclusions: A therapeutic vaccine therapy containing HBsAg/HBcAg represents a promising therapeutic approach for CHB patients Therapeutic efficacy of 5 vaccinations with therapeutic vaccine containing HBsAg/HBcAg-based vaccine by nasal route is almost comparable with 48-weeks treatment with Peg-IFN. Follow up study of this phase III clinical trial would yield important information about the role of nasally-administered HBsAg/HBcAg-based therapeutic vaccine in CHB.

761 COMPARISON OF SERUM HBsAg DECLINES DURING TENOFOVIR DISOPROXIL FUMARATE (TDF) TREATMENT IN DIFFERENT CHRONIC HEPATITIS B (CHB) SUB-POPULATIONS

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Background and Aims: High virologic and biochemical response achievable with TDF has been well described in both HBeAg positive and HBeAg negative chronic HBV infections. The HBsAg loss rate with TDF for chronic HBV infection varies according to the patient’s HBeAg status. The aim of this analysis was to investigate HBsAg decline in different CHB sub-populations initially treated with TDF in 3 late phase studies: HBeAg-negative CHB (102), HBeAg-positive active CHB (103), Immune-Tolerant (IT) CHB (101).

Methods: HBsAg levels were determined by Abbott Architect Assay (dynamic range 0.05–250 IU/mL) in 763 CHB patients across different sub-populations: HBeAg negative (250 in TDF, 125 in ADV-TDF), active HBeAg positive (174 in TDF, 90 in ADV-TDF), IT (64 and 60, respectively, in TDF and FTC/TDF). Absolute and categorical declines of HBsAg values were evaluated every 24 weeks through 4 years of continuous treatment. Factors predictive of HBsAg decline and ultimately of HBsAg loss were investigated by univariate and multivariate analyses.

Results: Significantly more patients with HBeAg-positive active CHB had a ≥1 log IU/mL decline than patients with IT CHB (p=0.0014 at week 192) and HBeAg-negative CHB (p<0.001 at week 192) [Fig. 1]. In the 488 subjects initially treated with TDF, the HBsAg decline of ≥1 log at week 24 was the strongest predictor of HBsAg loss: PPV of 45% and NPV of 97% in the active HBeAg positive patients. Overall, 31/488 (6.3%) showed an HBsAg decline ≥1 log at week 24. 29/31 were patients with HBeAg-positive active CHB. ALT (OR 1.01, p<0.0001), HBeAg status (positive vs negative OR 31.2, p=0.0019), and HBsAg level (≥50000 vs ≤50000 IU/mL; OR 3.5, p=0.0052) at baseline were the strongest factors associated with achieving the HBsAg decline ≥1 log at week 24 in a multivariate model.

Conclusions: Different kinetics of HBsAg reduction were observed in CHB sub-populations, with the highest decline in HBeAg-positive CHB patients. A therapeutic vaccine containing HBsAg/HBcAg represents a promising therapeutic approach for CHB patients Therapeutic efficacy of 5 vaccinations with therapeutic vaccine containing HBsAg/HBcAg-based vaccine by nasal route is almost comparable with 48-weeks treatment with Peg-IFN. Follow up study of this phase III clinical trial would yield important information about the role of nasally-administered HBsAg/HBcAg-based therapeutic vaccine in CHB.

Figure: HBsAg decline ≥1 log_{10} in CHB populations.
patients and the lowest in HBeAg-negative patients. An HBsAg reduction of ≥1 log at week 24 was predictive of HBsAg loss in patients with HBeAg-positive active CHB.

762 ADDITIONAL PEG-INTERFERON IN HBeAg-POSITIVE HIV CO-INFECTED PATIENTS ON cART INCLUDING TENOFOVIR: THE ANRS HB01 EMVIEPI STUDY

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Background and Aims: Tenofovir is the preferred treatment for chronic Hepatitis B in HIV co-infected patients on combined antiretroviral therapy (cART). However, the rate of HBe seroconversion in those with positive hepatitis B e antigen (HBeAg) remains low. An immuno-modulatory molecule such as Peg-interferon (Peg-IFN) could be beneficial in these patients.

Methods: Pilot study of one year Peg-IFN add-on (180 g/week) in HBeAg positive-HIV co-infected patients on cART including Tenofovir and Emtricitabine or Lamivudine for at least 6 months. The primary endpoint was HBV sustained response defined by Peg-IFN could be beneficial in these patients.

Results: 51 patients, with a median age of 46 (range: 32–65) years, including 49 (96%) men took part. Median duration of HIV and HBV therapy was 10.3 (0.6–22) and 9.5 (0.5–16) years, respectively. Median baseline CD4 count was 506 (175–1316) mm³, HIV viral load was <50 copies/ml in 49 (96%) patients. ALT level was normal in 76%, HBV DNA undetectable in 73%, and 24% (12/49) had METAVIR fibrosis stage F3 or F4. Before Peg-IFN, median duration of Tenofovir was 39.5 (6–82) months. Nine (18%) patients stopped Peg-IFN prematurely, mainly because of anemia or psychiatric disorders. Undetectable HBV and HIV viral loads were achieved in 90% of patients at W48 (end of Peg-IFN) and in 88% at W72. Median HBsAg decreased from 3.68 at entry to 3.33 and 3.38 log10 IU/ml at W48 and W72 and was not predictive of an HBV sustained response at W72.

Table: Intent-to treat HBV serological evolution

<table>
<thead>
<tr>
<th></th>
<th>During Peg-IFN (W0-W48)</th>
<th>W72</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg loss: n (%)</td>
<td>12/51 (24%)</td>
<td>8/51 (16%)</td>
</tr>
<tr>
<td>Anti-HBe seroconversion: n (%)</td>
<td>6 (12%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>HBV sustained response: n (%)</td>
<td>NA</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>HBsAg loss: n (%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

Conclusions: Despite the loss of HBeAg during treatment in one quarter of the patients, the addition of Peg-IFN did not allow to increase the rate of HBe seroconversion in HBeAg positive HIV co-infected patients.

763 ENTECAVIR EFFECT ON LIVER FUNCTION IN FIELD PRACTICE: INTERIM ANALYSIS OF THE ITALIAN Master-Entas COHORT STUDY OF PATIENTS WITH CHRONIC HEPATITIS B


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Background and Aims: Entecavir effect on liver function and complications of liver cirrhosis has not been assessed in registration as well as in real life studies. Here we evaluated entecavir activity in patients with decompensated liver cirrhosis in a large cohort of patients undergoing entecavir treatment in field practice.

Methods: 300 patients prospectively enrolled from 2009 by 36 Italian centers were evaluated for liver function assessed by serum bilirubin, albumin and prothrombin time. In 132 (44%) patients with liver cirrhosis decompensation episodes and complications were recorded and Child and MELD scores available for 95/132 patients were measured during follow-up.

Results: Cumulative rates of undetectable hepatitis B virus DNA (determined by PCR assay) were 62.8%, 83.1%, 85% and 89% at 6, 12, 18 and 24 months in the whole cohort, while in patients with cirrhosis a slower decline was observed showing 47.7%, 69.4%, 86.8% and 88.1% at 6, 12, 18 and 24 months of follow-up. Bilirubin, albumin and prothrombin time were recorded in 150–98 patients (baseline–24 months). Baseline bilirubin levels above 2 mg/dl were present in 16% of cases, albumin below 3.5 g/dl in 24% and INR above 1.7 in 8% of cases, showing progressive improvement during follow-up. At 24 months, 6% had bilirubin above 2 mg/dl, 13% albumin below 3.5 and one patient INR above 1.7. (bilirubin p < 0.02, albumin p < 0.05, prothrombin time p < 0.02) Sixty-seven/95 cirrhotic patients were Child-A, 24 Child-B and 4 Child-C. Child score improved under treatment showing a significant decrease at month 18 from baseline (p < 0.05).

Twenty-three cirrhotic patients were decompensated with ascites, and 3 patients had hepatic encephalopathy at baseline. A progressive reduction of ascites episodes was observed during follow-up with ascites still present in half of the cases (11 patients) at 6 months. Finally in the whole patient cohort 4 variceal bleeding cases occurred and 18 new diagnosis of HCC were reported.
Fourteen patients died, 11/14 because of liver failure. Four patients underwent OLT.

**Conclusions:** Entecavir treatment in field practice is associated with an improvement of liver function in HBV patients with chronic hepatitis and liver cirrhosis.

**764 ENTECAVIR EFFECTIVENESS IN DAILY CLINICAL PRACTICE, INTERIM ANALYSIS OF THE ITALIAN Master-Entas COHORT STUDY OF PATIENTS WITH CHRONIC HEPATITIS B**


**Background and Aim:**

**Methods:** Three-hundred patients enrolled from 2009 in 36 Italian clinical centers were evaluated for the cumulative rates of undetectable hepatitis B virus DNA (HBV-DNA determined by PCR assay) levels, alanine aminotransferase (ALT) normalization, hepatitis B e antigen (HBeAg) serocconversion, drug resistance mutations (sequencing or line probe assay) and monitored for hepatitis B e antigen (HBeAg) seroconversion, drug resistance mutations (sequencing or line probe assay) and monitored for

**Results:** Median age was 55 years (24–83) and median time to the first visit was 3.5 months. Forty-four per cent (132/300) had an improvement of liver function in HBV patients with chronic hepatitis and liver cirrhosis.

**Conclusion:** The study of Entecavir treatment in field practice confirms its efficacy in suppressing HBV replication with rare drug resistance mutations and its safety in patients with chronic hepatitis and liver cirrhosis.

**765 TWELVE-MONTHS ENTECAVIR LONGITUDINAL CHANGES IN LIVER FIBROSIS, ACTIVITY AS PER FibroTest-FibroMax AND LIVER STIFFNESS MEASUREMENTS IN CHRONIC HEPATITIS B:

**STENOTAS IMPACT ON FIBROSIS REGRESSION**


**Background:** Fibrosis-regression rate in treated chronic hepatitis B (CHB) patients was similar using Fibrotest (Biopredictive) or liver biopsy, while for liver stiffness measurements (LSM) by Fibroscan (Echosens) there was a possible overestimation related to necroinflammatory activity (NIA) (AVT 2010).

**Aim:** To prospectively evaluate the histological impact of a strong inhibitor of HBV-replication, entecavir motherapy at 0.5mg per day, using non-invasive methods, i.e. FibroMax (including Fibrotest, Actitest, Steatotest for estimating fibrosis, activity and steatosis) and LSM.

**Methods:** 133-CHB monoinfected, NUC-naive patients were preincluded in 19 centers in France. Data was recorded at baseline (M0), six, and 12-months (M6, M12): viral load, Fibromax [panel of scores (0–1)] and LSM (0–75 kPa). Applicability (App) was defined as after exclusion of unreliable LSM and failures. Viral response (VR) was defined as undetectable HBV-DNA. Statistics included repeated measures ANOVA (Bonferroni Multiple-Comparison Tests).

**Results:** 116 patients were included [5 lost of follow-up, 9 missing, 3 non-App Fibrotest (acute flare-up ALT>600U/L)]. Characteristics were: age 44 (19–82) yrs; 73% males; 70% anti-HBe( ); 46% Caucasian; 2.6% alcohol>20g/day; median viral load=4.6 logIU/ml; App-LSM 81% (55/68). 31% (N=36) had advanced fibrosis (AF; F2/F3/F4-METAVIR) and 11% (N=12) cirrhosis as per Fibrotest; 46% (N=53) significant NIA (A1A2A3-METAVIR) as per Actitest; 26% (N=21) had M0 steatosis>1% as per Steatotest. 88 patients achieved M6, 61 M12 with 64% M6-VR and 84% M12-VR. Significant NIA as per Actitest regressed from M0 0.58 (0.03) to M6 0.27 (0.03, P<0.0001) and M12 0.27 (0.03, P<0.0001 vs M0). The same was true for AF as per Fibrotest: M0 0.67 (0.02) vs M6 0.56 (0.02, P=0.0001) and M12 0.54 (0.02, P=0.002 vsM0). Among AF-patients without M6 fibrosis-regression, 43% had baseline steatosis>5% as per Steatotest compared to 0% (p=0.04) in AF-patients that regressed fibrosis. As per AF-LSM no regression was observed vs M0 at M6 [8.5 (1) vs 10.1 (1) kPa, P=0.28] but at M12 [6.3 (0.4) kPa, P=0.009 vs M0]. M6 regressions of significant NIA and AF as per Actitest and Fibrotest were observed regardless the VR (vs non-VR) 32% vs 40% (p=0.30) and 38% vs 50% (p=0.74), respectively.
Conclusions: After six and twelve months of entecavir treatment, advanced fibrosis and activity as presumed by Fibrotest-Actitest were significantly reduced, regardless of the viral response. Fibrosis-regression as per liver stiffness measurement was observed only after twelve-month treatment. Patients without fibrosis-regression after 6-months treatment had more baseline steatosis.

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HEPATOCELLULAR CARCINOMA (HCC) RISK IN HBeAg-NEGATIVE CHRONIC HEPATITIS B (CHBe−) WITH OR WITHOUT CIRRHOSIS TREATED WITH ENTECAVIR: RESULTS OF THE NATIONALWIDE HepNet.Greece COHORT STUDY

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Background and Aims: HCC may still develop in patients starting with lamivudine, while the HCC rates in patients under newer antivirals are unclear. We estimated the incidence and evaluated predictors of HCC in a large nationwide prospective cohort of CHBe− patients treated with entecavir. The CHBe− patients from the same cohort (HepNet.Greece) who started lamivudine were used for comparison.

Methods: We included 321 adult CHBe− patients (age: 53±14 years, males: 69%) who were treated with entecavir for ≥12 months [antiviral(s)-naïve: 86%, lamivudine/advemovir exposure without resistance: 23%/1%] and had no HCC during the first 6 months. Men follow-up was 30±18 months. CHBe− without cirrhosis, compensated and decompensated cirrhosis were diagnosed in 75%, 20% and 5% of 281 patients (disease severity unclassified: 40%). Lamivudine group included 818 CHBe− patients selected by the same criteria (therapy ≥12 months and no HCC within first 6 months).

Results: In the entecavir group, HCC developed in 4/321 (1.2%) patients at a median of 1.5 (range: 1.0–4.5) years, while the cumulative HCC incidence was significantly higher in cirrhotic than non-cirrhotic patients (1–, 3–, 5-year: 0%, 3%, 17% vs 1%, 1%, 1%; P=0.024, log-rank). The probability of HCC under entecavir was also higher in older patients [HR per year: 1.10 (1.06–1.13), P=0.001], but it was not significantly associated with gender or baseline ALT and HBV-DNA. Entecavir compared to lamivudine group patients had similar age, gender and cirrhosis distribution, but significantly lower baseline ALT and HBV-DNA levels and shorter follow-up, as well as a trend for lower HCC incidence (1–, 3–, 5-year: 0.7%, 3.8%, 5.6% vs 0.3%, 1.1%, 4.8%; P=0.056, log-rank). However, in multivariable Cox regression analysis including all 1139 patients, the HCC risk was independently associated with older age [HR per year: 1.10 (1.06–1.13), P=0.001], male gender [HR: 2.88 (1.28–6.50), P=0.011] and cirrhosis [HR: 2.00 (1.10–3.66), P=0.024], but not with type of initial therapy.

Conclusions: Data from this large nationwide study indicate that the HCC risk remains increased in entecavir treated CHBe− patients with cirrhosis, particularly of older age. The HCC risk does not seem to be significantly reduced with entecavir compared to antiviral therapy starting with lamivudine, at least during the first 3 years of treatment.
EFFECTIVENESS OF TENOFOVIR FOR CHRONIC HEPATITIS B IN FIELD PRACTICE – 2 YEAR INTERIM RESULTS FROM THE PROSPECTIVE GERMAN MULTICENTER NON-INTERVENTIONAL STUDY (GEMINIS)


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Background and Aims: Tenofovir DF (TDF) represents in clinical studies a very effective and tolerable CHB treatment and is a recommended first-line therapy. Knowledge on long-term efficacy and safety in daily practice is yet still limited.

Methods: 400 monoinfected TDF-naïve CHB patients were prospectively enrolled into this observational study. 2 year data are reported here.

Results: At BL, mean age was 45 years, 69% male, 76% Caucasian, 11% cirrhotic, 69% HBeAg−, 46% treatment-naïve, 92% received TDF monotherapy, 14% had liver biopsies; comorbidities: diabetes (16%), arterial hypertension (14%), cardiovascular (11%). Median HBV-DNA at baseline was 6.9exp4 in naïve, 2.6exp2 in pretreated patients. Virologic response at 24 months (HBV-DNA <169 IU/ml): HBeAg+ patients ( naïve/pretreated) 88%/94% (BL: 12%/41%), HBeAg− patients 94%/97% (BL: 10%/52%), in ADV+LAM (n = 57) and in ETV (n = 41) pretreated patients 100%/96% (BL: 61%/46%), respectively. 20% with HBeAg seroconversion, HBsAg-loss in 5.1% of HBeAg+. No virologic breakthrough (confirmed >1log increase from nadir) and no resistance reported to date. 4 patients added another drug (naïved added LAM, LAM, ETV, 1 LAM/ETV-experienced added ETV), 7 patients switched to another drug (6 ETV, 1 LAM) all due to adverse reactions (fatigue, headache, nausea). TDF retention rate was 92%, >7 day TDF pause was documented in 24 patients, primarily for non-compliance (n = 7), stay abroad/vacation (n = 6). Mean±SD ALT [U/L] reduction BL-month 24: 78±121–36±22; ALT elevations in HBV-DNA-suppressed patients are more likely in obese (BM≥30 kg/m²). Serum CrCl (107±32–102±31 ml/min) and phosphorous (1.11±0.2–1.07±0.2 mmol/L) remained stable. No frequent AR reported. 4 renal events were detected, all in patients with prior long-term LAM +/- ADV therapy, and comorbidities: diabetes (2x), renal insufficiency (2x), cirrhosis (1x). Prior to the occurrence of these 4 AR, TDF had not been dosed according to recommendation. 5 pregnancies during TDF were reported, in 4 TDF was given in all trimesters and in 1 2nd/3rd, all newborns were HBsAg− and healthy.

Conclusions: After 2 years in routine practice TDF poses a very effective treatment for CHB. TDF profoundly suppressed HBV replication in the majority of naïve and pretreated patients, the safety profile was favorable. TDF should be dosed according to SnPC.

FROM COMBINATION-THERAPY TO MONO-THERAPY IN TREATMENT EXPERIENCED CHB PATIENTS WITH VIRAL RESISTANCE OR PARTIAL RESPONSES: FIRST RESULTS OF AN INTERNATIONAL MULTICENTER COHORT STUDY

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Background and Aim: Long-term complete viral suppression is a major goal to prevent disease progression in patients with HBV. Aim of this ongoing cohort study is to investigate the safety and efficacy of mono-therapy with entecavir (ETV) or tenofovir (TDF) following a long-term rescue combination-therapy with ETV plus TDF in 22 chronic hepatitis B (CHB) patients who were only partial responders or multidrug resistant.

Methods: Open label cohort study, investigator initiated, from 5 European centres. Patients were only included with suppressed viremia (LLoD <69 IU/ml) for >12 months during ETV plus TDF rescue combination treatment. ALT, HBV-DNA, qHBsAg were measured at baseline and every 3 months and resistance tests determined.

Results: 22 patients (15 HBeAg+), median age 48 years, 17 males, previously treated with a median of 5 lines of antiviral therapy (range 4–8), 8/22 (36%) with advanced liver disease, were included. Reason for switch from combination-therapy to mono-therapy was simplification in 21 cases and desire to have children in one case. Median ALT at baseline was 0.7 ULN (range 0.36–1.24). Median ETV plus TDF treatment duration was 31 months, median treatment duration of subsequent TDF mono-therapy (n = 19) was 23, for ETV (n = 3) 11 months, respectively. HBV-DNA remained suppressed during mono-therapy in 20 patients, in two patients there was a low level viremia detectable (maximum 265 IU/ml). One patient was on ETV with lamivudine experience, one cirrhotic patient on TDF, both with negative resistance testing. ALT levels remained stable in all patients, no hepatic flares occurred. The probability for a continuous HBV DNA suppression was not reduced in patients with adefovir or lamivudine resistance or in patients with advanced liver disease. One patient lost HBsAg after 10 months on TDF mono-therapy, one cirrhotic patient developed an HCC. Quantitative HBsAg levels were not significantly different from end of combination therapy and end of observation mono-therapy.

Conclusions: Mono-therapy with ETV or TDF after successful rescue combination therapy with ETV plus TDF in CHB patients harboring viral resistance patterns or showing only partial virologic responses to previous therapies was efficient, safe, and well tolerated in patients with or without advanced liver disease.

STOPPING LONG-TERM NUCLEOS(T)IDE ANALOGUE THERAPY BEFORE HBsAg LOSS IN HBeAg NEGATIVE CHB PATIENTS: FOLLOW-UP OF LONG TERM RESPONDERS


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Background and Aims: Long-term treatment with nucleos(t)ide analogues (NUC) is highly effective but HBsAg loss is a rare
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event in HBeAg-negative patients. Small pilot trials have challenged the question of sustained remission after discontinuation of long-term NUC-therapy in some patients. We recently reported on high relapse rates (72%) after treatment discontinuation in 32 HBeAg-negative patients (AASLD 2011). Here we report on the long-term outcome of patients without HBV relapse after stopping antiviral treatment after 37–80 months.

**Methods:** 9/32 patients without relapse were identified by retrospective data base search. These patients were prospectively followed (median 24 months).

**Results:** All patients were HBeAg-negative, 7/9 male, median age 43 years, genotype A or D. Three patients had received lamivudine, two adefovir, one telbivudine, and three entecavir. At stopping treatment all responder patients showed qHBsAg levels of <1000IU/ml, six lost HBsAg off therapy (at months 6, 9, 12, 14, 20, 28) and three of these developed anti-HBs (16, 18, 26 months after treatment termination). All nine patients showed an ongoing reduction of qHBsAg levels, demonstrated long-term normal or close to normal ALT-levels, with HBV-DNA ranging from undetectable levels to 6.9x10^7 log IU/ml. No patient displayed apparent liver disease progression by regular fibroscans, whereas a trend towards improvement (albeit not significant) could be detected.

**Conclusion:** Stopping long-term NUC therapy in HBeAg-negative CHB patients with non-advanced liver disease might be an option for patients with HBsAg titer <1000IU/ml. These patients develop a high rate of HBsAg loss off-therapy and suggest that most of these patients may have developed some degree of immunological control of HBV during and off-treatment. Better immunological characterization of CHB patients with an indication for antiviral treatment is urgently needed to identify predictive markers to stop long-term NUC-therapy in some patients.

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**SEQUENCE ANALYSIS OF THE HBV PreC/C REGION BY ULTRA-DEEP PYROSEQUENCING AS A PREDICTOR OF ADEFOVIR TREATMENT OUTCOME IN PATIENTS WITH HBeAg-POSITIVE CHRONIC HEPATITIS B**

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One of the main goals of HBV antiviral therapy in patients with HBeAg-positive chronic hepatitis B is the HBe seroconversion with sustained inhibition of viral replication. Whether and when such patients receiving nucleos(t)ide analogues can stop treatment without exposing the patient to a relapse is unknown. Thus, predictive markers are needed, both at baseline to evaluate the risk of treatment failure and during therapy to predict treatment success and evaluate the likelihood of a viral rebound at its withdrawal. We studied a cohort of 156 treatment-naive patients with chronic HBeAg-positive chronic hepatitis B treated with adefovir dipivoxil and followed for a maximum of 180 weeks. The sequence of the HBV PreC/C domain was characterized in sequential samples obtained from each patient before and during adefovir treatment. Sequence analysis was generated by means of ultra-deep pyrosequencing (UDPS) using Genome Sequencer FLX (Roche Molecular systems/454) analyzed by Pyropack® (in-house software). From 337 serial samples from the 156 patients, we generated 1.05 Mb of sequences. UDPS analysis was substantially more sensitive to characterize changes within the PreC/C region than the reverse hybridization-based Line Probe Assay, used for comparison in a subset of samples. Using UDPS data, we found no signature sequence at baseline that predicted the subsequent therapeutic outcome of the patients on adefovir therapy. Dynamic studies of the PreC/C region on treatment showed that amino-acid substitutions at positions 1762, 1764 and 1896 of the core promoter and preC regions, respectively, were the only ones associated with a lower probability of an HBe seroconversion. In conclusion, we used ultra-deep pyrosequencing, a highly sensitive new technology, to assess whether signature sequences in the PreC/C region could help tailor antiviral treatment of HBeAg-positive chronic hepatitis B. We did not identify PreC/C signature sequences with a sufficiently high predictive value on treatment outcome and do not recommend sequencing this region to guide clinical practice.

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**SERUM HEPATITIS B SURFACE ANTIGEN LEVELS DURING FIVE YEARS ENTECAVIR THERAPY IN ASIAN CHRONIC HEPATITIS B PATIENTS**

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**Background:** Change in hepatitis B surface antigen (HBsAg) levels during long-term nucleoside analogue therapy in chronic hepatitis B (CHB) has not been well investigated.

**Aim:** To determine the serologic, biochemical, virologic responses and resistance profile of continuous entecavir up to 5 years.

**Methods:** 222 (70.7% male, median age 45 years) CHB patients started on entecavir between July 2005 and November 2007 were recruited. The rates of HBV DNA detectability, hepatitis B e antigen (HBeAg) seroconversion, alanine aminotransferase (ALT) normalization, and entecavir signature mutations up to year 5 were determined. Serum HBV DNA and HBsAg levels were measured by Cobas Taqman and Elecsys II assay respectively. Resistance profile was determined by a line probe assay (LiPA) for patients with detectable HBV DNA.

**Results:** 222, 188, 173, 170 and 156 patients were followed up for 1, 2, 3, 4 and 5 years respectively. The rates of HBV DNA undetectability, HBeAg seroconversion for HBeAg-positive patients and ALT normalization (for patients with elevated baseline ALT) at year 5 were 97.4%, 55.7% and 91.4% respectively. 1 patient developed HBsAg seroclearance at year 2. 2 patients developed entecavir signature mutations at years 3 and 5 (rt180M, rt204V, rt180M and rt180M, rt204V, rt184I/L/F/M, rt202G, rt250V respectively), resulting in a cumulative resistance of 1.2% up to year 5. Among patients with 5 years of follow-up (n=156), the median annual HBsAg decline was 0.116 (range −0.176 to 0.980) log IU/mL/year with HBsAg-positive patients having a greater median HBsAg decline when compared to HBeAg-negative patients (0.141 and 0.097 log IU/mL/year respectively, p=0.021). Patients with significant HBsAg decline, defined as >0.25 log IU/mL/year (n=31), when compared to patients without significant HBsAg decline, was associated with younger median age (43.5 and 49.3 years respectively, p=0.014), HBeAg-positivity (54.8% and 34.7% respectively, p=0.039), and higher median baseline HBV DNA levels (7.99 and 6.22 log IU/mL respectively, p<0.001).

**Conclusion:** Entecavir for 5 years achieved excellent rates of virologic suppression and low rates of resistance. Serum HBsAg levels decreased gradually during treatment, with a higher rate of decline seen in younger HBeAg-positive patients.

Acknowledgement: This study was supported by an unrestricted grant from Bristol-Myers Squibb.
Cessation of nucleoside analogue therapy in hepatitis B e antigen-negative chronic hepatitis B (CHB) is controversial. It is uncertain whether serum hepatitis B surface antigen (HBsAg) levels can predict virologic kinetics after treatment cessation.

**Methods:** Entecavir was stopped in HBeAg-negative patients treated for at least 2 years with no co-existing or decompensated liver disease. All patients had undetectable HBV DNA levels on at least 3 separate occasions 6 months apart before treatment cessation. Serum HBsAg (Elecys II, lower limit of detection 0.05 IU/mL), HBV DNA (Cobas Taqman, lower limit of detection 20 IU/mL) and liver biochemistry were monitored at every 6–12 weeks for 1 year. Entecavir was restarted if virologic relapse, defined as HBV DNA >2,000 IU/mL occurred. The primary endpoint was sustained virologic remission, defined as HBV DNA persistently ≤200 IU/mL.

**Results:** 184 patients (median age 54 years, 67.9% male) were recruited. The median baseline HBsAg level was 892 (range 2.3–24,100) IU/mL. Median duration of entecavir therapy before cessation was 3.05 (range 2.02–5.95) years. At the time of writing, 158 (85.9%) and 104 (56.2%) patients have been followed up for 12 and 24 weeks respectively. The cumulative rates of virologic relapse, calculated using the Kaplan–Meier method, were 11.2% at week 12 and 78.1% at week 24 respectively. Among patients with 24 weeks of follow-up (n=104), patients achieving sustained virologic remission (n=9) had a significantly longer median duration of entecavir treatment before therapy cessation (4.05 and 3.03 years respectively, p=0.088), a higher probability of undetectable viremia at week 12 (88.9% and 17.9% respectively, p<0.001) and a trend towards a lower median baseline HBsAg level (701 and 936 IU/mL respectively, p=0.088).

**Conclusion:** Close monitoring of HBsAg levels helps classify flares during peginterferon therapy and predicts treatment response. Base on this interim analysis, a longer duration of prior nucleoside analogue therapy and off-treatment HBV DNA undetectability at week 12 were associated with a higher chance of sustained virologic remission after treatment cessation. Subsequent analysis would determine if baseline and off-treatment HBsAg levels could predict virologic remission after treatment cessation. Acknowledgement: This study was supported by an unrestricted grant from Roche Diagnostics.
**POSTERS**

**775** VIROLOGICAL RESPONSE DOES NOT LOWER LIVER DISEASE PROGRESSION AMONG CHRONIC HEPATITIS B CIRRHOTIC PATIENTS TREATED WITH LONG-TERM NUCLEOS(T)IDE-ANALOGUE

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**Background:** A large number of chronic hepatitis B patients with cirrhosis are on long-term nucleos(t)ide-analogue therapy. The impact of anti-viral treatment on liver disease progression among these cirrhotics remains to be characterized.

**Method:** CHB cirrhotics treated with long-term LAM-ADV combination therapy or ETV monotherapy in our center were enrolled and followed for outcomes. Liver disease progression was defined as liver decompensation, development of hepatocellular carcinoma and death. Cumulative probability of virological and clinical outcomes was evaluated by Kaplan–Meier analysis, log-rank test and Cox-regression analysis.

**Results:** A total of 264 cirrhotics (compensated disease 87.5%) fulfilled enrollment criteria, of which 143 and 121 were treated with LAM-ADV and ETV respectively. In LAM-ADV group, 57 (39.9%) were HBeAg-positive, mean HBV-DNA was 5.8 (5.5–6.1) log IU/mL, mean LAM-ADV duration was 41.4 (14.5–68.3) months and mean follow-up was 72.8 (31.5–114.1) months. In ETV group, 48 (39.7%) were HBeAg-positive, mean HBV-DNA was 4.4 (4.1–4.7) log IU/mL, mean ETV duration was 33.3 (15.2–51.4) months and mean follow-up was 45.7 (16.7–74.6) months. Among LAM-ADV group, cumulative probability of (a) virological response were 59.9%, 92.2%, 95.6% and (b) liver disease progression was 4.5%, 17.7%, 29.2%, at year 1, 3 and 5 respectively. Among ETV group, cumulative probability of (a) virological response was 74.5%, 94.8%, 100% and (b) liver disease progression was 7.8%, 12.5%, 17.7% at year 1, 3 and 5 respectively. The probability of liver disease progression was not influenced by virological response (p = 0.174). When stratified by treatment group, virological response again did not impact on the cumulative probability of liver disease progression (LAM-ADV group: p = 0.173 and ETV group: p = 0.433). Among virological responders, there was also no difference between LAM-ADV combination and ETV monotherapy group (p = 0.199). Cox-regression showed baseline decompensated disease status was a significant predictor of disease progression (HR 4.96; 95%CI 2.61–9.44; p < 0.01) whereas virological response had no impact (p = 0.196).

**Conclusion:** Among cirrhotics treated with long-term LAM-ADV combination therapy or ETV monotherapy, despite the excellent anti-viral efficacy, there was still significant probability of liver disease progression. LAM-ADV combination or ETV-monotherapy induced virological response did not lower the probability of liver disease progression in these patients.

**776** ESTABLISHMENT OF A POTENT ANTI-HBsAg RESPONSE AND DURABLE IMMUNLOGICAL CONTROL OF VIREMIA WITH SHORT TERM IMMUNOTHERAPY AFTER REP 9AC-INDUCED HBsAg SEROCLEARANCE IN CHRONIC HBV INFECTION

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**Background:** HBsAg is known to block cytokine responses and its persistent circulation during immunotherapy may block immune stimulation in patients with chronic HBV. REP 9AC is a nucleic acid-based polymer (NAP) that clears serum HBsAg by blocking HBV subviral particle formation and release. The ability of add-on immunotherapy after REP 9AC-induced HBsAg seroclearance to establish a permanent immunological control of chronic HBV infection was examined.

**Methods:** Add on immunotherapy to existing REP 9AC therapy was initiated in patients experiencing HBsAg seroclearance with persistent serum HBV DNA. Immunotherapy consisted of thymosin α1 (Zadaxin™) or pegylated interferon α-2a (Pegasys™). Virologic responses were monitored by measuring serum HBV DNA (Roche Cobas™), serum HBsAg and serum anti-HBs (Abbott Architect™).

**Results:** REP 9AC-induced HBsAg seroclearance was achieved in most patients with 8–16 weeks of treatment and was accompanied by the appearance of moderate free anti-HBs titers (50–60 mIU/mL) and reductions in serum HBV DNA (to 1000–3000 CPM). Addition of Zadaxin™ or Pegasys™ to REP 9AC therapy resulted in rapid and profound increases in anti-HBs titters after as little as 6 weeks of immunotherapy to titers comparable to or greater than those observed in healthy patients with a strong vaccine response. In 8 of 9 patients, all treatment was halted after 13–27 weeks of immunotherapy exposure. In these patients, anti HBs titters off-treatment are currently comparable to or exceed those observed in uninfected subjects with a strong HBV vaccine response up to 4 months off treatment. Additionally, serum HBV DNA is also suppressed or is declining in all of these patients off-treatment. Currently, serum HBV DNA levels have reached <500 cpm (2 patients), <150 cpm (1 patient) or <116 cpm (LLOQ) in the remaining 5 patients.

**Conclusions:** REP 9AC-induced HBsAg seroclearance appears to potentiate the immunostimulatory effects of either Zadaxin™ or Pegasys™ in all patients, suggesting that circulating HBsAg may play a direct role in blocking the stimulation of immune function by immunotherapy. Short term exposure to immunotherapy in the absence of HBsAg may induce permanent immunological control of HBV infection in most patients.

**777** INCIDENCE AND CLINICAL CONSEQUENCES OF REDUCED TUBULAR PHOSPHATE RE-ABSORPTION (TmPO4/GFR) IN NAÏVE CHRONIC HEPATITIS B PATIENTS TREATED WITH TENOFOVIR: A TWO-YEAR FIELD STUDY

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Reduced tubular re-absorption of phosphate, a specific and sensitive marker of kidney tubular damage, has been reported in HIV-positive patients receiving tenofovir (TDF)-containing regimens. However, the prevalence and incidence of this disorder among untreated and TDF-treated chronic hepatitis B (CHB) patients, are unknown.

**Methods:** 106 consecutive NUC-naïve CHB patients (55yr, 75% males, 80% HBeAg-negative, 30% cirrhics, serum creatinine 0.88 mg/dl, eGFR by MDRD 85 ml/min) with at least 6 months of TDF therapy were assessed for tubular phosphate re-absorption (TmPO4/GFR) by the Walton and Bijvoet normogram, and phosphate levels at baseline and every 3–4 months for 2 years. Phosphate <2.3 and <2.0 mg/dl identified grade 2 and 3 hypophosphatemia, respectively. TmPO4/GFR between 0.60–0.80 or <0.60 mmol/L, confirmed in two consecutive controls, identified grade 1 or grade 2 hypophosphataemia, respectively.

**Results:** Before treatment, 36 (34%) patients had a reduced TmPO4/GFR, which was already severe in 7 (7%) cases while 3 (2.5%) patients had grade 2 hypophosphatemia. During a median of 21 (range 6–41) months of TDF treatment, median TmPO4/GFR
declined from 0.89 to 0.79 mmol/L (p = 0.031) whereas phosphate levels remained unchanged (3.2 vs 3.1 mg/dL, p = 0.27). TmPO4/GFR significantly declined with the first 12 months and then stabilized. Six of the 29 (21%) patients with reduced baseline TmPO4/GFR progressed to grade 2 after a median of 3 months, whereas among the 70 patients with baseline normal TmPO4/GFR, 20 (29%) developed a reduced TmPO4/GFR (16 patients to grade 1, 4 patients to grade 2) after a median time of 6 months (range 3–18). Overall during TDF treatment, TmPO4/GFR remained unchanged in 73 (69%) patients, improved in 7 (7%) but worsened in 26 (24%). Among 103 patients with normal phosphate at baseline, 4 (4%) showed a decline of phosphate levels (grade 2 in 2 patients). Overall, 5 (5%) patients required TDF dose reduction, 4 because of eGFR decline (at months 3, 6, 9 and 19) whereas one for grade 2 TmPO4/GFR decline (<0.50 mmol/L).

Conclusions: Hypophosphatemia affects approximately one third of untreated patients and worsens in approximately one quarter of naïve patients treated with TDF, yet without causing significant hypophosphatemia or clinical consequences.

778 IMPROVED HBsAg LOSS RATE IN CHRONIC HEPATITIS B PATIENTS TREATED WITH PROLONGED PEGINFERON α-2a COMBINED WITH NUCLEOS(T)IDE ANALOGUE
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Aims: To investigate the improved HBsAg response in chronic hepatitis B (CHB) patients treated with prolonged peginterferon α-2a combined with nucleos(t)ide analogue.

Methods: One hundred and fifty-two CHB patients treated with peginterferon α-2a plus nucleos(t)ide analogue from Jan 2007 to Sep 2012 were retrospectively investigated. The mean treatment duration was 79 weeks (range 24–120 weeks). Serum HBsAg levels at baseline, week 12, 24, 36, 48, 72, 96 and 120 of treatment were detected.

Results: The cumulative HBsAg loss rates of patients treated at 48-week, 96-week and 120-week with peginterferon α-2a plus nucleos(t)ide analogue were 8.55% (13/152), 26.97% (41/152) and 29.61% (45/152), respectively. The time to HBsAg loss was 74.93±27.21 weeks. Most HBsAg loss occurred during week 49–96. The HBsAg level at week 12 of treatment in 107 patients who obtained HBsAg loss was 1.91±1.27 log10 IU/mL, which was significantly lower than baseline (3.22±0.82 log10 IU/mL) (t=5.759, P <0.01). However, the HBsAg level at baseline in 107 patients who did not obtain HBsAg loss was 3.57±0.96 log10 IU/mL and HBsAg levels reduction at week 12, 24, 48, 96 and 120 were 0.24±0.38, 0.45±0.60, 0.83±0.84, 1.29±1.05 and 1.66±1.66 log10 IU/mL, respectively (Figure 1). As expected, the HBsAg level at week 12 of treatment was not significantly different from that at baseline in this group of patients (t=1.529, P = 0.128), while that at week 96 was significantly decreased compared with week 48 (t=2.166, P = 0.033).

Conclusion: Prolonged peginterferon α-2a combined with nucleos(t)ide analogue treatment could increase the cumulative HBsAg loss rate. In patients who do not obtain HBsAg loss, prolonged therapy could reduce the HBsAg level and improve the anti-viral response. The reduction of HBsAg level of week 12 from baseline could be a predictor of HBsAg clearance.

779 EFFECTS OF HEPATITIS B VIRUS QUASISPECIES AND REVERSE TRANSCRIPTASE VARIANTS ON TREATMENT RESPONSIVENESS TO ENTECAVIR
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Background and Aims: Entecavir therapy reduces hepatitis B virus (HBV) DNA to an undetectable level in around 60–80% of patients after 1 year of treatment, but HBV DNA may remain detectable in the remaining patients. We aimed to determine whether baseline HBV reverse transcriptase (rt) sequence polymorphisms and quasispecies complexity and diversity are associated with the differences in treatment response.

Methods: Pre-treatment HBV rt sequence from 305 entecavir-treated patients were determined by DNA sequencing. Sequencing data were associated with their virological outcome, as defined by optimal response (undetectable HBV DNA at year 1) or partial response (HBV DNA >60 copies/mL). Quasispecies complexity and diversity were determined using MEGA 5.0 software.

Results: Seventeen rt variants were more frequently detected in the partial responders (n = 64; 21%) than in the optimal responders (all P <0.05). Multivariate analysis revealed that high baseline HBV DNA, hepatitis B e antigen (HBeAg)-positivity and rt124N were associated with partial entecavir response. Compared with the partial responders, the optimal responders had a higher quasispecies complexity at nucleotide and amino acid levels (P =0.036 and 0.087, respectively) and higher quasispecies diversity, as reflected by a greater genetic distance at both nucleotide and amino acid levels (P =0.019 and 0.032, respectively) and a greater number of synonymous substitutions per synonymous site (dS; P =0.015) and number of non-synonymous substitutions per non-synonymous site (dN; P =0.039).

Conclusions: High baseline HBV DNA, HBeAg-positivity and rt124N were associated with partial entecavir response at year 1. Baseline HBV quasispecies complexity and diversity were higher in the optimal responders than in the partial responders.

780 A CLINICAL STUDY ON ANTI-HBV-DC-MTL COMBINED WITH TELBIVUDINE IN THE HBeAg POSITIVE CHRONIC HEPATITIS B VIRUS INFECTION
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Background and Aims: To observe the clinical efficacy of anti-HBV-DC and anti-HBV-MTL (anti-HBV-DC-MTL), the dendritic cells (DC) and mixed T lymphocyte (MTL) originating from peripheral blood mononuclear cells (PBMC) sensitized by HBsAg, in combination
with telbivudine, in HBeAg positive chronic hepatitis B virus (HBV) infection.

**Methods:** 46 HBeAg positive chronic HBV infection including 34 males and 12 females aged 17–50 years were recruited in the study including 15 chronic HBV carriers, 16 chronic hepatitis B (CHB) patients with ALT>2ULN and 15 CHB patients with ALT>2ULN. PBMCs were obtained from 50ml of heparinized peripheral blood through density gradient centrifuge. Anti-HBV-DC was proliferated from adherence PBMCs under the induction by GM-CSF and IL-4, and sensitized with hepatitis B vaccine containing 50μg HBsAg. Anti-HBV-MTL was proliferated from no-adherence PBMCs under the induction by IL-2, IL-12 and anti-HBV-DC. Anti-HBV-DC was harvested on day 7 and injected, half hypodermically and half intravenously, to the patient once every two weeks for 12 times totally. Anti-HBV-MTL was harvested on day 14 and injected intravenously to the patient once every two weeks for 12 times totally. Telbivudine was taken 600mg daily. Quantitative HBV (TRFIA) and HBVDNA and hepatic functions were evaluated at week 0, 4, 12, and 24.

**Results:** Mean of HBsAg, HBeAg and HBVDNA decreased significantly, while mean of HBeAb increased obviously after therapy of 4, 12 and 24 weeks. At week 4, 12 and 24, HBeAg negative conversion rate were 36.36% (20/55), 40.00% (22/55) and 45.46% (25/55) respectively in all patients, HBeAg positive conversion rate were 25.46% (14/55), 36.36% (20/55) and 38.18% (21/55), HBeAg seroconversion rate were 21.82% (12/55), 34.56% (19/55) and 38.18% (21/55), HBVDNA negative conversion rate were 32.73% (18/55), 36.36% (20/55), and 56.36% (31/55), ALT normalization rate were 19.44% (7/36), 55.56% (20/36) and 72.22% (26/36), and HBeAg seroconversion rate was 10.53% (2/19), 15.79% (3/19) and 15.79% (3/19) in chronic HBV carriers, 22.22% (4/18), 33.33% (6/18) and 33.33% (6/18) in patients with ALT≤2ULN, and 33.33% (6/18), 55.56% (10/18) and 66.67% (12/18) in patients with ALT>2ULN. The rate of adverse effect was 3.33% observed in re-infusion of anti-HBV-DC vaccine.

**Conclusions:** Anti-HBV-DC vaccine in combination with lamivudine and thymosin-α1 can be considered as a safe and efficient approach for HBeAg positive patients, which may effectively inhibit the viral replication, lower rapidly HBsAg, HBeAg and HBVDNA, improve the production of HBeAb, and increase the HBeAg seroconversion rate.

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**A CLINICAL TRIAL ON ANTI-HBV-DC VACCINE COMBINED WITH LAMIVUDINE AND THYMOSIN-α1 IN THE HBEAG POSITIVE PATIENTS OF CHRONIC HEPATITIS B VIRUS INFECTION**

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**Background and Aims:** To observe the clinical efficacy of anti-HBV-DC vaccine, the dendritic cells (DC) originating from peripheral blood mononuclear cells (PBMC) sensitized by HBsAg, in combination with lamivudine and thymosin-α1, in HBeAg positive patients of chronic hepatitis B virus (HBV) infection.

**Methods:** 55 HBeAg positive patients including 40 males and 15 females aged 15–54 years were recruited in the study including 19 chronic HBV carriers, 18 chronic hepatitis B (CHB) patients with ALT>2ULN and 18 CHB patients with ALT>2ULN. PBMCs obtained from 50 ml of heparinized peripheral blood through density gradient centrifuge and adherence method were proliferated under the induction by GM-CSF and IL-4, and sensitized with the stock of hepatitis B vaccine containing 30μg HBsAg on day 5 and with hepatitis B vaccine commercially available containing 20μg HBsAg on day 6. anti-HBV-DC vaccine was harvested on day 7 and injected, half hypodermically and half intravenously, to the patient once every two weeks for 12 practices applications totally. Lamivudine was taken 100 mg daily, and thymosin-α1 1.6 mg was injected hypodermically twice a week. Quantitative HBV (TRFIA) and HBVDNA and hepatic functions were evaluated at week 0, 4, 12, and 24.

**Results:** Mean of HBsAg, HBeAg and HBVDNA decreased significantly, while mean of HBeAb increased obviously after therapy of 4, 12 and 24 weeks. At week 4, 12 and 24, HBeAg negative conversion rate were 36.36% (20/55), 40.00% (22/55) and 45.46% (25/55) respectively in all patients, HBeAg positive conversion rate were 25.46% (14/55), 36.36% (20/55) and 38.18% (21/55), HBeAg seroconversion rate were 21.82% (12/55), 34.56% (19/55) and 38.18% (21/55), HBVDNA negative conversion rate were 32.73% (18/55), 36.36% (20/55), and 56.36% (31/55), ALT normalization rate were 19.44% (7/36), 55.56% (20/36) and 72.22% (26/36), and HBeAg seroconversion rate was 10.53% (2/19), 15.79% (3/19) and 15.79% (3/19) in chronic HBV carriers, 22.22% (4/18), 33.33% (6/18) and 33.33% (6/18) in patients with ALT≤2ULN, and 33.33% (6/18), 55.56% (10/18) and 66.67% (12/18) in patients with ALT>2ULN. The rate of adverse effect was 3.33% observed in re-infusion of anti-HBV-DC vaccine.

**Conclusions:** Anti-HBV-DC vaccine in combination with lamivudine and thymosin-α1 can be considered as a safe and efficient approach for HBeAg positive patients, which may effectively inhibit the viral replication, lower rapidly HBsAg, HBeAg and HBVDNA, improve the production of HBeAb, and increase the HBeAg seroconversion rate.

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**THE aa15–17 AMINO ACID SEQUENCE IN THE TERMINAL PROTEIN DOMAIN OF HBV POLYMERASE AS A VIRAL FACTOR AFFECTING IN VIVO REPLICATION ACTIVITY OF THE VIRUS**

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**Aims:** The viral factors affecting sustained response after discontinuation of treatment with nucleoside analogs in patients with viral hepatitis B are uncertain. Thus, the amino acid sequences responsible for the replication activity of HBV were evaluated in vivo.

**Methods:** The subjects were 203 patients with HBV infection who had been treated with nucleoside analogs. Therapy was discontinued when the following criteria were fulfilled in the patients: HBe antigen-negative and serum HBV DNA titer <2.1 Log copies/mL for at least 1 year, with core-related antigen titer <3.0 Log IU/mL. Serum HBV-DNA levels were measured every 2 or 4 weeks for 48 weeks following the treatment discontinuation, and the full amino acid sequences of the HBV detected in the patients' sera were compared in relation to the rate of increase of the serum HBV-DNA levels following the treatment discontinuation.

**Results:** A total of 34 patients (16.7%) fulfilled the criteria for treatment discontinuation, and the serum HBV-DNA titers increased to more than 4.0 Log copies/mL in 26 of these patients (76.5%); within 4 weeks in 5 patients (group A), between 4 and 12 weeks in 13 patients (group B), and later than 12 weeks in 8 patients (group C). The amino acid sequences of HBV were evaluated in 22 of these patients, and were found to differ among the 3 groups, especially in the terminal protein domain of polymerase; mutations from acidic to neutral amino acids between aa15 and aa17 showing DDE motifs were absent in group A, while these were present in 1 of 12 patients in group B, and 5 of 6 patients in group C.
Conclusion: Amino acid sequences between aa15 and aa17 in the terminal protein domain of polymerase may affect the in vivo replication activity of HBV.

783 EARLY SERUM HEPATITIS B VIRUS LARGE SURFACE PROTEIN LEVEL: A STRONG PREDICTOR OF VIROLOGICAL RESPONSE FOR PEGIFN, BUT NOT FOR ENTECAVIR IN HBeAg-POSITIVE CHRONIC HEPATITIS B

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Background and Aims: Quantitative hepatitis B surface antigen (HBsAg) is emerging as useful tools for predicting antiviral effect, but the detection includes all three forms of circulating particles in the serum. The study aims to evaluate the usefulness of serum hepatitis B virus large surface protein (LHBs) levels for predicting antiviral treatment effect.

Methods: A total of 62 hepatitis B e antigen (HBeAg) positive patients were enrolled: 21 patients received peginterferon (Peg-IFN) treatment and 41 patients received entecavir (ETV) treatment. Quantification of LHBs, HBsAg and HBVDNA was carried out at baseline and during antiviral therapy (weeks 4, 12, 24, 36 and 48). Virological response (VR) was defined as an undetectable HBVDNA level (<1000 copies/mL) after 48 weeks of therapy. Patients who exhibited HBeAg loss or seroconversion at week 48 were regarded as serological response (SR).

Results: The serum LHBs concentration positively correlated with HBVDNA and HBsAg (r=0.635 and 0.588, respectively). During week 48 of Peg-IFN and ETV therapy, LHBs and HBVDNA level decreased significantly in a biphasic manner and HBsAg level tended to decrease slowly. In Peg-IFN group, the cutoff of 88.46 U/mL in serum LHBs at week 4 gave the best area under the receiver operating characteristic curve (AUC = 0.96) with sensitivity, specificity, positive predictive value and negative predictive value of 100%, 85.7%, 88.9% and 100% in association with VR. The predictive model incorporating LHBs, HBsAg and HBVDNA can discriminate VR at baseline (AUC = 0.79) and show association with SR at week 12 (AUC = 0.80).

Conclusions: On-treatment quantification of serum LHBs could be a useful parameter for predicting VR in patients with Peg-IFN. Combining LHBs, HBsAg and HBVDNA can predict VR and SR more effectively and earlier.

08c. VIRAL HEPATITIS C: CLINICAL (THERAPY)

784 VIRAL KINETICS MODELING TO PREDICT cEVR AND AID IN DOSE SELECTION DECISIONS FOR PHASE-2/3 CLINICAL TRIALS

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Background and Aims: Sovaprevir is a potent, linear, non-covalent, and specific HCV NS3 protease inhibitor. Chronically-infected HCV GT-1 subjects are currently being treated with sovaprevir-based combination therapies. To help with dose selection in phase-2/3 clinical trials, viral kinetics modeling and simulations methodology was developed for predicting cEVR (<10 IU/mL). These predictions were made by modeling the RNA decay observed after monotherapy with sovaprevir or IFN and using the estimated viral kinetics parameters in simulations. The cEVR was also predicted for sovaprevir doses that had not been explored during monotherapy trials. Presented here are the observed and predicted cEVR for sovaprevir and P/R combination therapy.

Methods: RNA decay data after monotherapy with sovaprevir was modeled as previously described (Science 1998, 282, 103). Estimated viral kinetics parameters were then analyzed to determine their value range and probability distribution patterns in the patient population. For sovaprevir doses unexplored during monotherapy, but used in combination therapy, drug efficiency was estimated by correlating C_\text{min} observed with different doses and the drug efficiency of 400 mg QD sovaprevir (0.5918±0.0122). At the patient population was generated in silico, and viral kinetics parameters were randomly assigned while staying consistent with the observed probability distribution patterns and value ranges. Antiviral efficiencies of sovaprevir and P/R were assumed to be additive. Subsequently, incidence of wild type and sovaprevir resistant mutants observed in vitro were estimated after 12 weeks of therapy.

Results: Drug efficiency estimates of 200 mg and 800 mg QD sovaprevir were 0.9884±0.0088 and 0.9978±0.0020, respectively. Predicted cEVR in the per-protocol population after 12 weeks of therapy with P/R in combination with 200 mg, 400 mg or 800 mg QD sovaprevir was 97.6 (±0.5)% , 97.7 (±0.4)% and 99.8 (±0.2) as compared to observed 100%, 94% and 100%, respectively.

Conclusions: Methodology development was successful for predicting cEVR in HCV GT-1 infected subjects after combination treatment with sovaprevir and P/R; and, the predicted and observed cEVR’s with 200 mg or 400 mg or 800 mg sovaprevir in combination with P/R were comparable. Such predictions can potentially aid with future dose selection of direct acting antivirals for phase-2/3 clinical trials.

785 CONTINUED LOW UPTAKE OF TREATMENT FOR HCV IN A LARGE COMMUNITY-BASED COHORT OF INNER CITY RESIDENTS

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Background and Aims: Despite advances in HCV therapy, treatment uptake remains low, particularly among people who inject drugs. The aim of this study was to evaluate HCV treatment uptake and associated factors in a large community-based inner city cohort in Vancouver, Canada.

Methods: The Community Health and Safety Evaluation (CHASE) study is a cohort study of inner city residents recruited from January 2003 to June 2004. HCV status and information on prescriptions for HCV treatment were retrospectively and prospectively determined through data linkages with provincial virology (January 1992 to December 2009) and pharmacy databases (January 2003 to March 2010). Logistic regression analyses were used to identify factors associated with HCV treatment uptake.

Results: Among 2,913 participants, HCV antibody testing was performed in 2,405 and 64% were HCV antibody-positive. Individuals with spontaneous clearance (18%, n=277) were excluded from further analyses of HCV treatment uptake. Among the remaining 1,236 participants (mean age 42), 71% were male and 25% of Aboriginal ethnicity. The majority reported injecting
(60%) and non-injecting drug use (87%) in the six months prior to cohort enrolment. The most commonly reported injecting and non-injecting drugs were cocaine (82%) and crack cocaine (82%), respectively. Overall, 6% of HCV antibody-positive individuals (77 of 1,256) initiated treatment for HCV infection between January 2003 and March 2010. HCV treatment uptake increased from 0.2 (95% CI 0.0, 0.7) per 100 person-years in 2003 to 1.6 (95% CI 0.9, 2.6, \( P = 0.045 \)) per 100 person-years in 2009 (Figure). In adjusted logistic regression analyses, Aboriginal ethnicity [adjusted odds ratio (AOR) 0.23; 95% CI 0.10, 0.51] and crack cocaine use (AOR 0.61; 95% CI 0.37, 0.99) were associated with a decreased odds of receiving HCV treatment, while methamphetamine injecting (AOR 0.16; 95% CI 0.02, 1.18) trended towards a lower odds of receiving HCV treatment.

**Conclusion:** Although HCV treatment uptake has increased over time, there are still extremely low rates of treatment initiation in this large community-based cohort of inner city residents with a high prevalence of HCV infection and access to universal healthcare.

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**Hepatitis C triple therapy induced anemia. Is treatment with erythropoietin justified?**


**Introduction:** Chronic Hepatitis C (CHC) triple therapy including protease inhibitors (PIs) increases the incidence of anemia, leukopenia and thrombocytopenia compared to pegylated interferon and ribavirin therapy. Administration of erythropoietin (EPO), ribavirin dose reductions and blood transfusion are some therapeutic tools used to alleviate the anemia. The aim of this study is to analyze variations in endogenous levels of EPO during CHC treatment with PIs in relation to hemorrhoglobin concentration (Hb) and blood cell counts in order to estimate the usefulness of exogenous EPO administration.

**Material and Methods:** 24 CHC patients were treated with PIs, 13 with Telaprevir and 11 with Boceprevir. Routine clinical variables (Hb, white blood cells, neutrophils and platelets) and EPO plasma concentration (determined by ELISA) were analyzed during triple therapy (0, 4 and 12 weeks in Telaprevir and 0, 4, 8 and 12 weeks in Boceprevir). The results were statistically analyzed using Wilcoxon test, logistic regression and AUC-ROC analyses.

**Results:** While baseline Hb levels (15.8 g/dL) decreased progressively and significantly at 4, 8 and 12 weeks of treatment (12.5, \( p < 0.001 \); 12.4, \( p < 0.005 \), and 10.9, \( p < 0.001 \), respectively), endogenous EPO levels increased significantly compared to baseline (7.9 mIU/mL): 47.05, \( p < 0.001 \); 77.78, \( p < 0.001 \) and 87.31, \( p < 0.005 \) respectively). Interestingly, baseline Hb concentration was a predictor of early anemia (Hb <12.5 g/dL after 4 weeks of PI therapy, AUC-ROC>0.75) while baseline EPO levels were a predictor of leukopenia (WBC <3 miles/mm3) and thrombocytopenia (platelets <120 miles/mm3) at week 4 of treatment with PIs (AUC-ROC>0.75, in both cases).

**Conclusions:** During the treatment of CHC with PIs, increased levels of endogenous EPO (>5-fold from the baseline) do not imply increases in Hb. Considering the adverse effects of exogenous EPO, its cost, and its unclear benefit in the light of our findings, routine use of exogenous EPO in triple therapy induced anemia may be unwarranted. In addition, the remarkable accuracy of baseline Hb and endogenous EPO levels as predictors of early (within 4 weeks of PIs treatment) anemia, leucopenia and thrombocytopenia in these patients may be useful prognostic markers of adverse hematologic reactions associated with triple therapy.

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**786 PREVALENCE OF PERSONALITY DISORDER IN SPANISH PENITENTIARY POPULATION. SUBANALYSIS OF PERSEO STUDY**


**Aim:** To evaluate the prevalence of personality disorders (PD) in prison inmates starting treatment for chronic hepatitis C (CHC).

**Methods:** A prospective, multicenter epidemiological study in 25 Spanish prisons. Prevalence of PD in inmates starting treatment for CHC with peginterferon alfa-2a (Pegasys®) plus ribavirin in 2011 was studied. The Personality Diagnostic Questionnaire-4+ (PDQ-4+) was used for diagnosis of PD. It consists of a self-administered questionnaire and a short interview of clinical significance and is an instrument validated in Spain for diagnosis PD according to DSM-IV criteria. The PDQ-4+ was administered before starting treatment for CHC. Predictor factors for developing PD were assessed by collecting sociodemographic (age, sex, time since first admission in prison) and epidemiological (drug use, intravenous drug use [IVDU] and HIV co-infection, HCV diagnosis date, fibrosis grade, viral load [VL] in IU/mL and HCV genotype) variables.

**Results:** Of the 257 patients included, 195 (75.9%) were studied because the PDQ-4+ was incomplete or invalid in 62 cases. There were no statistically significant differences between patients with and without an evaluable PDQ-4+.

Mean age was 40 years, 92.3% men, 87.1% Caucasian, 79.1% IVDU, and 25.3% HIV-coinfected. Among coinfected subjects, there were more cases of IVDU (95.2% vs 73.7%; \( p = 0.001 \)), longer IVDU (23.6
Prevalence of PD was 72.3% (95% CI: 66–78.6%) with the following distribution: 36.9% in group A, 55.9% in group B and 32.3% in group C. By individual disorders, the most prevalent were antisocial (46.7%), borderline (30.8%) and paranoid disorder (29.2%). 51.3% had more than one PD. There were no statistically significant differences between patients with and without PD according to age, sex, HIV coinfection, drug use or IVDU, but differences were found according to genotype (lower prevalence of PD in genotype 4; 56.8% vs 74.7%; \( p = 0.03 \)).

Conclusion: The prevalence of PD in prison inmates starting treatment for CHC with peginterferon alfa-2a plus ribavirin is very high. The most frequently observed PD were antisocial, borderline and paranoid disorder.

788 RAPID VIRAL RESPONSE IN PRISON INMATES TREATED FOR CHRONIC HEPATITIS DUE TO HCV WITH PEGINTERFERON alfa-2a PLUS RIBAVIRIN. SUBANALYSIS OF PERSEO STUDY


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Aim: To study RVR in prison inmates treated for chronic hepatitis due to HCV.

Methods: A prospective multicenter study in 25 Spanish prisons in 2011. RVR was studied in inmates treated with peginterferon alfa-2a plus ribavirin. HCV viral load (VL) in IU/mL was described at treatment week 4 in log10 units versus baseline. Complete RVR (CRVR) was defined as absence of HCV VL at treatment week 4±1 and partial RVR (PRVR) was considered as a ≥1 log reduction in HCV VL versus baseline VL. Variables: age, sex, alcohol consumption, IVDU, HIV coinfection, baseline HCV VL, genotype, and personality disorder. HCV VL was considered high if >400,000 IU/mL. To evaluate variables associated with RVR, a univariate and multivariate analysis were performed using logistic regression, calculating the adjusted odds ratio with 95% confidence intervals.

Results: A sample of 227 patients was analyzed with baseline HCV VL determination in the last year and quantification of HCV-RNA available at treatment week 4. Mean age was 40 years, 92.5% men, 80.6% with history of IVDU, 26.4% HIV-coinfected and 56.4% had PD. High baseline HCV VL in 61.7%, 42.7% genotype 1 and 16.3% genotype 4. The mean reduction in VL was 4 log. VL decreased 5 log in monoinfected patients genotype 2–3 and between 3 and 3.5 log in coinfected or monoinfected patients with genotypes 1–4 (\( p < 0.001 \)). A RVR was obtained in 89.4% (53.7% CRVR and 35.7% PRVR). CRVR was more frequent in genotypes 2–3 (83.9%) and less frequent in 1–4, particularly if baseline VL was high. CRVR was associated with:

\begin{itemize}
  \item a. age [higher in age less than 30 years; \( p = 0.006; \) AOR: 15.0];
  \item b. genotype [higher in genotypes 2–3 (\( p < 0.001; \) AOR: 14.1)];
  \item c. HIV [higher in monoinfected (\( p < 0.001; \) AOR: 4.2)];
  \item d. IVDU [higher if no IVDU in last year (\( p = 0.004; \) AOR: 14.5)]; and
  \item e. baseline VL [higher if VL not elevated (\( p = 0.046; \) AOR: 2.1)].
\end{itemize}

Considering genotype 3 versus all others, it was observed that non-3 genotypes had a 7.7 higher risk of not achieving a CRVR or PRVR.

Conclusion: The RVR obtained in prison inmates treated with peginterferon alfa-2a plus ribavirin was very high, particularly in patients with genotype 3.

789 SERUM INTERLEUKIN-6 LEVELS DURING TREATMENT CORRELATE WITH RESISTANCE TO TELAPREVRIBASED TRIPLE THERAPY IN CHRONIC HEPATITIS C

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Background: Interleukin-6 (IL-6), a pleiotropic cytokine, is elevated in various types of chronic liver disease, and was reported to be associated with hepatocarcinogenesis, insulin resistance and iron metabolism. To determine whether IL-6 affects response to the newest antiviral treatment, we investigated serum IL-6 dynamics during telaprevir (TVR)-based triple therapy and final virological outcome.

Patients and Methods: We analyzed 172 chronic hepatitis C (CHC) patients who were treated with TVR-based triple therapy. Clinical and laboratory parameters including genetic variations near the IL28B gene (rs8099917) were assessed. Drug adherence was monitored in each patient and calculated as percentages of actual total dose administered of a standard total dose. We analyzed correlation of these parameters and treatment outcomes using univariate and multivariate analyses. Serum IL-6 levels were measured at baseline and at day1, 2 and 4, and 1, 2, 4, 8, 12, 16 and 20 weeks, and the end of treatment. Moreover, we investigated IL-6 and its signaling in differences to anti-viral response between IFN-resistance JFH1 and IFN-sensitive HCV-2b/JFH1 chimeric virus by using HCV cell culture system.

Results: Rapid viral response (RVR) rate was 79%, and sustained viral response (SVR) rate was 80%. Univariate analyses showed that the treatment response was significantly correlated with IL28B polymorphism as well as gender, fibrosis, pre-treatment viral load and serum creatinine. We compared time-dependent changes of serum IL-6 levels between patients with SVR and those without SVR, and found that on-treatment serum IL-6 levels remained significantly lower after temporary elevation in patients with SVR than those without. In contrast, increased IL-6 levels (∼2-folds) were related to the resistance to TVR-based triple therapy. In vitro, expression levels of SOCS3 and its inducer, IL-6 in IFN-resistant cells were higher than those in IFN-sensitive cells. Expression levels of endoplasmic reticulum stress proteins were also significantly higher in IFN-resistant cells.

Conclusion: Our results suggest that serum IL-6 is correlated to treatment resistance to TVR-based triple therapy, and that the IFN resistance may be induced by IL-6-induced up-regulation of SOCS3. IL-6 and related signaling system may provide new targets for difficult-to-treat CHC patients and prevention of hepatocarcinogenesis.
**Hepatitis C Treatment Outcomes and Predictors of Response in Treatment-Naive Patients Treated with Peginterferon alfa/Ribavirin in Real-World Italian Clinics Compared With Other Countries: PROPHESYS Sub-Analysis**

**Background**: PROPHESYS was a large, non-interventional, multinational cohort study of patients treated for chronic hepatitis C, with three cohorts in 19 countries. 22.4% of patients (n = 1604) were from Italian centers, allowing comparison of real-life practice and treatment outcomes in Italy vs. other countries.

**Methods**: PROPHESYS 1 included patients prescribed PegIFN alfa-2a/RBV, while patients in PROPHESYS 2 and 3 received either PegIFN alfa-2a or PegIFNα-2b/RBV. 1604 Italian HCV mono-infected, treatment-naive patients enrolled in PROPHESYS 2 were compared with 5559 patients from the other 18 countries enrolled in PROPHESYS 1–3. Analysis populations were (a) all patients treated and (b) those with sufficient follow-up data (i.e., excluding patients with HCVRNA <50 IU/mL at end of treatment [EOT], without HCVRNA test result showing relapse and missing HCVRNA test ≥140 days after EOT for reasons not related to efficacy/safety).

**Results**: Compared with other Italian patients, more Italian patients were White/Caucasian, had a lower incidence of cirrhosis and used fewer concomitant medications (Table). There were no substantial differences between Italy and other countries in premature withdrawals from treatment due to safety/efficacy/other reasons (28.6% vs. 30.8%) or dose modifications of PegIFN (13.0% vs. 11.6%) and RBV (14.6% vs. 14.3%) due to pre-specified AEs/laboratory abnormalities. On-treatment and sustained virological response (SVR24) rates tended to be higher in Italy, especially in G2 and 3 patients (Table).

**Conclusions**: In Italian patients from the PROPHESYS study, week 2 and 4 virological responses were highly predictive of SVR. SVR24 rates in Italian G2/3 patients were higher compared with patients from other countries, which can be partly explained by differences in disease characteristics.

**Acknowledgement**: Funded by Roche SpA

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**Table (abstract 790)**

<table>
<thead>
<tr>
<th></th>
<th>Italy (PROPHESYS 2), n = 1604*</th>
<th>Other countries (PROPHESYS 1–3), N = 5559*</th>
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<tbody>
<tr>
<td></td>
<td>G1 (n = 777)</td>
<td>G2 (n = 478)</td>
</tr>
<tr>
<td>Male, %</td>
<td>52.0</td>
<td>47.3</td>
</tr>
<tr>
<td>Age, years, median (range)</td>
<td>50.0 (19–76)</td>
<td>57.0 (18–77)</td>
</tr>
<tr>
<td>White/Caucasian, %</td>
<td>98.8</td>
<td>99.2</td>
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<tr>
<td>Liver fibrosis, %</td>
<td></td>
<td></td>
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<tr>
<td>Bridging fibrosis/cirrhosis</td>
<td>11.8</td>
<td>8.2</td>
</tr>
<tr>
<td>No cirrhosis</td>
<td>51.7</td>
<td>43.5</td>
</tr>
<tr>
<td>Not assessed/missing</td>
<td>36.4</td>
<td>48.3</td>
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<tr>
<td>Concomitant medications, %</td>
<td>54.7</td>
<td>47.5</td>
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<tr>
<td>Virological response, %</td>
<td></td>
<td></td>
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<tr>
<td>Week 2†</td>
<td>5.5</td>
<td>23.8</td>
</tr>
<tr>
<td>Week 4 (RVR)</td>
<td>27.8</td>
<td>86.8</td>
</tr>
<tr>
<td>End of treatment</td>
<td>60.5</td>
<td>92.9</td>
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<tr>
<td>SVR24†</td>
<td>44.9</td>
<td>81.4</td>
</tr>
<tr>
<td>SVR24 in pts w/bridging fibrosis/cirrhosis, %</td>
<td>33.7 (n = 92)</td>
<td>71.8 (n = 39)</td>
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<tr>
<td>Patients w/ sufficient follow-up data</td>
<td></td>
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<tr>
<td>SVR24</td>
<td>48.2</td>
<td>84.7</td>
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<tr>
<td>PPV of wk 2 for SVR24†, %</td>
<td>91.7</td>
<td>91.1</td>
</tr>
<tr>
<td>PPV of RVR for SVR24, %</td>
<td>84.8</td>
<td>89.7</td>
</tr>
</tbody>
</table>

*Includes patients with G1–6 and unknown genotype. †Not all study sites included a week 2 measurement.

†Patients with incomplete follow-up data were imputed as treatment failures.
Table 1 (abstract 791). SVR according to on treatment virologic response at TW4 (≥1 log drop) and TW8 through EOT (undetectable)

<table>
<thead>
<tr>
<th></th>
<th>TW4</th>
<th>TW8</th>
<th>TW12</th>
<th>EOT</th>
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<tbody>
<tr>
<td></td>
<td>BOC RGT</td>
<td>BOC+PR</td>
<td>BOC RGT</td>
<td>BOC+PR</td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virologic response</td>
<td>73% (77/105)</td>
<td>83% (85/103)</td>
<td>53% (56/105)</td>
<td>62% (64/103)</td>
</tr>
<tr>
<td>SVR</td>
<td>79% (61/77)</td>
<td>84% (71/85)</td>
<td>89% (50/56)</td>
<td>88% (56/64)</td>
</tr>
<tr>
<td>Previously untreated patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virologic response</td>
<td>68% (252/368)</td>
<td>69% (254/366)</td>
<td>57% (208/366)</td>
<td>56% (204/366)</td>
</tr>
<tr>
<td>SVR</td>
<td>81% (203/252)</td>
<td>79% (200/254)</td>
<td>88% (184/208)</td>
<td>90% (184/204)</td>
</tr>
</tbody>
</table>

Results: Baseline characteristics were generally comparable between groups; however, more relapse patients than previously untreated patients had METAVIR F3/F4 fibrosis (18%–22% vs 9%–11%). Among patients receiving BOC RGT, similar proportions of previously untreated and relapse patients had ≥1 log decline at week 4, undetectable HCV-RNA at week 8, week 12 and at the end of treatment (EOT) (Table 1). SVR was 63% and 69% in previously untreated and relapse patients, respectively. Virologic response rates at week 4, week 8, week 12, and at EOT were also similar between previously untreated and relapse patients receiving BOC + PR. The SVR was 66% and 75%, respectively, in previously untreated and relapse patients receiving BOC + PR. Rates of SVR according to on-treatment response were similar in previously untreated and relapse patients, regardless of BOC regimen (Table 1).

Conclusion: BOC + PR and BOC RGT are effective treatment regimens, achieving comparable rates of virologic response among previously untreated patients and those with relapse following previous PR.

Background: The N-CORE study showed that extending treatment with peginterferon alfa-2a (40KD) plus ribavirin (PegIFN alfa-2a/RBV) from 24 to 48 weeks increases sustained virological response (SVR24) rates and decreases relapse rates among HCV G2/3 patients who have detectable viraemia at week 4 of treatment and adhered to study procedures. This sub-analysis explored the benefit of treatment extension across key patient subgroups.

Methods: N-CORE is a multicentre, randomised, open-label study. HCV G2/3 patients treated with PegIFN alfa-2a/RBV who did not achieve a rapid virological response (RVR) at week 4 but did achieve an early virological response at week 12 (HCV RNA <15 IU/mL or ≤2-log_{10} drop) were randomised at week 24 to either stop treatment at week 24 or continue treatment to week 48. This analysis includes data from the following populations: intent-to-treat (ITT; all patients randomised) and the study completer (SC; patients who completed treatment and had [a] 24 weeks' follow-up with evaluable HCV RNA or [b] detectable HCV RNA at any time post-treatment). Information on IL28B genotype was not collected as part of this study.

Results: SVR24 rates were higher with 48 weeks of treatment for the SC population compared with 24 weeks of treatment (p = 0.0231), but not in the ITT population (p > 0.05). Relapse rates were lower with 48 weeks of treatment (22%) compared with 24 weeks of treatment (41%) for the SC population (p = 0.0216). Within each individual arm not all patient subgroups showed the typical pattern of baseline predictors of SVR. This may reflect the fact that only non-RVR patients entered the randomised treatment period. In both analysis populations, across various subgroups, SVR24 rates were higher in non-RVR patients randomised to 48 weeks versus 24 weeks (Table).

Conclusion: Extension of treatment with PegIFN alfa-2a/RBV from 24 to 48 weeks increased SVR24 rates in G2/3 patients who have detectable viraemia at week 4 of treatment across various patient subgroups, particularly those with advanced fibrosis.

Acknowledgement: Funded by F. Hoffmann-La Roche Ltd.
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REAL-LIFE DATA OF TELAPREVIR-BASED TRIPLE-THERAPY IN PATIENTS WITH CHRONIC HEPATITIS C GT1 IN GERMANY – AN INTERIM ANALYSIS


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Background and Aims: Telaprevir (TVR)-based triple therapy in patients (pts) with chronic Hepatitis C (HCV) in daily practice in Germany is investigated in this non-interventional study to evaluate the implementation of futility rules, response-guided therapy, safety and efficiency.

Methods: Prospective study, investigating TVR-based therapy in therapy-naïve and pretreated pts with chronic HCV GT 1. Data from the first 400 pts starting treatment (50% of the planned total) at 67 sites, followed up for a maximum of 24 weeks (W) of treatment.

Results: 70.3% of pts were pretreated (41.3% prior relapser, 32.7% non-responder) and 16% of pts reported cirrhosis at baseline (BL). 42.0% of pts had HCV RNA levels <8000 IU/mL at BL. 78.9% of pts (79.4% therapy-naïve; 78.3% pretreated) showed rapid virological response (RVR). Adherence to futility rules (stop if HCV-RNA >1000 IU/mL at W4) was found in 5 of 7 pts: two pts continued treatment until W12. At W12, 90.6% of pts had undetectable HCV RNA. 73.3% of therapy-naïve pts and 76.0% of pretreated pts were HCV-RNA negative at both W4 and W12. 12 pts (3.0%) with RVR at W4 suffered a breakthrough. Most pts (86.8%) had adverse events (AE) during the first 12W, 11.5% serious adverse events (SAE). AEs were mostly mild (62.0%) or moderate (35.1%), including rash (32.2%, mostly rated as mild or moderate) and anemia (30.1%). HB decrease <12g/dl (female) or <13g/dl (male) was reported in 88.5% of pts. Mean HB levels decreased from 14.3g/dl at BL to 10.4g/dl at W12; HB levels <8.5g/dl within the first 24W of treatment were present in 19.6% of anemia cases and 8.4% required transfusion. 14 pts (3.5%) received erythropoetin. 14 (3.8%) anemia cases and 13 (15.3%) rash cases were considered as SAE.

Conclusions: This interim analysis confirms the antiviral efficiency of TVR-based triple therapy in GT1 HCV in a real life setting showing eRVR rates of more than 70% in treatment naïve and pre-treated patients. Adherence to futility rules was confirmed in most patients. As observed in clinical trials, adverse events were reported frequently.

* % based on number of pts with HCV-RNA measurements

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THE EFFECT OF CODAminISTRATION OF THE PROTON-PUMP INHIBITOR OMEPRAZOLE ON THE PHARMACOKINETICS OF DAclatasvir IN HEALTHY SUBJECTS

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Background: Daclatasvir (DCV; BMS-790052) is a highly selective, first-in-class, HCV NSSA replication complex inhibitor. DCV exhibits pH-dependent solubility, which decreases from 20mg/mL at pH2 to 0.015mg/mL at pH7. Since it is anticipated that DCV will be coadministered with acid-suppressing medications potentially reducing DCV exposure, this study aimed to assess the effect of the proton-pump inhibitor omeprazole on the pharmacokinetics of DCV.

Methods: This was a randomized, open-label study in healthy subjects (N=24). Subjects received a single dose of DCV 20mg or 60mg on Day 1 (Treatments A/B) followed by a 48h washout period.

These DCV doses were selected since they represented the lowest and highest doses undergoing clinical evaluation when this study was conducted, and it was expected that DCV exposures would vary across this dose range. Omeprazole 40mg QD was administered from Days 3–9 (Treatment C), with a concomitant single dose of DCV 20mg or 60mg on Day 8 (Treatments D/E). Subjects received the same DCV dose on both days. DCV pharmacokinetics were assessed for 48h post-dose on Days 1 and 8. Adjusted ratios of geometric means and 90% confidence intervals (CI) were estimated.

Results: Adjusted ratios of geometric means and 90% CIs are presented in the table. Coadministration of DCV 20mg with omeprazole did not affect overall DCV exposures. AUC(τ) and C24 were reduced by approximately 16% and 8%, respectively, when DCV 60mg was coadministered with omeprazole. Both doses of DCV with/without omeprazole were well tolerated.

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adjusted geometric mean ratio (90%CI)</th>
<th>Cmax</th>
<th>AUC(0-T)</th>
<th>AUC(τ)</th>
<th>C24</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCV 20mg</td>
<td>(Treatment D vs A)</td>
<td>0.800</td>
<td>1.004</td>
<td>1.021</td>
<td>1.133</td>
</tr>
<tr>
<td>DCV 60mg</td>
<td>(Treatment E vs B)</td>
<td>0.643</td>
<td>0.821</td>
<td>0.840</td>
<td>0.915</td>
</tr>
</tbody>
</table>

Conclusions: Coadministration of DCV with omeprazole resulted in reduced bioavailability of DCV; a 16% reduction in AUC(τ) was observed at the 60mg dose selected for phase 3 evaluation. However, this reduction in DCV exposure was not clinically significant and dose adjustment is not required when DCV is coadministered with omeprazole. These findings are comparable to those observed when DCV is coadministered with the H2-receptor agonist famotidine.

795

REAL WORLD COSTS OF TELAPREVIR-BASED TRIPLE THERAPY, INCLUDING COSTS OF MANAGING ADVERSE EVENTS, AT THE MOUNT SINAI MEDICAL CENTER, NY: $147,000 PER EOT


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Aims: The addition of telaprevir to IFN/RBV improved SVR rates for genotype 1 HCV, but also increased adverse events (AEs) and costs. We estimated the total management cost of triple therapy in a real-world setting.

Methods: Pre-treatment, on-treatment, and post-treatment costs were calculated using 2010 US estimates from Medicare, Agency for Healthcare Research and Quality (AHRQ), and Red Book WAC, adjusting for inflation. Resource utilization was based on standard practices and data on 134 patients who initiated telaprevir use at Mount Sinai Medical Center (5/2011–12/2011). End-of-treatment (EOT) data were available; SVR data are pending. FIB-4 scores ≥3.25 indicated advanced fibrosis/cirrhosis.

Results: Median age of the 134 patients was 57 years (IQR=51–61), 91 were male, 23 were black, 16 had HCV/HIV co-infection, 48 had advanced fibrosis/cirrhosis; 75 (56%) had an EOT. Median cost of standard triple therapy (telaprevir, IFN/RBV and routine care) was $77,020 ($66,045–$92,980) per patient. Median total cost of treatment (triple therapy plus AE management) was significantly higher: $82,500 ($67,967–$98,138), p = 0.02. On an intention-to-treat basis, median total cost per EOT was $147,321 ($121,321–$175,176). Seventy-seven patients (57%) had AE-attributable costs; 49% received epoeto-in-a and 12% had a treatment-related hospitalization. For the 58 patients who completed 48 weeks of treatment, the
median total cost was $100,609 ($93,412–$112,772). Total cost was significantly lower for the 13 patients who completed response-guided therapy; $76,488 ($74,627–$90,034), p < 0.01. Total cost was greater for patients with F3-F4 fibrosis than for patients with F0–F3 fibrosis: $93,413 versus $79,390, but the difference was not significant, p = 0.15. Median total cost for the 19 patients who discontinued due to AE was $52,158 ($27,179–$75,565), and it was $67,465 ($32,600–$75,935) for the 42 patients with on-treatment virologic failure.

**Conclusions:** The median total cost of 48 weeks of telaprevir-based triple therapy was $100,609, including costs of preparing the patient for treatment, AE management, and post-treatment SVR testing. The median total cost per EOT was $147,321. Response-guided therapy reduced costs, while advanced fibrosis did not significantly impact them. Reductions in AE and viral resistance are needed to optimize the clinical and economic effectiveness of HCV treatment (NIH:DA031095, DK903137; Gilead).

### 796 FACTORS ASSOCIATED WITH SUSTAINED VIROLOGICAL RESPONSE IN HCV/HIV PATIENTS RETREATED WITH Peg-INTERFERON–a2a-360 µg/WK AND RIBAVIRIN HIGH DOSE: RESULTS OF THE ANRS-HC-20 ETOC STUDY

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**Background:** The era of peg-interferon (P)/ribavirin (R) is not over for HIV/HCV patients. The current controversy around “drug-to-drug” interactions between HCV direct acting antiviral (DAAs) and HIV treatment is one of the future restraint which could limit use of triple therapy. Predictive factor in non responders patients will be useful to guide future treatments.

**Patients and Methods:** HIV/HCV patients (genotype 1 and genotype 4) who experimented non response (–2 log drop of HCV viral load at week (W)12) to a well conducted anti HCV treatment (P (180 µg/W) + R full dose) for at least 12W were included. The design was 6 months with Pa-2a (360 µg/W) + R (18 µg/kg/d) followed by a “viral response guided” following period (P (180 µg/W) + R). Results: 49 patients (38M/11F, G163%, G437%, 635±273CD4/mm³, 2/3 with liver fibrosis≥F3) were included. At W12, 13/49 (26%) patients had a complete early virological response (EVR), 16/49 (33%) patients had a partial EVR (more than a 2 log HCV viral load drop) and 20/49 (41%) failed to reach EVR and stopped the treatment. Twenty patients (41%) reached W24 with a negative HCV PCR and continued the treatment with Pa-2a180 µg/W. Among them, 10 (50%) reached a sustained virological response (SVR) at W96 (final SVR of 10/49 (20%) patients). Side effects were not more frequent in this study than in “usual-dosed” treatments (6/49 (12%) patients had to stop the treatment for side effect). Hematological growth factors were widely used (27%EPO and 8%G-CSF). Predictive factors associated with SVR were female gender, IL28B CC (vs IL28B CT+TT). HCV viral load, CD4 cell count at inclusion, liver fibrosis and genotype 1 (vs 4) were not predictive of SVR.

**Discussion:** In these “hard to treat” patients with high rate of advanced liver disease, only IL28B genotype and gender were linked to SVR. Combined with predictive factors of SVR, this treatment combination could be an interesting option in such “hard to treat” patients, harbouring all the negative predictive factors to HCV clearance. If combined with new DAAs, it could increase SVR and avoid the emergence of resistance.

### 797 HIGH PREVALENCE OF CHRONIC HEPATITIS C IN 8009 PATIENTS WITH MIGRATION BACKGROUND LIVING IN GERMANY


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**Background:** The prevalence of HCV antibodies (anti-HCV) in the general German population has been estimated to be around 0.5%. Meanwhile nearly 20% of the German population has a migration background. Since there is a growing body of data demonstrating an increase in the burden of migration-related chronic viral hepatitis in Western societies we aimed to assess the anti-HCV prevalence in patients with a migration background.

**Methods:** Between 09/2008 and 12/2010 anti-HCV status of 8009 patients with migration background was documented by 42 centers in Germany. In addition demographic data, HCV genotype, and AST/ALT levels were recorded and retrospectively analyzed.

**Results:** A total of 1348 patients were found to be anti-HCV positive. In 16 general health practices and 5 hospital departments of internal medicine anti-HCV prevalence was found to be 5.8% (156/2670) and 6.3% (57/905). A higher prevalence of 25.0% (711/2849) and 26.7% (422/1582) in practices specialized in hepatology or drug substitution may reflect selection of patients with high risk for HCV infection. Countries of origin were GUS-countries (50%), Poland (8%), Turkey (8%), other European countries (17%), Asian countries (10%), Africa (5%) and other (2%). The mean age of these patients was 43 years, 35% were female and 5% were co-infected with HIV. HCV genotypes (G) were documented from 1047 patients and showed the following distribution: G1 59% (subtype 1a 38%, subtype 1b 50%, unknown subtype 12%), G2 8%, G3 27%, G4 5% and other 1%. Abnormal ALT, AST and gamma-GT levels were found in only 56%, 45% and 43% of patients. Regarding the time point of HCV infection information was available from 679 patients. Interestingly, 68%/32% of patients reported HCV transmission after/before immigration due to intravenous drug use (60%/28%) and blood transfusion (17%/42%).

**Conclusions:** There is a high prevalence of chronic hepatitis C in patients with migration background living in Germany. Remarkably, the majority of patients seem to acquire HCV infection after immigration. Intravenous drug abuse is the main risk factor for HCV transmission in this population.
POSTERS

798 EFFICACY OF TELAPREVIR DOSED DAILY VERSUS EVERY 8 HOURS BY IL28B GENOTYPE: RESULTS FROM THE PHASE III OPTIMIZE STUDY


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Background and Aims: OPTIMIZE established the non-inferior efficacy of telaprevir (TVR, T) twice daily (bid) versus every 8 hours (q8h) in combination with peginterferon/ribavirin (PR) across a range of patient characteristics. One stratification factor of the study was IL28B genotype. Here we provide detailed characterization of study results across IL28B genotype groups.

Methods: OPTIMIZE was a randomized, open-label, multicenter, Phase III trial in treatment-naïve patients with chronic HCV genotype 1 infection (NCT01241760). In total, 740 patients were randomized to either TVR 1125 mg bid (N=369) or TVR 750 mg q8h (N=371) in combination with PR.

Table: Baseline characteristics

<table>
<thead>
<tr>
<th>IL28B genotype</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>q8h</td>
<td>N=106</td>
<td>N=207</td>
<td>N=56</td>
</tr>
<tr>
<td>bid</td>
<td>N=108</td>
<td>N=207</td>
<td>N=56</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>28</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>Race (%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BL VL (x10^5 IU/mL)</td>
<td>88</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>HCV viremia (x10^5 copies/1-1)</td>
<td>61</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Fibrosis stage (F3–F4)</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

Results: The percentage of patients by IL28B genotype was: CC=29%, CT=56%, TT=16%. Genotype distribution was balanced equally by treatment arm (Table). Based on multivariate analysis, female sex, black race, lower baseline (BL) HCV viral load (VL), and cirrhosis were significantly associated with lower odds of CC genotype. The efficacy of TVR bid versus q8h was similar regardless of IL28B genotype.

799 EFFICACY AND SAFETY OF TRIPLE-THERAPY WITH PegINTERFERON-RIBAVIRIN, AND BOCEPREVIR AS COMPASSIONATE-USE IN SPANISH PATIENTS WITH HEPATITIS C GENOTYPE 1 WITH SEVERE FIBROSIS: INTERIM-ANALYSIS AT 12 WEEKS


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Background and Aims: In 2011, the Spanish Agency for Medicines and Health Products authorised the compassionate use of boceprevir in patients with hepatitis C and severe fibrosis. This authorisation generated a registry directed to safety. We report the safety and effectiveness profiles of treatment with boceprevir in combination with peginterferon alfa/ribavirin in patients genotype 1 hepatitis C with severe fibrosis (F3 (bridging fibrosis) – F4 (cirrhosis)) in biopsy or FibroScan® >9.5 Kpa), who received 12 weeks of treatment.

Methods: Prospective multicentre national registry including patients with hepatitis C genotype 1, both naïve and treatment experienced, who had bridging fibrosis or cirrhosis treated with peginterferon alfa-2a or alfa-2b, ribavirin, and boceprevir as compassionate use in accordance with the SPC.

Results: For the 102 patients the mean age was 54 years. 64% were male, 18/82% 1a/1b genotype and baseline viral load of 6.2 log (mean 3,470,712 ± 4,148,524). 85% of the patients had F4 and 22% had oesophageal varices, 19% were treatment naïve and 81% had undergone prior treatment with PegIFN+ribavirin (31% prior relapsers, 36% partial responders and 33% null responders). 19% of the patients had baseline levels <90,000 platelets. Up to week 12, 33 patients (32.4%) developed at least one serious adverse event (SAEs 46) (Table 1).
Table 1. Adverse events in 102 patients

<table>
<thead>
<tr>
<th>Event</th>
<th>Patients with at least one event, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serious adverse events (SAEs)</td>
<td>33 (32.4%)</td>
</tr>
<tr>
<td>Premature discontinuation</td>
<td>33 (32.4%)</td>
</tr>
<tr>
<td>Due to SAEs</td>
<td>10 (9.8%)</td>
</tr>
<tr>
<td>Discontinuing patient care</td>
<td>3 (2.9%)</td>
</tr>
<tr>
<td>Virological failure</td>
<td>20 (19.6%)</td>
</tr>
<tr>
<td>Death</td>
<td>2 (1.96%)</td>
</tr>
<tr>
<td>Septic shock, Multi-organ failure secondary to pneumonia</td>
<td>8 (7.8%)</td>
</tr>
<tr>
<td>Infection Grade 3-4</td>
<td>5 (4.9%)</td>
</tr>
<tr>
<td>Hepatic decompensation (Grade 3/4)</td>
<td>4 (3.9%)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>29 (28.4%)</td>
</tr>
<tr>
<td>Hg &lt;10.0 g/dL</td>
<td>3 (2.9%)</td>
</tr>
<tr>
<td>Hb &lt;8.0 g/dL</td>
<td>26 (25.5%)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>9 (8.8%)</td>
</tr>
<tr>
<td>Ribavirin dose adjustment</td>
<td>27 (26.4%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>44 (43.1%)</td>
</tr>
<tr>
<td>N &lt;500/mm³</td>
<td>5 (4.9%)</td>
</tr>
<tr>
<td>Use G-CSF</td>
<td>2 (2.0%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>18 (17.6%)</td>
</tr>
<tr>
<td>Platelets &lt;50,000</td>
<td>1 (0.98%)</td>
</tr>
<tr>
<td>Platelets &lt;25,000</td>
<td></td>
</tr>
</tbody>
</table>

In the Intent to Treat analysis (ITT) the percentage of patients with HCV RNA <100 IU/ml (undetectable) at week 12 was 68% (58%), 79% (74%) for treatment naïve patients, 81% (77%) for relapers, 77% (63%) for partial responders and 37% (22%) for null responders. In the Per-Protocol analysis (patients initiating boceprevir (n = 87)) the response was 79% (68%), 83% (78%) for treatment naïve patients, 84% (80%) for relapers, 96% (79%) for partial responders and 50% (30%) for null responders.

**Conclusions:** The triple therapy of boceprevir with PEG-IFN/RBV in patients with severe fibrosis is very effective in negatively HCV viremia at 12 weeks (68%), but is associated with serious adverse events in more than 33% of the patients.

**801**

**UTILITY OF ITPA GENOTYPING TO PREDICT EARLY SEVERE ANEMIA IN CHRONIC HEPATITIS C PATIENTS RECEIVING TRIPLE THERAPY**

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**Introduction:** Inosine triphosphatase (ITPA) polymorphisms, such as rs1127354 (C/A) and rs7270101 (A/A) are associated with a deficiency of this enzyme and a lower risk of developing anemia in patients with chronic hepatitis C (CHC) treated with interferon and ribavirin. There is little information regarding the role of ITPA genotyping in triple therapy including protease inhibitors (PIs), whose most significant adverse effect is anemia.

**Aims:** To determine ITPA polymorphisms in patients under triple therapy including the PIs boceprevir (BOC) and telaprevir (TVP) and correlate them with the risk of early anemia.

**Material and Methods:** Blood samples from 68 CHC patients genotype 1 were analyzed. All the patients were treated with triple therapy including TVP (N = 41) and BOC (N = 27). Both ITPA polymorphisms were genotyped by real-time PCR and melting curves (LightCycler, Roche). Anemia was defined based on two criteria: decrease in Hb ≥ 3 g/dL and a Hb < 10 g/dL at week 4 of PI treatment.

**Results:** ITPA non-deficient polymorphisms (homzygous for the major allele) were detected in 39 patients (57%) and polymorphisms with some ITPA deficit (one or more minor alleles) were detected in 29 cases (43%). The table shows the results at week 4 of PI treatment.

<table>
<thead>
<tr>
<th>rs1127354</th>
<th>rs7270101</th>
<th>ITPA deficiency</th>
<th>Number of cases</th>
<th>Hb decline ≥ 3 g/dL at week 4</th>
<th>Hb &lt; 10 g/dL week 4</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>A/A</td>
<td>0%</td>
<td>39 (77%)</td>
<td>30 (77%)</td>
<td>12 (30%)</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>A/C</td>
<td>40%</td>
<td>16 (24%)</td>
<td>4 (25%)</td>
<td>30 (19%)</td>
<td></td>
</tr>
<tr>
<td>A/C</td>
<td>A/A</td>
<td>70%</td>
<td>7 (10%)</td>
<td>1 (14%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>A/C</td>
<td>A/C</td>
<td>90%</td>
<td>6 (9%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Genotypes with ITPA deficiency ≥70% were less likely to develop anemia than those <40% deficiency (p < 0.05). Only 1 patient (8%) with >70% deficiency presented a drop in Hb > 3 g/dL, versus 34 cases (62%) with deficits <40%. Hb < 10 g/dL was detected in 15 patients (27%) and all of them had genotypes with deficits <40% (p < 0.05). No significant differences were observed between the two PI administered.

**Conclusion:** The ITPA genotyping enables identification of CHC patients with a lower risk of anemia under PI therapy. Early detection of ITPA polymorphisms may identify patients at high risk of early severe anemia, who may require more frequent monitoring and a further reduction of ribavirin dose.
Conclusions: In CHC liver fibrosis improves significantly in those patients who achieve SVR after antiviral therapy. New antiviral therapies should be considered in older patients without SVR because they are at high risk of liver fibrosis progression.

802 FOLLOW-UP AFTER CESSION OF THERAPY WITH DIRECT ACTING ANTIVIRALS IN CHRONIC HEPATITIS C PATIENTS – WHO IS AT RISK OF LATE RELAPSE?

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Results of antiviral therapy for chronic hepatitis C (CHC) with the direct acting antivirals (DAA) are reported variably across the clinical trials putting emphasis on virological response at different time-points after stopping therapy.

Aims: To assess proportion of patients achieving end of treatment (EOT) response and SVR at 4, 12, 24 and 48 weeks after therapy cessation and the role of variable factors such as previous therapy status, cirrhosis, genotype 1 subtype, IL28b genotype, treatment regimen (protease inhibitor alone or boosted with ritonavir, NS5B polymerase and NS5A inhibitors) and dose reduction.

Patients: 63 CHC genotype 1 (40 naïve and 23 therapy experienced (TE)) (median age 48y, 27% cirrhosis) were treated with DAA in combination with pegylated interferon and ribavirin (median duration 24 weeks) within the following clinical trials (naïve: Roche NV20536 and NV22776; Gilead US 196–0112 with GS-9190+GS-9256; BI1220.5; VX-950: Optimise and PPI-461–102 and for TE: VX-950 Realize, TMC435: Aspire and Promise and BI1220.5) available in a single centre between January 2009–October 2011.

Results: Overall EOT was 84% (85% for naïve and 83%TE), SVR4 68% (73% naïve and 61%TE), SVR12 65% (70% naïve and 57%TE), SVR24 63% (68% naïve and 57%TE) and SVR48 was 62% (65% naïve and 57%TE). 4 patients relapsed after achieving SVR (2 by follow up week 12 (FUW12), 1 at FUW24 and 1 by FUW48). Patients with cirrhosis have similar responses as non-cirrhotic patients for EOT (76% vs. 82%), SVR4 (65% vs. 70%), SVR12 (59% vs. 67%), SVR24 and SVR48 (both 59% vs. 63%). There were no significant differences between genotype 1α vs. 1β for EOT (86% vs. 81%), but this was different for SVR4 (72% vs. 63%, p = 0.03) and SVR12 (69% vs. 59%, p = 0.02) and similar for SVR24 (66% vs. 59%) and SVR48 (62% vs. 59%), suggesting that genotype 1α was more often associated with post-treatment relapse after FUW4. Treatment regimen and dose reduction did not impact on differences in therapy responses. IL28b genotype CC was associated with EOT 100% and 93% for SVR4, SVR12, SVR24 and SVR48 respectively and was highly predictive of response after FUW4 (PPV 93%).

Conclusions: Relapse after FUW4 is rare in DAA treated patients (6%). Genotype 1α and non-CC IL28b genotypes were more frequent in patients with relapse after achieving SVR.

803 RELATIONSHIP BETWEEN TRANSMINASE LEVELS AND PLASMA PHARMACOKINETICS FOLLOWING ADMINISTRATION OF MK-5172 WITH PEGYLATED INTERFERON α-2b AND RIBAVIRIN (PR) TO HCV GENOTYPE (G) 1 TREATMENT-NAÏVE PATIENTS

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Background and Aims: MK-5172 is an HCV NS3/4A protease inhibitor with a high barrier to resistance. 332 treatment-naïve G1-infected patients were randomized to receive boceprevir+PR or MK-5172 100-, 200-, 400-, or 800-mg QD + PR for 12 weeks followed by 12 (RVR achieved) or 36 weeks (no RVR) of PR. Therapy was highly effective with ≥85% achieving SVR12. While ALT/AST levels normalized by week 4, they increased to >2X and >5X upper limit of normal (ULN) in a minority of patients in a dose-dependent fashion (Table). Analyses were conducted to explore the relationship between plasma pharmacokinetics (PK) and late ALT/AST elevations.

Methods: Patients in a PK sub-study provided MK-5172 plasma samples at select visits through Week 12. PK/safety correlations were analyzed, including PK endpoints for steady-state trough and C2hr and two safety endpoints (late ALT/AST >2×ULN or >5×ULN). Logistic PK/safety regression analyses were conducted to assess the probability of the safety endpoint occurring at a given PK parameter value; the PK predictability for each endpoint was determined by performing Receiver Operating Characteristic and Negative Predictive Value analyses.

Results: Geometric mean (GM) trough and C2hr values were well-correlated with both safety endpoints. A GMtrough and GM2hr of 56 nM and 1470 nM, respectively, were predicted to have a ≤10% mean probability of ALT/AST >2×ULN occurring, and are ~2- and ~4-fold above the upper 90% Confidence Interval (CI) for the 100-mg dose. A GMtrough and GM2hr of 128 nM and 4675 nM, respectively, are predicted to have a mean probability of ≤5% of >5×ULN occurring, and are ~4- and ~13-fold above the upper 90% CI for the 100-mg dose.

Conclusions: Plasma MK-5172 PK is well-correlated with key ALT/AST safety endpoints. Combined with high SVR rates for the 100-mg dose, the PK/Safety analysis supports the low risk for a hepatic safety signal and continued development of MK-5172 at a dose of 100-mg QD.

<table>
<thead>
<tr>
<th>MK-5172 QD dose</th>
<th>100 mg</th>
<th>200 mg</th>
<th>400 mg</th>
<th>800 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVR12 (%)</td>
<td>90%</td>
<td>91%</td>
<td>90%</td>
<td>88%</td>
</tr>
<tr>
<td>Late ALT/AST%</td>
<td>&gt;2×ULN</td>
<td>2%</td>
<td>9%</td>
<td>19%</td>
</tr>
<tr>
<td>&gt;5×ULN</td>
<td>0%</td>
<td>2%</td>
<td>6%</td>
<td>9%</td>
</tr>
</tbody>
</table>

804 EXPOSURE–RESPONSE ANALYSES OF ASUNAPREVIR IN COMBINATION WITH DACLATASVIR = PEGINTERFERON/ RIBAVIRIN AMONG PATIENTS WITH GENOTYPE 1 CHRONIC HCV INFECTION: DOSE SELECTION FOR PHASE 3 CLINICAL TRIALS

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Background: Asunaprevir (ASV) is a selective HCV NS3 protease inhibitor with activity against genotypes (GT) 1 and 4, which is currently in clinical development in combination with daclatasvir (DCV), a highly selective, first-in-class NS5A replication complex inhibitor. To guide ASV dose selection for phase-3, a population pharmacokinetic (PopPK) model was developed and exposure–response and viral-kinetic (VK) models were evaluated.

Methods: Interim data from three phase-3 studies in US/EU and Japanese patients of ASV in tablet doses ranging from 200–1200 mg/day with peginterferon-alfa-2a and ribavirin (alfa/RBV; AI447016; treatment-naïve) or ASV with DCV±alfa/RBV (AI447011/AI447017; null-responders and interferon-ineligible naïve) were pooled. PopPK analyses were performed using nonlinear mixed-effects methodology. Adverse event binary response and time-to-event data were correlated to exposure estimates from the PopPK model using linear logistic regression and Cox-regression models. A novel two-strain VK model was
developed, calibrated using emerging response data, and used to predict virologic responses.

**Results:** PopPK modeling suggested modest time-dependent increases in ASV clearance. Steady-state AUC was 37% higher among Japanese patients. Age was a covariate explaining 16.6% variability in clearance. Higher ASV AUC correlated with greater incidence of ≥Grade 2 adverse liver events; however, at the 200 mg BID dose relative risk for these events was similar to placebo in Japanese patients and those aged >60 years (odds ratios: 1.09 versus 1.06 for non-Japanese; 1.08 versus 1.06 for age ≤60 years). Concomitant administration of DCV or alfalfa/RBV did not affect the timing or risk of liver events. VK modeling and simulation successfully predicted on-treatment response in AI447016, AI447017 and AI447011 populations. Sustained virologic response (SVR) was projected to be lower in GT1b interferon-ineligible and null-responders receiving ASV 200 mg QD (70%) versus BID (76%) in combination with DCV alone. ASV 200 mg QD and BID in combination with DCV+alfalfa/RBV were projected to achieve similar SVR rates among GT1a null-responders (83.3–84.5%); however, 200 mg BID could theoretically improve SVR rates.

**Conclusions:** An integrated quantitative analysis supported an equivalent tablet dose of ASV 200 mg BID for phase-3 to maximize antiviral response and minimize adverse events. The ASV 100 mg BID softgel formulation used in phase-3 studies approximates the 200 mg BID tablet exposures.

**805 WEEK 4 AND 12 EFFICACY OF TELAPREVIR (TVR) IN COMBINATION WITH PEGINTERFERON alfa 2a (RIBAVIRIN (P/R)) IN TREATMENT EXPERIENCED PATIENTS WITH GT-1 UNDER REAL LIFE CONDITIONS**

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**Introduction:** Under conditions of clinical trials the addition of TVR to P/R resulted in significantly high rates of week 4 and 12 responses in previously treated patients with chronic HCV GT-1 infection [1]. Is this reproducible in real life?

**Methods:** Between October 2011 and August 2012 1280 GT-1 patients (pts) treated with TVR containing triple therapy were included in the non-interventional study PAN conducted by the Association of German Gastroenterologists in Private Practice (bng) and Roche. Data of 537 treatment experienced pts with simultaneous start of TVR and PEG/RBV and with data up to week 12 were analyzed. Precision of measuring HCV RNA at week 4 or 8 was set to ±3d. Extended rapid virologic response (eRVR) was calculated for pts with both data of week 4 and 12 (n=312).

**Results:** 290 pts were relapers, 231 non responders, 74 of them with classification as partial responders and 46 as nullresponders. The remaining pts discontinued former therapy due to intolerability or personal reasons. Demographic mean data were: age 51.4yrs, male gender 63.3%, BMI 26.7 kg/m², ALT 97.2 IU/L. 19.7% of pts had platelets <140x10⁹/μL and 21.0% liver cirrhosis (at least one result of sonography, histology, elastography or clinical appearance). 72.4% of pts had high viral load (>400,000 IU/ml), distribution of GT-1 subtypes was 24.6% 1a, 53.4% 1b, 0.6% other and 21.4% unknown. Since 25.0% and 13.0% of pts in week 4 and 12 had no valid HCV RNA as defined, table shows adjusted data of virological responses. Rates of anaemia <10 g/dl and <8.5 g/dl were 23.7% and 9.3%. 16.8% of pts got ribavirin dose modification. AEs reported with more than 20% were fatigue, skin disorder / rash, pruritus, anaemia and nausea. 12.1% of pts completely discontinued therapy until week 12.

**Conclusion:** Although real life patients were not selected according to in- and exclusion criteria, virological efficacy of triple therapy with TVR/P/R in treatment experienced patients until week 12 is very similar to the pivotal trial.

**Table:** Virological efficacy of week 4, week 12 and weeks 4 and 12 combined.

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Relapse (pts)</th>
<th>Non responder (pts)</th>
<th>Of them</th>
<th>null responder (pts)</th>
<th>Total of treatment experienced (pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVR (wk 4)</td>
<td>70.7%</td>
<td>52.8%</td>
<td>69.2%</td>
<td>35.2%</td>
<td>61.6%</td>
</tr>
<tr>
<td>eRVR (wk 4)</td>
<td>47.9%</td>
<td>68.3%</td>
<td>78.0%</td>
<td>52.6%</td>
<td>78.5%</td>
</tr>
<tr>
<td>eRVR (wk 4+12)</td>
<td>71.3%</td>
<td>31.1%</td>
<td>63.3%</td>
<td>36.7%</td>
<td>61.5%</td>
</tr>
</tbody>
</table>

Each virological response is given as LLOD (<1200 IU/ml).

**Reference(s)**


**806 MANAGEMENT AND OUTCOMES OF ANEMIA IN THE INTERNATIONAL TELAPREVIR EARLY ACCESS PROGRAM, FOR PATIENTS WITH HEPATITIS C GENOTYPE 1 INFECTION**

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**Background and Aims:** Anemia is a common adverse event during standard treatment for HCV infection. HEP3002 is an ongoing, open-label, early access program of telaprevir in 16 countries, for patients with genotype 1 hepatitis C with severe fibrosis or compensated cirrhosis.

**Methods:** This interim analysis included 16 weeks data from the first 609 patients treated with telaprevir, pegylated interferon-alpha and ribavirin (RBV) for 12 weeks, followed by PR. Use of iron supplements, erythropoietin (EPO) and blood transfusions were allowed. Anemia included the clinically significant adverse event terms (SSC) of anemia or hemoglobin reduction. All analyses were on the intent to Treat (ITT) population.

**Results:** Mean age was 54 years and mean weight 79 kg; 67% were Male and 98% Caucasian, 66% had HCV RNA levels ≥800,000
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IU/mL, 45%55% had severe fibrosis/cirrhosis, 28% had genotype 1a. Up to Week 16, 59% of patients developed grade 1–4 anemia (Hb <11 g/dL or >2.5 g/dL reduction), with 31% severe cases (Hb <9 g/dL or >4.5 g/dL reduction); 206 patients (34%) dose reduced ribavirin, 146 (24%) received EPO, 70 (11%) were transfused, 8 (1%) received iron-based products and 19 (3%) discontinued treatment for anemia. 122 patients (20%) had both a reduction in ribavirin dose and either EPO or a blood transfusion. Results by baseline disease stage are shown below:

<table>
<thead>
<tr>
<th>Outcome</th>
<th>F3 patients (n = 273)</th>
<th>F4 patients (n = 335)</th>
<th>Total (n = 609)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 3 anemia SSC event</td>
<td>55 (20%)</td>
<td>100 (30%)</td>
<td>155 (26%)</td>
</tr>
<tr>
<td>Grade 4 anemia SSC event</td>
<td>15 (5%)</td>
<td>20 (6%)</td>
<td>35 (6%)</td>
</tr>
<tr>
<td>Discontinuation for anemia</td>
<td>3 (1%)</td>
<td>16 (5%)</td>
<td>19 (3%)</td>
</tr>
<tr>
<td>Anemia as Serious AE</td>
<td>11 (4%)</td>
<td>22 (7%)</td>
<td>33 (5%)</td>
</tr>
<tr>
<td>RBV dose reduction</td>
<td>80 (29%)</td>
<td>126 (38%)</td>
<td>206 (34%)</td>
</tr>
<tr>
<td>EPO use</td>
<td>53 (19%)</td>
<td>93 (28%)</td>
<td>146 (24%)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>26 (10%)</td>
<td>44 (13%)</td>
<td>70 (11%)</td>
</tr>
</tbody>
</table>

Conclusions: In this telaprevir early access program for patients with severe fibrosis or compensated cirrhosis, Grade 3 or 4 anemia was reported in 31% of patients, but discontinuation for anemia was rare (3%). Anemia was more common for patients with F4 stage disease at baseline.

807 TRIPLE THERAPY IN CHRONIC HEPATITIS C GENOTYPE 1. LIMITATIONS IN CLINICAL PRACTICE. SPANISH MULTICENTER STUDY

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The triple therapy (TT) in treatment of chronic hepatitis C genotype 1 (CHC-1) virus infection, represents a paradigm shift in the management of these patients. However, the access to this therapy is limited for several reasons.

Objectives:

a. Identify the reasons for not starting TT.

b. Describe the demographic, clinical, virological and histological characteristic of patients who TT were prescribed.

c. Relate the healthcare environment and the probability of receiving TT.

Methods: We performed an observational, cross-sectional, multicenter study, in 19 Spanish hospitals with different health care environment (Level III: 5, Level II: 10, Level I: 4), from July to September 2012 We included all patients with CHC-1 who attended the outpatient clinic during this period. We excluded patients with another genotype and coinfected.

Results: We included 660 patients that the inclusion criteria were performed. The basal characteristic were: 63% male, median aged 54.5 + 12.1 years, 54.2% was previously treated with interferon-ribavirin and median basal viral load was 6.53 log10). The TT was contraindicated in 159 cases (24%); (age >70 years in 66 patients, 23 decompensated cirrhosis, 20 serious organic or mental illness, 13 active drug users and 29 for several reasons). In the remaining 501 patients, despite the absence of contraindications, therapy is indicated only in 211 subjects (31.5% of the total patients). The reasons for not treatment in the 290 remaining patients were: patient refusal (82 cases, 28.2%), government restrictions (84 cases, 28.9%) and the TT was postponed pending further therapies (55 cases, 18.9%). In the remaining 69 cases (23.9%) miscellaneous causes were registered. In the univariate analysis, TT indication was statistical significant associated with host-dependent factors p <0.05 (young age, urban habitat; high academic level, significant liver fibrosis) and health environment dependent factors p <0.05 (Level III hospital, specialised nurse and physician experience in TT).

Conclusions: Despite the high efficacy of triple therapy, it is performed only in a minority of patients with CHC-1. About 25% of patients were treated because of contraindications but more than 50% of CHC patient the TT especially was not indicated because hospital environment and stage of the disease.

808 MODELING HCV KINETICS DURING INTRAVENOUS SILIBININ MONOTHERAPY IN THE PERI-TRANSPLANT PERIOD

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Background and Aims: Modeling of HCV kinetics during the peri-transplant period has been limited due to the lack of available safe antigens. Here we sought to characterize and model HCV kinetics during intravenous silibinin (SIL) monotherapy in the peri-transplant period.

Methods: Frequent HCV samples were measured daily for 14 to 21 days before liver transplantation in 9 patients receiving SIL treatment (Marino et al. J Hepatol; in press). A mathematical model that includes hepatocyte proliferation (Gastroenterology. 2009, 136(4):1402–9) was used to model the HCV kinetic data. Using a stochastic random search within a parameter-space of chronic HCV patients and knowledge of parameter boundaries required for their individual observed viral decline pattern, we obtained about 100 possible parameter sets for each patient. Then we performed non-linear fitting to estimate viral host parameters and SIL’s mode of actions (MOA) in each patient.

Results: Apart from one null responder, three distinct viral decline patterns were identified:

i. biphasic (n = 1, Fig. 1, circles).

ii. triphasic with a continuous final decline (n = 2, Fig. 1 squares) and

iii. triphasic with a final viral plateau starting at about 12 days post-treatment initiation (n = 5, Fig. 1, diamonds).

The mean second (or final) phase slope of viral decline (Fig. 1) was high 1.3±0.5 log10/wk, ie, 2-fold higher than typically observed during 2nd phase of (pegylated)-interferon-alpha±ribavirin treatment in non-cirrhotic subjects. Model fits suggest that mean death/loss rate of infected cells is n = 1.2±0.8 day−1 and that SIL blocks both viral production/secretion and infection with overall mean effectiveness of 92±5%.

Conclusions: In cirrhotic patients awaiting liver transplantation, SIL achieved significant decline in viral load ie., 2.7±0.8 log10, lower than baseline at end of days 14 or 21 under SIL monotherapy. The model predicts that SIL effectively blocks both viral infection and viral production/release with high effectiveness of 92±5%.
**Background:** In HCV-infected G1 patients, Boce- and Telaprevir-containing regimens have significantly improved sustained virologic response (SVR) rates. In patients with advanced fibrosis, these regimens have shown to be cost-effective compared to Peg-Interferon/Ribavirin. We compared, in naive patients without severe fibrosis (F0/F1/F2), immediate vs. delayed treatment initiation considering that previr regimens are standard of care but that safer and more efficacious Directly Active Agents (DAAs) will be soon available (in particular oral combinations).

**Methods:** We used a mathematical model to estimate quality adjusted life years (QALYs), cost and incremental cost-effectiveness ratio (ICER) of five treatment initiation strategies in patients with F0/F1/F2 fibrosis in France in 2012. We compared different strategies under alternative scenarios regarding new DAAs availability (Table). SVR was considered to be 90% with new DAAs vs. 80% for “previr” containing regimens in F0/F1/F2 patients, with a 20%-reduction in F3/F4 patients. Tolerance (2–10% serious adverse events based on fibrosis) and costs were assumed to be similar for new DAAs and “previr” regimens.

**Results:** In F0/F1/F2 patients, immediate treatment initiation with current “previr” regimens is less efficacious and more expensive when compared to a strategy that treat ≥F3 patients before 2015 and all patients by new DAAs ≥2015 (€4100 to 8400 additional costs/patient) (Table). The latest strategy is very cost-effective in F0/F1 patients at diagnosis, when compared to the strategy that always treat ≥F2 patients: ICER = 20,000€/QALY (F0) or 40,000€/QALY (F1). In F2 patients at diagnosis, treating ≥F3 patients before 2015 and all patients by new DAAs ≥2015 is associated with the lowest costs and highest efficacy. Results assumed a perfect diagnosis of fibrosis, and the absence of loss of follow-up in delayed treatment strategies. They were robust to variations in age or alcohol prevalence at diagnosis, new DAAs availability time (2017 vs. 2015), costs (“previr” costs ×1.5), or efficacy (40%-reduction in F3/F4 patients). However, in F2 patients with co-morbidities such as alcoholic patients, immediate treatment initiation became cost-effective.

**Conclusion:** Because safer and more efficacious DAAs will be soon available, delaying treatment in naive G1 patients without severe fibrosis is effective and cost-effective.

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**809 IMMEDIATE OR DELAYED TREATMENT INITIATION WITH “PREVIR” CONTAINING REGIMENS IN HCV-INFECTED NAIVE GENOTYPE 1 (G1) PATIENTS WITHOUT SEVERE FIBROSIS? A COST-EFFECTIVENESS ANALYSIS (ANRS N°12188)**

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**Table (abstract 809)**

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Cost* (€)</th>
<th>QALY (years)</th>
<th>ICER (€/QALY)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F0 at diagnosis, mean age = 47:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat when ≥F2 (previr ≥2015; new DAAs ≥2015)</td>
<td>35,600</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>Treat when ≥F3 (previr ≥2015; new DAAs ≥2015)</td>
<td>38,300</td>
<td>19.4</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;2015 treat when ≥F3 (previr), ≥2015 treat regardless to fibrosis stage (new DAAs)</td>
<td>39,600</td>
<td>20.1</td>
<td>20,000&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>2015 treat when ≥F2 (previr), ≥2015 treat regardless to fibrosis stage (new DAAs)</td>
<td>39,700</td>
<td>19.8</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treat immediately</td>
<td>48,000</td>
<td>19.8</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>F1 at diagnosis, mean age = 51:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat when ≥F2 (previr ≥2015; new DAAs ≥2015)</td>
<td>39,800</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>&lt;2015 treat when ≥F3 (previr), ≥2015 treat regardless to fibrosis stage (new DAAs)</td>
<td>40,600</td>
<td>18.8</td>
<td>4,000&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;2015 treat when ≥F2 (previr), ≥2015 treat regardless to fibrosis stage (new DAAs)</td>
<td>41,800</td>
<td>18.7</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treat when ≥F3 (previr ≥2015; new DAAs ≥2015)</td>
<td>4,200</td>
<td>18.1</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treat immediately</td>
<td>48,800</td>
<td>18.4</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>F2 at diagnosis, mean age = 54:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2015 treat when ≥F3 (previr), ≥2015 treat regardless to fibrosis stage (new DAAs)</td>
<td>4,630</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Treat when ≥F3 (previr ≥2015; new DAAs ≥2015)</td>
<td>50,400</td>
<td>16.3</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treat immediately</td>
<td>50,500</td>
<td>16.8</td>
<td>Dominated1&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Costs and QALYs are discounted (3%). †Dominated = more expensive and less efficacious.

<sup>1</sup>Very cost-effective strategy because ICER < French GDP (€32,200).
INTERPLAY BETWEEN ON-TREATMENT PREDICTORS AND DONOR/RECIPIENT (D/R) IL28B STATUS IN PREDICTING SUSTAINED VIROLOGICAL RESPONSE (SVR) IN HCV-1,4 LIVER TRANSPLANT (LT) RECIPIENTS TREATED WITH PEG-INTERFERON + RIBAVIRIN (PR)

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Background and Aim: Antiviral therapy with PR in HCV-LT recipients after liver transplantation (LT) can improve graft and patient outcomes. Donor and/or recipients (D/R) clinical, virological and genetic features have been identified as predictors of SVR but their impact on clinical practice is controversial.

Methods: From 2000, 72 consecutive LT recipients (58 HCV-1,4) with significant HCV recurrence on protocol liver biopsy (Ishak score grading≥2 and/or staging≥2) in absence of whatever contraindication to PR regimen were treated with Peg-IFNa plus ribavirin (GFR-based dose) intended for 48 weeks. IL28B rs12979860 genotype was tested in all D/R PBMC by standard methods. The PR dose adjustments were based on patient tolerability/side effects.

Results: Overall, 48/58 HCV-1,4 received PR for at least 24 weeks and completed the post-treatment follow-up (5 discontinued and 5 are ongoing): 81% males, 43% HCC at LT, median R age 54 years, D age 56 years; R-IL28B CC=29%, CT=50%, TT=21%; D-IL28B CC=54%, CT=33%, TT=13%; Maintenance immunosuppression was cyclosporine <50 years donor graft (p=0.37) and in those under 50 years donor graft (p=0.37) and in those older vs. younger than 50 years donor graft (p=0.37) and in those under cyclosporine vs. tacrolimus (p=0.36) immunosuppression. After splitting recipients according to RVR and cEVR (yes vs. no) D/R-IL28B status (CC vs. CT/TT) did not emerge as a predictor of SVR.

Conclusions: D/R-IL28B status did not predict PR treatment outcome in HCV-1,4 LT recipients. The achievement of a cEVR was the strongest predictor of SVR in our cohort of patients.
Background: Pegylated interferons (Peg-IFN) alpha 2a and alpha 2b show different pharmacokinetic properties, that affect absorption, serum half-life and excretion. Notwithstanding, both subtypes are still used indifferently for the treatment of hepatitis C in traditional dual combination as well as with newer agents. In this study, we assessed whether standard doses of Peg-IFN alpha 2a and 2b affect in a differential manner the early HCV viral kinetics.

Methods: Patients with liver biopsy-proven, HCV-RNA+, chronic active hepatitis C underwent antiviral treatment with PR between 2007 and 2011 were studied. Histology grading and staging, IL28B status and viral genotype were analysed. HCV-RNA quantitation was performed by Cobas Taqman 5 minutes before treatment start and subsequently after 48/72 hours, 7, 14 and 28 days.

Results: 187 patients were studied (median age 55 yrs, males 57%, cirrhosis 29%). 85 patients (45%) received Peg-IFN alpha 2a, 102 (55%) Peg-IFN alpha 2b. The two groups were homogeneous for HCV genotype and well balanced for IL28B genotype distribution (Table 1).

Table 1
genotype | Peg-IFN alpha 2a (N=85) | Peg-IFN alpha 2b (N=102) |
--- | --- | --- |
| Genotype 1, N (%) | 47 (55%) | 55 (54%) |
| Genotype 2/3, N (%) | 38 (45%) | 47 (46%) |
| IL28B CC, N (%) | 9 (26.4%) | 26 (34%) |
| IL28B CT, N (%) | 19 (56%) | 38 (50%) |
| IL28B TT, N (%) | 6 (17.6%) | 12 (16%) |

IL28B genotyping was available in 34 and 76 patients in the 2a and 2b groups, respectively.

Table 2 shows the median HCV-RNA drop (log10 IU/mL) at different time points according to the Peg-IFN subtype used.

Table 2
<table>
<thead>
<tr>
<th>Peg-IFN alpha 2a (N=85)</th>
<th>Peg-IFN alpha 2b (N=102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline viremia</td>
<td>6,003</td>
</tr>
<tr>
<td>Drop at 48/72 h</td>
<td>1,215</td>
</tr>
<tr>
<td>Drop at 7 days</td>
<td>1,253</td>
</tr>
<tr>
<td>Drop at 14 days</td>
<td>2,005</td>
</tr>
<tr>
<td>Drop at 28 days</td>
<td>3,043</td>
</tr>
</tbody>
</table>

A rebound in viremia at day 7 of treatment was observed in 29% and 66% of patients treated with Peg-IFN alpha 2a and 2b, respectively (p = 0.014).

Conclusions: The early kinetics of HCV-RNA decline is different according to the Peg-IFN subtype used. This should be taken into account in response-guided treatment decision making.

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TELAPREVIR SERUM LEVELS DURING TREATMENT FOR HCV GT1 INFECTION ARE ASSOCIATED WITH EXTENDED RAPID VIROLOGIC RESPONSE BUT DO NOT AFFECT HEMOGLOBIN DECLINE

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Background and Aim: The current standard of care for the treatment of HCV genotype 1-infection is a triple combination therapy that includes an HCV protease inhibitor (e.g. telaprevir; TVR) and the peginterferon/ribavirin backbone. In the TVR registration trials, response rates were significantly higher compared to peginterferon/ribavirin alone. However, the rate of side effects, including anemia, was also significantly increased. We aimed to investigate the impact of TVR serum levels on treatment response and anemia development in patients undergoing TVR-based triple-therapy.

Methods: Thirty-seven patients (mean age: 54±13; male gender: 59.5%; treatment-naive: 16%; cirrhosis: 65%) with HCV genotype 1-infection were treated with a TVR-based triple-therapy regimen according to the current EASL guidelines. TVR serum levels were analyzed 4 hours after intake by liquid chromatography electrospray-ionization-tandem mass spectrometry at week 2 of antiviral therapy. On-treatment HCV-RNA response was assessed at week 4, 12 and 24 by real-time PCR.

Results: An extended rapid virologic response (eRVR; defined as negative HCV-RNA at week 4 through week 12) was achieved in 15/37 patients (40.5%). Significant anemia (defined as hemoglobin decline >3 g/dl at week 4) occurred in 43% of patients. Mean ±SD TVR serum levels at week 2 were 3.36±0.21 log10ng/ml and did not differ over time (when assessed at weeks 4, 8 and 12). TVR serum levels at week 2 were significantly higher in patients who achieved an eRVR compared to those who did not achieve an eRVR (3.44±0.17 vs. 3.30±0.22 log10ng/ml; p = 0.036). In contrast, high TVR serum levels were not associated with week 4 anemia (absolute hemoglobin reduction; hemoglobin decline >3 g/dl).

Conclusions: TVR serum levels are associated with on-treatment response. However, TVR serum levels are not significantly associated with distinct anemia, which occurs in almost half of TVR-treated patients.
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and statistical analysis was performed with SPSS 19.0 (level of significance of \( p < 0.05 \)).

**Results:** No difference was found comparing TNF-\( \alpha \) G-308A promoter polymorphism (GA, AA or GG genotype carriers) with all the clinical, histological and laboratory data of the 156 patients studied. A trend to an increase of sustained response (SR) was observed in patients carrying TNF-\( \alpha \) GA or AA genotypes (SR = 87%, NR+RR = 13%), when compared to GG genotype (SR = 61.5%, NR+RR = 38.5%), \( p = 0.054 \). Comparing the two groups (SR vs NR+RR), any difference was found in terms of HCV genotype, liver fibrosis, steatosis and all the clinical parameters studied.

**Conclusions:** These data seems to suggest an independent role of TNF-\( \alpha \) G-308A promoter polymorphism on the type of response to standard antiviral therapy in chronic HCV infection. The allele A of TNF-\( \alpha \) G-308A promoter polymorphism influenced sustained virological response.

**815 FACTORS ASSOCIATED WITH INTOLERANCE TO PEGINTERFERON \( \alpha \)-RBV IN TREATMENT-NAÏVE, CIRRHOTIC/NON-CIRRHOTIC HCV GENOTYPE 1-INFECTED PATIENTS: ANALYSIS OF DATA FROM THE MULTINATIONAL PROPHESYS COHORTS**

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**Aim:** To estimate rates of intolerance to PegIFN/RBV and identify factors associated with intolerance in genotype 1 (G1) patients who received PegIFN/RBV in the multinational PROPHESYS cohorts.

**Methods:** Rates of intolerance to PegIFN/RBV were calculated for treatment- naïve, G1 patients stratified by known cirrhosis status (yes/no) who received PegIFN/RBV in the non-interventional PROPHESYS cohorts. Treatment intolerance was defined as premature discontinuation for: adverse events or laboratory abnormalities (AE/LA); other reasons (failure to return, refused treatment etc); ≥35% reductions in PegIFN/RBV dose for AE/LA; or death.

**Results:** A total of 3351 patients were included (mean age, 48yr; 53% male; 89% white; mean weight 75kg; mean HCV-RNA, 6.0-log10 IU/mL). A similar proportion of cirrhotic (317/1088, 29.1%) and non-cirrhotic patients (647/2263, 28.6%) had intolerance to PegIFN/RBV. Kaplan–Meier estimates of the rate of intolerance by genotype 1 (G1) patients stratified by known cirrhosis status (yes/no) who received PegIFN/RBV in the non-interventional PROPHESYS cohorts. Treatment intolerance was defined as premature discontinuation for: adverse events or laboratory abnormalities (AE/LA); other reasons (failure to return, refused treatment etc); ≥35% reductions in PegIFN/RBV dose for AE/LA; or death.

**Results:** A total of 3351 patients were included (mean age, 48yr; 53% male; 89% white; mean weight 75kg; mean HCV-RNA, 6.0-log10 IU/mL). A similar proportion of cirrhotic (317/1088, 29.1%) and non-cirrhotic patients (647/2263, 28.6%) had intolerance to PegIFN/RBV. Kaplan–Meier estimates of the rate of intolerance by genotype 1 (G1) patients stratified by known cirrhosis status (yes/no) who received PegIFN/RBV in the non-interventional PROPHESYS cohorts. Treatment intolerance was defined as premature discontinuation for: adverse events or laboratory abnormalities (AE/LA); other reasons (failure to return, refused treatment etc); ≥35% reductions in PegIFN/RBV dose for AE/LA; or death.

**Conclusions:** Cirrhosis at baseline was not associated with higher rates of intolerance to PegIFN/RBV compared with non-cirrhotic patients; however, markers of cirrhosis (higher AST, lower ALT, lower platelets) were associated with intolerance. Intolerance to PegIFN/RBV was associated with lower SVR-24 rates and higher relapse rates in both cirrhotic and non-cirrhotic patients.

**816 RIBAVIRIN DOSE REDUCTION DURING TELAPREVIR CONTAINING TRIPLE THERAPY DOES NOT AFFECT EARLY VIROLOGIC RESPONSE IN NON-RESPONDERS AND RELAPSERS WITH ADVANCED LIVER FIBROSIS**


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**Background and Aims:** Anemia is well known adverse event related to triple therapy with telaprevir (TVR), peginterferon (P) and ribavirin (R). As demonstrated previously, ribavirin dose reduction (RDR) does not affect efficacy of triple therapy in naïve and low-fibrotic patients. However it was not confirmed in non-responders and relapers with advanced fibrosis. The aim of this study was to demonstrate effect of RDR in such a difficult to treat population receiving TVR+P/R in real-life settings of 16 sites involved in named patients program (AdvEx study).

**Methods:** The study was carried out in 205 chronic HCV genotype 1 infected, P/R experienced patients (127 males, 78 females, aged 20–75 years) and included 98 (48%) prior P/R null-responders (NR), 27 (13%) partial-responders (PR) and 80 (39%) relapers (REL). All patients had liver cirrhosis or bridging fibrosis (F3/F4) confirmed by liver biopsy or elastography and received TVR plus P/R at standard doses. The interventions to counteract anemia were: RDR and/or blood transfusion. Erythropoietin administration was not allowed. At the moment of this ITT analysis, week 12 data were available.

**Results:** Complete early virologic response (cEVR) rate was 83% (NR-75%, PR-86%, REL-92%). RDR was applied in 91 (44%) and blood transfusion ±RDR in 20 patients (10%) without difference in cEVR rate (79% vs. 83%). There was no statistically significant difference between cEVR rates in patients who received full ribavirin dose (all-88%; NR-84%, PR-79%, REL-94%) or RDR (all-82%; NR-74%, PR-92%, REL-93%), but it was significantly lower if RDR was carried-out within initial 4 weeks (50%) compared to RDR between week 4 and 12 (83%, \( p = 0.03 \), particularly among NR (44% vs 75%, \( p = 0.04 \)). None of 7 subjects who discontinued ribavirin and TVR achieved cEVR. Patients with the lowest on-treatment Hb \( <12g/dL \) (86%) achieved significantly higher cEVR rate than those with Hb \( \geq 12g/dL \) (66%, \( p = 0.019 \)).

**Conclusions:** In difficult to treat population of treatment experienced patients with advanced liver fibrosis ribavirin dose
reduction did not affect cEVR rate. However it decreased significantly if ribavirin dose reduction was performed within initial 4 weeks of treatment. Anemia seems to be a positive predictor of cEVR in this population.

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**RANDOMISED CONTROLLED TRIAL OF 24 AND 48 WEEKS OF PEGYLATED INTERFERON alfa 2a PLUS RIBAVIRIN IN PATIENTS WITH GENOTYPE 3 HCV AND CIRRHOSIS**

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**Introduction:** Chronic infection with genotype 3 HCV is regarded as ‘easy-to-cure’. However patients with genotype 3 HCV and cirrhosis respond poorly. We evaluated extending therapy in patients with genotype 3 HCV and advanced fibrosis.

**Methods:** This was a multicentre study. Patients with genotype 3 HCV and advanced fibrosis (liver biopsy showing Ishak fibrosis stage 4, 5 or 6 or radiological features of cirrhosis) were randomised to receive either 24 or 48 weeks of Pegylated interferon alfa 2a 180 μg per week and 800 mg ribavirin. Virological responses were assessed using sensitive PCR based assays with a lower limit of quantification of less than 30 IU/ml. The trial was powered to detect a 20% increase in sustained virological response (SVR) and a total of 140 patients were required.

**Results:** 13 UK sites screened 159 patients – 141 were randomised. Four did not commence therapy (excluded from the modified intent to treat analysis presented). Mean age was 48 years (28–74) Results of the initial analysis (missing data = failure) are shown. There was no significant difference in the two arms (p>0.05 for all analyses). Treatment was reasonably well tolerated with side effects in line with previous studies. Analysis of light weight patients who received a higher dose of ribavirin per Kg did not indicate that higher doses of ribavirin were beneficial. Interim analysis of IL-28 genotyping suggests no effect of IL-28 on response.

**Table:** Response in patients with G3 HCV and cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>24 weeks</th>
<th>48 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number randomised</td>
<td>71</td>
<td>66</td>
</tr>
<tr>
<td>Number (%) without RVR</td>
<td>37 (52%)</td>
<td>33 (50%)</td>
</tr>
<tr>
<td>Number (%) without RVR who achieved SVR</td>
<td>10 of 37 (27%)</td>
<td>7 of 33 (21%)</td>
</tr>
<tr>
<td>End of treatment response</td>
<td>55 (77%)</td>
<td>45 (68%)</td>
</tr>
<tr>
<td>Sustained virological response</td>
<td>36 (50%)</td>
<td>34 (51%)</td>
</tr>
<tr>
<td>Withdrew in first 24 weeks</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Withdrew in second 24 weeks</td>
<td>NA</td>
<td>10</td>
</tr>
</tbody>
</table>

**Conclusion:** In patients with genotype 3 HCV and cirrhosis extending therapy beyond 24 weeks is of no value. It will be interesting to see whether extension of therapy in patients with direct acting antiviral agents improves response rates in ‘hard-to-cure’ populations.

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**HCV-TARGET: A LONGITUDINAL, OBSERVATIONAL STUDY OF NORTH AMERICAN PATIENTS WITH CHRONIC HEPATITIS C (HCV) TREATED WITH BOCEPREVIR OR TELAPREVIR**


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Phase III trials of boceprevir and telaprevir for HCV provided important information on treatment response, although data is lacking for certain groups, especially those with “difficult to cure” characteristics such as African Americans and cirrhosis. The aim of HCV-TARGET is to evaluate the effects of triple therapy in the broader population now being treated in the United States and Canada, including those underrepresented in clinical trials.

**Methods:** The HCV-TARGET consortium of academic and community investigators utilizes novel, standardized source data abstraction and a common database to enroll sequential patients treated with regimens that include boceprevir and telaprevir. Demographic, clinical, adverse event, and virological data are collected throughout treatment and post-treatment follow-up. Whole blood for DNA and serum from specified time points are stored at a central biorepository.

**Results:** In this ongoing study, 1068 participants have been enrolled to date of whom 816, at varying stages of treatment (telaprevir 77%, boceprevir 23%), are included in this preliminary analysis. The majority are male (60%), Caucasian (76%), and between ages 40–64 years (84%), African Americans comprised 20% of participants and 7% of patients were older than 65 years. Cirrhosis, defined by biopsy or clinical criteria, was present in 35%. Genotype 1a/1b/not subtyped was 61%, 22%, 11%, respectively, and 49% were treatment naive. Adverse events (AE) requiring medical intervention or dose modification to the antiviral regimen occurred in 76%. Anemia, occurring in 56% with nadir hemoglobin ≤8.5 g/dl (12%) or 8.5–10 g/dl (26%), was managed with ribavirin dose reduction (45%), EPO (15%), and/or transfusion (8%). Rash was noted in 29% while 1% discontinued treatment due to rash. A new decompensating event (ascites, encephalopathy, variceal hemorrhage) occurred in 11 (4%) cirrhotic patients. Treatment was prematurely discontinued due to AE in 5% of non-cirrhotic and 10% of cirrhotic patients. Data collection is ongoing and SVR data will be presented.

**Conclusions:** HCV-TARGET encompasses the North American experience with boceprevir and telaprevir across a broad spectrum of clinical practices in a cohort enriched with African American patients and cirrhosis. Continuing analyses will inform clinicians regarding these previously underrepresented populations and best practices for managing adverse events.
819 IMPROVED PHARMACODYNAMICS AND PHARMACOKINETICS AFTER INTRAVENOUS APPLICATION OF PEG-INFERNON alfa-2a 180 μg IN CHRONIC HEPATITIS C GENOTYPE 1 NULLRESPONDERS TO PEG-INFERNON/ribavirin. THE IVAN STUDY

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Background: Mechanisms of PEG-IFNα nonresponsiveness are not completely understood. Peg-IFNα plasma concentrations show great variability after subcutaneous (s.c.) application in some patients. Inadequate PEG-IFNα drug levels may be one factor of reduced response to combination treatment which might be adjusted by intravenous (i.v.) application. Aim of our study was to evaluate pharmacodynamics and pharmacokinetics of i.v. vs. s.c. administration of PEG-interferon alfa-2a.

Methods: Prospective, randomized, open-label, multicenter, cross over, phase 1 study. 29 genotype1 patients with prior null response to PEG-IFNα/ribavirin (21 male (72%), mean age 54 years (range 38–69), median liver stiffness 14kPa (range 4.6–41), IL28B response to PEG-IFNα/ribavirin (21 male (72%), mean age 54 years (range 38–69), median liver stiffness 14kPa (range 4.6–41), IL28B rs12979860 T allele carriers 93%, were randomized to 4 treatment arms: PEG-IFNalfa-2a 180μg once weekly for two weeks either s.c. (A) or i.v. (B), whereas arm C (s.c.) and D (i.v.) started twice weekly. After wash out of 6 weeks patients who started in s.c. arms (A/C) switched to i.v. regimen, and vice versa (B/D). Early pharmacodynamic and pharmacokinetic measurements were performed.

Results: Intravenous application of PEG-IFNalfa-2a lead to significantly stronger decline in HCVRNA at day1 as compared to s.c. administration (1.0 log10IU/mL (range −0.3–2.3) vs. 0.2 log10IU/mL (−0.43–0.76); p < 0.001). HCVRNA nadir achieved on treatment was 1.3 log10 in i.v. vs. 0.4 log10 in s.c. arms (p = 0.01). Rebound in mean HCVRNA levels was seen in all treatment arms. PEG-IFNalfa-2a through levels were significantly higher after i.v. (23.643 pg/ml (range 125–43.600) once weekly, 29.155 pg/ml (range 6.010–49.100) twice weekly) vs. s.c. (4.125 pg/ml (range 125–15.100) o.w., 4.333 pg/ml (range 850–11.500) t. w.) as were PEG-IFNalfa-2a AUC0–12h and AUC0–7d i.v. (AUC0–12h: 23.643 pg/ml*h (range 125–43.600) twice weekly) vs. s.c. (4.125 pg/ml*h (range 125–15.100) t. w.) with AUC0–12h and AUC0–7d i.v. (AUC0–12h: 6.010–49.100) twice weekly). PEG-IFNalfa-2a AUC0–12h and AUC0–7d i.v. (AUC0–12h: 6.010–49.100) twice weekly) vs. s.c. (4.125 pg/ml*h (range 125–15.100) t. w.) with AUC0–12h and AUC0–7d i.v. (AUC0–12h: 6.010–49.100) twice weekly). Peg-IFNα plasma concentrations show great variability after subcutaneous (s.c.). application in some patients. Inadequate PEG-IFNα drug levels may be one factor of reduced response to combination treatment which might be adjusted by intravenous (i.v.) application. Aim of our study was to evaluate pharmacodynamics and pharmacokinetics of i.v. vs. s.c. administration of PEG-interferon alfa-2a.

Conclusions: Intravenous administration of PEG-IFNalfa-2a lead to significantly more pronounced decline in HCVRNA and higher PEG-IFNalfa-2a AUC as compared to s.c. application in null responders implicating that null response might be explained by inadequate drug levels and can be overcome by changing the mode of application. These findings may have implications to improve PEG-IFNα backbone activity for null responders awaiting DAA triple regimens.

820 THERAPEUTIC DRUG MONITORING OF TELAPREVIR IN CHRONIC HEPATITIS C PATIENTS RECEIVING TELAPREVIR-BASED TRIPLE THERAPY IS USEFUL FOR PREDICTING VIROLOGICAL RESPONSE AND AVOIDING TOXIC DRUG-EXPOSURE

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Background and Aims: Telaprevir (TVR) in combination with pegylated interferon α (PEG-IFNa) and ribavirin (RBV) has resulted in improved sustained virological response (SVR) rates, compared to PEG-IFNa plus RBV therapy, but increased adverse effects such as anemia, malaise, and rash. A steady state after multiple doses of orally administered TVR is reached after 3 to 7 days of administration. An adequate plasma trough concentration (C-tough) of TVR is required to maintain antiviral activity, optimize the dosage, and avoid toxic drug-exposure. The aim of this pharmacokinetic study was to investigate the impact of the TVR C-tough on therapeutic response and adverse effects.

Methods: We analyzed the data of 70 chronic hepatitis C patients with genotype 1 infection (treatment naïve 31.4%, prior relapsers 41.4%, and prior non-responders 27.2%). All patients received 12-week triple therapy that included TVR (2250mg/day), PEG-IFNa2b (60–150μg/week) and RBV (600–1000mg/day) followed by a 12-week dual therapy that included PEG-IFNa2b and RBV. The C-tough of TVR was determined by a validated assay using high-performance liquid chromatography at days 3, 7, and 14 and every two weeks after during the first 12 weeks of treatment. The serum HCVRNA level was measured by COBAS TaqMan HCV test. The intention-treat-analysis was done for 12 weeks after the start of therapy.

Results: The rates of undetectable HCV RNA at weeks 4 (RVR) and 12 (SVR12W) were 71.8% and 84.3%, respectively. Of the patients with RVR, 88% achieved an SVR12W. The mean C-tough of TVR of the SVR patients at day 3, day 7, and week 8 (2804, 2747, and 2845ng/mL, respectively) was significantly higher than that of the non-SVR patients (1648, 1805, and 1522ng/mL, respectively) (all P < 0.05). A significant difference in the mean C-tough of TVR at week 4 (3419 and 2743ng/mL) (P < 0.05) was observed between patients with and without anemia (hemoglobin <100g/L) at week 8. The C-tough of TVR was not correlated with malaise or rash.

Conclusions: Therapeutic drug monitoring of TVR for patients receiving TVR-based triple therapy is useful in clinical practice for predicting virological response and avoiding toxic drug exposure.

821 INTERFERON (IFN)-FREE ALISPORIVIR (ALV) HAS A BETTER OVERALL SAFETY PROFILE COMPARED TO IFN-CONTAINING TREATMENT: A POOLED ANALYSIS OF THE ALV DEVELOPMENT PROGRAM

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Background: Alisporivir (ALV) is the most advanced pangenotypic cyclophilin inhibitor in development for treating chronic hepatitis C. This pooled analysis reviews the safety profile of ALV as IFN-free treatment vs combination therapy with IFN and peginterferon/ribavirin (P/R).

Methods: 2,153 patients were randomized to one of 4 studies; 1,664 received at least one dose of ALV. Population included G1
naive and non-responder patients (treated with ALV/P/R at several doses); and G2/J naive patients (treated with ALV ± IFN).

**Results:** Adverse events occurred at lower rate with IFN-free ALV vs ALV/P/R or P/R control. Serious Adverse Events occurred in only 1.9% of patients in IFN-free groups vs 7.7% in ALV/P/R and 4.7% in P/R control. IFN-free SAEs were all single events and only 1, vomiting/diarrhea, was suspected related to ALV. ALV/P/R most common SAEs were hypertension, pneumonia and pancreatitis. In the P/R arm most common SAEs were GI or general disorders. There were no grade 4 hematologic abnormalities with IFN-free ALV; rare grade 3 abnormalities (1 neutropenia, 3 thrombocytopenia) vs 19% and 27% Gr3 neutropenia, 2% and 12% Gr3 thrombocytopenia and 1% and 3% Gr3 anemia with P/R and ALV/P/R respectively. Pancreatitis occurred in 7 patients receiving ALV/P/R, 1 patient in P/R control arm and 1 patient on P/R 10 weeks after discontinuing ALV. Elevations of triglycerides >ULN were observed in 6 cases prior to onset of pancreatitis (>4.5 mmol/L in one patient and nearly/above 11 mmol/L in 2 patients). ALV has been shown to be associated with increase in triglycerides, exacerbated by co-administration of IFN. At Week 24 mean change from baseline was 1.06 and 0.97 mmol/L for ALV/P/R and IFN-free ALV groups vs 0.47 mmol/L for P/R. There were no cases of pancreatitis with IFN-free ALV.

**Conclusions:** IFN-free ALV treatment is a well-tolerated treatment and shows only modest changes in hematological parameters vs IFN containing treatment. IFN-free ALV is associated with less AEs and SAEs than IFN containing ALV regimens or P/R and no pancreatitis cases were reported with IFN-free ALV treatment. ALV treatment results in modest increase in TGs which is more marked when IFN is added.

**822 NEUTROPENIA COMORBIDITY IN HEPATITIS C INFECTION (HCV) AND RISK OF SERIOUS INFECTION AFTER INTERFERON TREATMENT**


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**Background and Aims:** Neutropenia is a dose-dependent side effect of interferon based therapies. To date, interferon-induced neutropenia has not been demonstrated to increase risk of infections in HCV patients. This study evaluated the comorbidity of neutropenia in the general population and the risk of serious infection, anemia, and depression in neutropenic, HCV patients treated with interferon.

**Methods:** U.S. administrative claims data from Optum Clinformatics Data Mart was used. Beneficiaries diagnosed with neutropenia between 2008–2009 with no prior diagnosis of neutropenia for 1 year of continuous enrollment prior were identified. Cases were matched 1:1 by age and sex to a random sample of non-neutropenic controls. Descriptive analyses of comorbidities and logistic regressions were conducted. A second analysis used data from 2000–2009. Beneficiaries with 2 HCV diagnostic codes (excluding hepatitis coma), at least 1 year of enrollment, a neutropenia diagnosis 12 months post-interferon/ribavirin initiation, 12 months eligibility pre- and post-neutropenia diagnosis date (index), and no depression diagnosis prior to index were identified. HCV controls without neutropenia were matched by HCV treatment initiation date and days supply. Risk ratios for serious infections based on ICD-9 codes using a validated definition, anemia, and depression were calculated for the year post-index.

**Results:** A total of 6,472 neutropenic patients were identified in the general population and matched to non-neutropenic controls.

Of the comorbidities evaluated, 89% (48/54) were significantly elevated in the neutropenic population compared to controls. Specifically, the neutropenic cohort had a higher odds of HIV [OR=2.6 (1.3–5.4)], HCV [3.1 (1.8–5.3)], and HCV treatment with interferon-based therapy [OR=4.5 (2.0–10.4)]. In the second analysis, 644 patients met entry criteria and were matched to controls. Compared to treated HCV patients without neutropenia, those diagnosed with neutropenia had 223% increased risk of anemia [RR 3.23 (95%CI=2.69–3.88)], 51% increased risk of serious infection [RR 1.51 (95%CI=1.12–2.05)], and no increased risk of depression.

**Conclusion:** Neutropenic patients with HCV treatment in the prior 12 months were at increased risk for anemia and serious infection. This increased risk of serious infection in a large retrospective database is contrary to prior published literature in smaller patient cohorts.

**823 MODELING EARLY VIRAL KINETICS WITH ALISPORIVIR: INTERFERON-FREE TREATMENT AND SVR PREDICTIONS IN HCV G2/J PATIENTS**

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**Background:** Alisporivir (ALV) is a cyclophilin inhibitor with pan-genotypic activity against hepatitis C virus (HCV). We characterized viral kinetics (VK) in 249 patients infected with HCV genotypes 2 or 3 during treatment with Alisporivir interferon-free regimens for six weeks and 800 mg ribavirin (RBV) daily.

**Methods:** We used a VK model that integrated pharmacokinetic (PK) and pharmacodynamic effects to analyze patient data as well as to predict the effect of different doses of ALV twice a day with RBV on the sustained virologic response (SVR) rate.

**Results:** The VK model was able to fit the individual viral load profiles of 214 (86%) patients by assuming that ALV blocked viral production. A mean antiviral effectiveness of 0.93, 0.86 and 0.75 in patients treated with 1000, 800 and 600 mg ALV QD, respectively was estimated. Patients receiving RBV had a significantly faster rate of viral decline, which was attributed in our model to an effect of RBV in increasing the loss rate of infected cells, δ (mean δ = 0.35 δ0 vs 0.21 δ0 in patients +/– RBV, respectively, P=0.0001). The remaining 35 patients (14%) had a suboptimal response (i.e. flat or increasing levels of HCV RNA after week 1), and their viral kinetic profile was not described using the model. The occurrence of this suboptimal response was higher in patients that received ALV monotherapy than those receiving ALV+RBV (21.5 vs 10.5%, P = 0.02).

Moreover, high body weight and low RBV levels were associated with suboptimal response (in patients receiving RBV). There was a trend for low exposure to ALV to be associated with suboptimal response as well, suggesting that high RBV and ALV exposures are important in reducing the suboptimal response rate. The model predicts 73% SVR following 400 mg ALV BID + 400 mg RBV BID for 24 weeks. The predicted SVR rate following response-guided therapy was 79%.

**Conclusion:** Alisporivir 400 mg BID plus RBV may represent an effective IFN-free treatment that is predicted to achieve high SVR rates in genotypes 2 or 3 patients. Response-guided therapy would further increase SVR. In addition, weight-based RBV dosing should be considered to prevent suboptimal exposure.
IMPACT OF IL28B GENOTYPE ON HCV TREATMENT DECISION IN A LARGE FRENCH COHORT

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Background: IL28B genotype is a strong predictor for treatment induced hepatitis C virus (HCV) clearance. The French guidelines recommend IL28B genotype in naive HCV patients. However, its impact on treatment decision in real life practice is unknown.

Patients and Methods: Since October 2011, we conducted a prospective, multicenter study aimed to evaluate IL28B genotype decision (SNP rs12979860) in 1000 French HCV, genotype 1 or 4 patients (G1/4). At baseline physician’s intention to treat in the year was collected. On November 1st 2012, when 904 patients were enrolled, treatment decisions were recorded and matched with initial intentions. We report analysis in patients with persistent chronic HCV infection and at least 3 months between IL28B genotype and treatment information.

Results: 433 patients were analyzed in this interim study: 53% were treatment naïve, 8% were G1, 37% were cirrhotic (F3/4); 15% were H–HCV coinfected. At baseline, physician’s intention to treat was NOT for 150 (35%) and YES for 283 (55%) patients. In comparison we noted in YES group less G4 (7% versus 13%, p=0.03), more G3 (43% versus 28%, p=0.004), less coinfected patients (10% versus 25%, p<0.0001), but no difference for naïve patients. This intention was respected for 79.3% (119/150) of patients in NOT group and for 70.6% (200/283) of patients in YES group (p=0.07). In this initial NOT group, finally physicians treated more IL28CC (39% versus 17%, p=0.02) than in untreated patients and more patients treated in liberal setting (68% versus 35%, p=0.001), but no difference according to HCV genotype, fibrosis or coinfection. In the initial YES group finally untreated patients in comparison with treated ones had more often no or moderate fibrosis F0/2 (67% versus 53%, p=0.03); also a trend of more untreated patients was observed after May 2012 (28% versus 18%, p=0.05), explained by the EASL decision in improving the patient profile.

Conclusion: Our study highlights no negative impact of IL28B genotype on HCV treatment intention in real live practice. Moreover, this test seems to be helpful for some clinicians in HCV treatment decision in improving the patient profile.

ANALYSIS OF THE OPTIMAL TVR DOSE PER WEIGHT JUDGING FROM ANEMIA DURING PEG-IFN/RBV/TVR COMBINATION THERAPY

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Background and Aims: The triple therapy including TVR has strong anti-HCV action, but mainly because of severe anemia the dose modification of ribavirin RBV or TVR is often necessary. We reported a possibility that in patients with low total clearance of ribavirin, the standard dosage of TVR (2250mg/day) may impair the total dose of RBV and clinical effect of triple therapy (2012 EASL). A purpose of this study is to examine the relation among TVR dosage per body weight (mg/kg) and anemia and clinical outcome.

Methods: We enrolled 25 treatment naïve patients, 44 relapers and 25 non-responders of previous PEG-IFN/RBV therapy with HCV genotype 1 and high viral load. The subject details were as follows: males 55.3%; IL28B at rs12979860 CC 53.2%, ITPA at rs1127354 CC 79.8%, median age 56.5 years old, body weight (BW) 61 kg, Hb 14.3 g/dl, platelet 17.7 x 10^4/mm3. The therapy protocol was 12 weeks of triple therapy and then 12 weeks of dual therapy. PEG-IFN and RBV were assigned by BW base and TVR was assigned fixed dose of 2250mg/day. In case of severe anemia, a dose modification of RBV was introduced. When the Hb level decreased to less than 8.5 g/dl, we discontinued all three drugs including TVR.

Results: The factors that contributed to a decline of Hb to less than 8.5 g/dl was TVR dose per weight (≥3370 mg/kg/370 mg/kg; P=0.003) and age (≥55y/o/≤54y/o: P=0.050) for the multivariate analysis. By ROC curve analysis, AUC of TVR dose per weight was 0.730 and cutoff value was 38.7 mg/kg. Cases with more than 38.7 mg/kg of TVR (high TVR mg/kg; N=44) showed higher TVR discontinuation rate (37.5%) compared with cases with less than 38.7 mg/kg (low TVR mg/kg; N=50) (5.6%) (P<0.001). As for outcome of therapy, limiting to non-CC cases, SVR rate were 42.1% (8/19) in high TVR mg/kg cases and 56% (14/25) in low TVR mg/kg cases (P=0.3612).

Conclusions: In triple therapy, high dosage of TVR per weight leads to severe anemia and discontinuation of TVR. Optimal TVR dose should be given in each case to achieve a better clinical outcome.
subgroups (Table). 56/103 (54%) patients with cirrhosis attained RVR (undetectable HCV RNA at week 4) and were treated for a total of 24 weeks. Of these 51/56 (91%) achieved eRVR (undetectable HCV RNA at weeks 4 and 12) and of these 51 patients 36 (71%) achieved SVR.

### Methods:

that to evaluate the effect of vitamin B12 on virological response hepatitis C virus (HCV) replication in invitro conditions. We aimed

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S. Hülagü, G. Sirin, O. Sentürk, A. Celebi, G. Dindar, H. Yılmaz.

### Conclusions:

respectively; anemia: 50% vs 44%, respectively). Similar for patients with and without cirrhosis (rash: 50% vs 53%, respectively. Total incidences of rash and anemia events were cirrhosis, and 8% (48/636) and 16% (104/636) of those without,

### Grade 3 or 4 adverse events (AEs) were reported in 41% (42/103) of patients with and 40% (252/636) of patients without cirrhosis (TVR phase). Serious adverse events (SAEs) and TVR discontinuations due to AEs occurred in 14% (14/103) and 21% (22/103) of patients with cirrhosis, and 8% (48/636) and 16% (104/636) of those without, respectively. Total incidences of rash and anemia events were similar for patients with and without cirrhosis (rash: 50% vs 53%, respectively; anemia: 50% vs 44%, respectively).

### Conclusions:

The relative efficacy of TVR bid versus q8h was similar regardless of fibrosis/cirrhosis stage, offering the potential of simplified TVR dosing to all patients, including those with cirrhosis.

### 827

**DOES VITAMIN B12 SUPPLEMENTATION IMPROVE SUSTAINED VIRAL RESPONSE RATES IN PATIENTS WITH CHRONIC HEPATITIS C?**

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### Background and Aims:

Vitamin B12 acts as a natural inhibitor of hepatitis C virus (HCV) replication in invitro conditions. We aimed that to evaluate the effect of vitamin B12 on virological response in hepatitis C virus infected patients in this study.

### Methods:

Thirty-two patients with Chronic Hepatitis C (CHC) were randomly assigned to receive pegylated interferon alpha-2a (Peg-IFN) plus ribavirin (Standard dual antiviral therapy;SDAT) or STAD plus vitamin B12 (STAD+B12). Viral response, as, undetectable serum HCV RNA was assessed 4 weeks after starting therapy (rapid viral response;RVR), 12 weeks after starting therapy (complete early viral response; eRVR) and 24 (for genotype 2) or 48 (for genotype 1) weeks after starting therapy (end of treatment viral response;ETVR), and 24 weeks after completing therapy (sustained viral response; SVR). Genotyping for the interleukin 28 B (IL-28 B) polymorphism was performed in all of the HCV genotype 1 patients (31/33).

### Results:

Distribution of genotype IL-28 B did not differ between the two groups. RVR and SVR difference were not detected between the two groups of patients. eRVR (0.07) and ETVR (0.09) were minimally higher in STAD+B12 group; but this was not statistically significant. The SVR rate was significantly higher in carriers of IL-28 B CC genotype polymorphism and in patients who have low baseline viral load.

### Conclusion:

Vitamin B12 supplementation does not significantly improves SVR rates, although cEVR and ETVR were minimally higher, but were not statistically significant, in STAD+B12 group.

### 828

**ROLE OF HEPATITIS C VIRUS SUBSTITUTIONS AND INTERLEUKIN 28B POLYMORPHISM ON RESPONSE TOPEGINTERFERON PLUS RIBAVIRIN IN A PROSPECTIVE STUDY OF RESPONSE-GUIDED THERAPY**

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### Background:

Recent studies have indicated that amino acid (aa) substitutions in the core region and NSSA interferon sensitivity-determining region (ISDR) of hepatitis C virus (HCV) as well as genetic polymorphisms in the interleukin-28B (IL28B) locus affect the outcome of interferon (IFN)-based therapies. We aimed to investigate the role of these factors on response to peginterferon plus ribavirin in a prospective study of response-guided therapy.

### Methods:

The aa sequences in core region and ISDR and rs12979860 genotypes were analyzed in 115 HCV-1 patients. The treatment was 24 weeks for patients achieving a rapid virological response (RVR), 48 weeks for those with an early virological response (EVR), and early terminated in those without an EVR.

### Results:

A sustained virological response (SVR) was achieved in 82% of 34 RVR patients, 45% of 74 EVR patients and 0% of 7 non-EVR patients. Logistic regression analysis showed that ISDR mutations (≥2) (odds ratio[OR]: 0.024), double core 70/91 mutations (OR: 0.136), and platelet counts≥150,000/μL (OR: 3.119) were independent pretreatment factors associated with SVR. Apart from rs12979860 CC genotype, low viral load and ISDR mutation (≥2) were significant factors predictive of RVR. Combination of rs12979860 genotype and baseline viral characteristics (viral load and core/ISDR mutations) could predict RVR and SVR with positive predictive value of 100% and 91%, and negative predictive value of 80% and 54%, respectively.

### Conclusion:

Pretreatment screening rs12979860 genotype and aa substitutions in the core region and ISDR could help identifying patients who are good candidates for peginterferon plus ribavirin therapy.

### 829

**IFN-BASED HCV-TREATMENT SEEMS TO REDUCE MUSCLE MASS**

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### Background and Aims:

The treatment of chronic Hepatitis C with Interferon is known to cause a wide variety of side effects such as diarrhea, nausea and a generally reduced well-being. In some cases the interferon dosage had to be reduced to stop patients lose weight. The aim was to clarify wether weight reduction is due to IFN-based HCV-treatment or due to some other factors in the study.

### Methods:

Thirty-two patients with Chronic Hepatitis C (CHC) were randomly assigned to receive pegylated interferon alpha-2a (Peg-IFN) plus ribavirin (Standard dual antiviral therapy;SDAT) or STAD plus vitamin B12 (STAD+B12). Viral response, as, undetectable serum HCV RNA was assessed 4 weeks after starting therapy (rapid viral response;RVR), 12 weeks after starting therapy (complete early viral response; eRVR) and 24 (for genotype 2) or 48 (for genotype 1) weeks after starting therapy (end of treatment viral response;ETVR), and 24 weeks after completing therapy (sustained viral response; SVR). Genotyping for the interleukin 28 B (IL-28 B) polymorphism was performed in all of the HCV genotype 1 patients (31/33).

### Results:

Distribution of genotype IL-28 B did not differ between the two groups. RVR and SVR difference were not detected between the two groups of patients. eRVR (0.07) and ETVR (0.09) were minimally higher in STAD+B12 group; but this was not statistically significant. The SVR rate was significantly higher in carriers of IL-28 B CC genotype polymorphism and in patients who have low baseline viral load.

### Conclusion:

Vitamin B12 supplementation does not significantly improves SVR rates, although cEVR and ETVR were minimally higher, but were not statistically significant, in STAD+B12 group.

### 828

**ROLE OF HEPATITIS C VIRUS SUBSTITUTIONS AND INTERLEUKIN 28B POLYMORPHISM ON RESPONSE TOPEGINTERFERON PLUS RIBAVIRIN IN A PROSPECTIVE STUDY OF RESPONSE-GUIDED THERAPY**

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### Background:

Recent studies have indicated that amino acid (aa) substitutions in the core region and NSSA interferon sensitivity-determining region (ISDR) of hepatitis C virus (HCV) as well as genetic polymorphisms in the interleukin-28B (IL28B) locus affect the outcome of interferon (IFN)-based therapies. We aimed to investigate the role of these factors on response to peginterferon plus ribavirin in a prospective study of response-guided therapy.

### Methods:

The aa sequences in core region and ISDR and rs12979860 genotypes were analyzed in 115 HCV-1 patients. The treatment was 24 weeks for patients achieving a rapid virological response (RVR), 48 weeks for those with an early virological response (EVR), and early terminated in those without an EVR.

### Results:

A sustained virological response (SVR) was achieved in 82% of 34 RVR patients, 45% of 74 EVR patients and 0% of 7 non-EVR patients. Logistic regression analysis showed that ISDR mutations (≥2) (odds ratio[OR]: 0.024), double core 70/91 mutations (OR: 0.136), and platelet counts≥150,000/μL (OR: 3.119) were independent pretreatment factors associated with SVR. Apart from rs12979860 CC genotype, low viral load and ISDR mutation (≥2) were significant factors predictive of RVR. Combination of rs12979860 genotype and baseline viral characteristics (viral load and core/ISDR mutations) could predict RVR and SVR with positive predictive value of 100% and 91%, and negative predictive value of 80% and 54%, respectively.

### Conclusion:

Pretreatment screening rs12979860 genotype and aa substitutions in the core region and ISDR could help identifying patients who are good candidates for peginterferon plus ribavirin therapy.
Methods: We examined 15 patients, nine male and six female, aged between 24 and 66 years. The patients have been treated with Peg-IFN (180 μg/week), Ribavirin and in some cases with Boceprevir or Telaprevir. During the treatment the patient’s weight has been monitored. Also the patients have been interviewed about other side effects and their general well-being. In order to find out whether patients lose rather fat, water or muscles our approach was to run a bioelectrical impedance analysis. For our analysis Akern BIA 101/s was used. The patients have been examined in the beginning, in week 4 and in week 12 of the therapy.

Results: Within all tested patients we found a loss of weight. The median weight of 76.8 kg was reduced to 74.4 kg which equates 3.2% weight loss. The phase angle decreases due to the catabolic stimulus we found as a result of the bioelectrical impedance analysis (PA 7.4 to PA 6.6) which means patients lose rather muscle-cells than fat-cells. In 3 patients with a telaprevir based triple therapy the reduction however was much higher. In the first four weeks of the treatment the phase angle in the Peg-IFN and Ribavirin group stays almost the same (medial PA 7.01 to PA 7.0), in the group with Telaprevir the phase angle decreases (PA 7.4 to PA 6.6).

Conclusion: A IFN-based HCV treatment results in a median weight loss of 3.2% in the first 12 weeks. The weight loss is due to a loss of muscle mass. In a telaprevir based triple therapy the muscle loss seems to be higher. Sports or a protein rich diet may reduce muscle loss.

830 CHOLESTEROL LEVELS AND PRESENCE OF DIABETES PREDICT EARLY VIROLOGICAL RESPONSE TO TRIPLE THERAPY WITH TELAPREVIR, PEG-INTERFERON alfa-2a 180 μg AND RIBAVIRIN IN CHRONIC HEPATITIS C

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Method: SVR rate in patients with genotype-2 (GT-2) chronic hepatitis C patients receiving PegIFN/RBV treatment in this study. There were 236 (76.4%) patients whose Hgb had decline more than 3 g/ml during the treatment. Those Hgb decline >3 after week 2 on treatment were easier to achieve SVR (93.7%) than those without decline of Hgb or decline before week 2 (86.3%). The predictor of SVR by logistic regression are patients whose Hgb decline more than 3 g/ml after 2 weeks on treatment (OR: 2.93, 95%CI: 1.13–7.64, p=0.028), those with RVR (OR: 5.36, 95%CI: 2.1–13.66, p<0.001), and with baseline higher platelet count (OR: 1.01, 95%CI: 1.00–1.02, p=0.01). By comparison of baseline characteristics between those on-treatment decline of hemoglobin <3 and ≥3, there were no differences in terms of age, baseline viral load, fibrosis status, and RVR rate. However, men are easier to have the decline of hemoglobin during treatment rather than women (p=0.001). Other factors related to decline of hemoglobin >3 during treatment are baseline creatinine and baseline hemoglobin.

Conclusion: The decline of hemoglobin >3 after 2 weeks on treatment, as well as higher baseline platelet count and RVR would
affect the SVR rate in GT-2 CHC patients received PegIFN/RBV treatment.

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**MONITORING OF FACTORS RELATED TO PLASMA CONCENTRATION OF RIBAVIRIN COULD IMPROVE RESPONSE TO ANTIVIRAL THERAPY IN CHRONIC HEPATITIS C GENOTYPE 1**

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**Objective:** We wanted to analyze which kinectics variables could be related to higher plasma concentration of Ribavirin (PCR) during the dual antiviral therapy in patients with chronic hepatitis C genotype 1 (CHC-1), which could be monitored during the first weeks of treatment in order to obtain higher possibilities of achieving SVR. The results could also be used for triple antiviral therapy.

**Design:** This prospective study included 103 patients with CHC-1, who were treated with 180 mcg/week of pegylated interferon plus ribavirin according to body weight during 48 weeks. We also recorded 6 baseline variables and kinetics factors (PCR at 1st month-therapy; values of urinary pH or HOMA-IR at 1st month-therapy; and the median corpuscular volume of erythrocyte (MCV) at 3th month-therapy.

**Results:** Patients with unfavorable CT/TT-ILE-28B genotype had a lower rate of SVR if they had lower PCR (2.5+1.2 vs 1.9+0.8 ng/mcl, OR 1.7 95%CI (1.0–3.1); p < 0.05. There was no difference in the PCR between patients with favorable ILE-28B genotype. High values of CrC were statistically associated with lower rate of SVR (74% versus 24% if CrC >140ml/min; odds ratio, 3.3; 95%CI, 1.8–5.9); P < 0.0001). Variables significantly associated with higher PCR on multivariate logistic regression analysis were F3–F4 liver fibrosis, baseline insulin resistance, increase of MCV (3th month-therapy), Urinary pH >6 and higher HOMA-IR (1st month-therapy).

**Table 1. Univariate association with levels of ribavirin**

<table>
<thead>
<tr>
<th>Features</th>
<th>PCR &gt;2 ng/ml (n=48)</th>
<th>PCR &lt;2 ng/ml (n=56)</th>
<th>RR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>23/20</td>
<td>44/12</td>
<td>3.2 (13–77)</td>
<td>0.010</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.2±9.8</td>
<td>171.4±7.6</td>
<td>1.0 (1.0–1.0)</td>
<td>0.020</td>
</tr>
<tr>
<td>Baseline urinary pH</td>
<td>5.86±0.76</td>
<td>5.55±0.79</td>
<td>1.8 (1.0–3.2)</td>
<td>0.040</td>
</tr>
<tr>
<td>Liver fibrosis (F0–F2/F3–F4)</td>
<td>25/18</td>
<td>44/12</td>
<td>2.6 (10–63)</td>
<td>0.03</td>
</tr>
<tr>
<td>Baseline hemoglobin (g/dl)</td>
<td>14.2±1.2</td>
<td>15.3±1.3</td>
<td>0.5 (0.3–0.7)</td>
<td>0.000</td>
</tr>
<tr>
<td>Decay of viral load at 1Mth (Log10 RNA) (−3.68±1.81) (−2.81±1.72)</td>
<td>0.7 (0.5–1.0)</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase MCV 3Mth (fl)</td>
<td>6.0±4.6</td>
<td>3.1±3.8</td>
<td>1.1 (1.0–1.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>Urinary pH 1 Mith</td>
<td>6.21±0.59</td>
<td>5.92±0.58</td>
<td>2.2 (10–48)</td>
<td>0.045</td>
</tr>
<tr>
<td>HOMA-IR &gt;4 (Yes/No) (for PCR &gt;2.5ng/ml)</td>
<td>9/10</td>
<td>55/65</td>
<td>3.8 (13–11.6)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**Table 2. Multivariate association with levels of ribavirin**

<table>
<thead>
<tr>
<th>Features</th>
<th>Odds Ratio (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>0.9 (0.1–0.6)</td>
<td>0.010</td>
</tr>
<tr>
<td>Liver fibrosis (F0–F2/F3–F4)</td>
<td>7.3 (1.1–46.8)</td>
<td>0.035</td>
</tr>
<tr>
<td>Decay Viral Load at 1Mth (Log10 RNA)</td>
<td>0.5 (0.3–0.9)</td>
<td>0.016</td>
</tr>
<tr>
<td>Increase MCV 3Mth (fl)</td>
<td>1.3 (1.1–1.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Urinary pH &gt;6 at 1Mth</td>
<td>5.1 (1.3–20.7)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

**Conclusions:** In patients with unfavorable ILE-28B genotype it is very important to achieve a plasma concentration of Ribavirin higher than 2 ng/mcl at 1st month of therapy to achieve SVR. Degree of increasing of MCV and urinary pH at 3th and 1st month-therapy respectively, could be useful tools to monitor dose of ribavirin during the antiviral therapy, specially for fibrosis F0–F2. We recommend to use the Lindahl’s formula based on clearance creatinin to adjust the pretreatment dose of Ribavirin, regardless of type of treatment (dual o triple antiviral therapy) in order to achieve the optimal daily dose of ribavirin according body weight and creatinin clearance.
Background: Alisporivir (ALV) is a host-targeting antiviral that impairs HCV replication complex formation by disrupting host cyclophilin A and HCV NS5A interaction. In the phase IIb VITAL-1 study, treatment-naïve genotype 2/3 HCV patients achieving RVR remained on interferon (IFN)-free ALV/ribavirin (RBV) treatment, with 89% of them achieving SVR24. This study examined transcriptional changes in peripheral blood mononuclear cells (PBMC) induced by treatment with either IFN-free ALV-containing regimes or IFN-containing regimes to gain more insight into mode of action of ALV.

Methods: VITAL-1 study collected PBMC from 105 patients at baseline and after 4 weeks of treatment. Total RNA was then extracted using RNeasy, and transcriptional changes were assessed using Affymetrix HG-U133plus2.0 microarrays. The PBMC-derived genomic data were analyzed using Partek Genomic Suite v6.5.

Results: Four weeks treatment with either IFN-containing (IFN/RBV or IFN/ALV) or IFN-free ALV (ALV or ALV/RBV) modulated expression of approximately 280 and 40 PBMC transcripts, respectively (p-value <0.05 and absolute foldchange >1.5 relative to pre-treatment levels). Notably, expression of interferon-stimulated genes (ISG) was significantly up-regulated in patients from IFN-containing (IFN/RBV or IFN/ALV) arms (p < 0.005). In contrast, significant down-regulation of ISG expression (p < 0.005) was observed in patients from IFN-free ALV arms (ALV or ALV/RBV), suggesting a potential “ISG desensitizing” effect by ALV. Longitudinal ISG expression changes correlated with viral load reduction in IFN-containing arms but not in IFN-free ALV-treated patients. Supporting these clinical findings, in vitro ALV treatment of hepatocyte cell lines did not up-regulate ISG expression, and there was no induction of endogenous interferon. In addition, no significant transcriptional changes of genes relating to lipid metabolism and mitochondrial function were identified.

Conclusions: HCV clearance with ALV, interferon-free treatment is associated with down-regulation of ISG expression after 4 weeks, in contrast to the significant up-regulation by IFN-containing regime, suggesting a distinct mechanism of action for ALV-based regimens. These findings indicate a potential benefit in treating poor responder patients, whose pre-treatment ISG levels are often elevated.
(1W to 8W). By a multiple regression analysis, RBV1W showed strong relation with maximum delta eGFR $[= 0.831\cdot eGFR0W - 2.160\cdot HB0W + 0.016\cdot RBV1W - 31.201 (r^2 = 0.7207, P < 0.0001)]$ and maximum delta Hb $[= 0.544\cdot HB0W + 0.001\cdot RBV1W - 4.319 (r^2 = 0.3797, P < 0.0001)]$. Furthermore, RBV1W was regulated by some factors $[RBV1W = 572\cdot gender (male=1, female=2) + 21.8\cdot Age + TVR\cdot mg/day + 129\cdot RBV (mg/kg) - 2940 (r^2 = 0.4344, P < 0.0001)]$.

**Conclusions:** In the PEG-IFN/RBV/TVR combination therapy, RBV1W is a good predictive marker of later renal dysfunction and anemia. By adjusting TVR and RBV dose, it is necessary to avoid the excessive elevation of RBV level to obtain good clinical effect.

### 836

**HCV ANTIVIRAL TREATMENT OUTCOMES COMPARED BETWEEN CLINICAL TRIAL PARTICIPANTS AND RECIPIENTS OF ROUTINE STANDARD OF CARE**

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**Aims:** Trial effect refers to the impact of enrollment in a clinical trial on treatment outcomes. Little literature exists evaluating the magnitude and direction of trial effect patients with chronic hepatitis C (HCV) undergoing antiviral therapy. We sought to investigate the impact of clinical trial participation on sustained virologic response (SVR) and other key measures of HCV treatment.

**Methods:** This was a single center, retrospective study of patients completing HCV antiviral therapy. Successful treatment was defined as achievement of SVR (HCV RNA negative 6 months post completion of therapy). Virological response and weeks 4 and 12 as well as treatment completion and adverse events were also evaluated. Patients receiving treatment as clinical trial participants were compared to patients receiving standard of care antiviral therapy in our outpatient clinic.

**Results:** 449 patients were included in our analysis (clinical trial participants=89, routine standard of care therapy=360). Patients were similar by age (trial=47, non-trial=45), sex (male: trial=74%, non-trial=72%), and ethnicity (white: trial=87%, non-trial=78%). Genotype 1 infection was more common in trial participants (trial: 83%, non-trial 53%; $p < 0.001$) and liver biopsy rates were higher in this group (trial=98%, non-trial=66%; $p < 0.001$). Psychiatric illness history was less frequent in trial participants (trial=30%, non-trial=53%; $p < 0.001$). Similar rates of SVR were seen between groups (trial=51%, non-trial 54%; $p = 0.72$), even when stratified for genotype (G1: trial=47%, non-trial 47%; $p = 0.78$) and trial DAA use (55%; $p = 0.27$). Trial patients were more likely to have interferon dose reductions (trial=18%, non-trial 6%; $p = 0.001$), and less likely to have treatment discontinuation for side effects (trial=8%, non-trial 18%; $p = 0.017$).

**Conclusions:** Our analysis did not identify any evidence of a trial effect resulting in improved or diminished SVR rates in HCV treatment recipients. Other potential positive and negative variables should be focused upon for patients deliberating between HCV clinical trial participation and receiving standard of care treatment.

### 837

**PREDICTION OF RESPONSE TO PEG-IFN/RBV THERAPY FOR CHRONIC HEPATITIS C GENOTYPE 2a AND 2b WITH HIGH VIRAL LOAD**

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**Background and Aims:** In Japan, HCV genotype 1b (HCV-1b) consists of about 70% of total HCV infection and most of the remaining patients are infected with HCV-2a (25%) or HCV-2b (5%). We previously reported that the viral sequence heterogeneity of part of NS5A, referred to as the IFN/RBV resistance-determining region (IRRD), the IFN sensitivity-determining region (ISDR), and interleukin 28B (IL28B) as a host factor are significantly correlated with the outcome of PEG-IFN/RBV treatment in HCV-1b patients. We aimed to investigate the impact of viral genetic variations within the NS5A and IL28B on PEG-IFN/RBV treatment outcome in HCV-2a and HCV-2b patients.

**Methods:** Sixty six patients with HCV-2a (35 patients) or HCV-2b (31 patients) who were treated with PEG-IFN/RBV for 24 weeks were analyzed.

**Results:** Of the 35 patients infected with HCV-2a, 23 (66%) and 28 (80%) patients achieved rapid virological response (RVR) and sustained virological response (SVR), respectively. Likewise, of 31 patients infected with HCV-2b, 21 (68%) and 20 (65%) patients achieved RVR and SVR, respectively. In HCV-2a patients, RVR was seen in 75% (21/28) of the SVR, whereas only in 29% (2/7) of the non-SVR. Significant correlation was observed between RVR and SVR ($p = 0.0331$) in HCV-2a patients, but not in HCV-2b patients. Multivariate analysis identified HCV-2a isolates with 4 or more mutations in IRRDR (IRRDR2a≥4) (odds ratio [OR] 0.3209, $p = 0.0485$) as significant determiner of RVR in patients infected with HCV-2a, and an N-terminal half of IRRDR having one or more mutations (IRRDR2b[N]≥1) (OR 28.6267, $p = 0.0077$) with HCV-2b. As for SVR, platelets (OR 0.1932, $p = 0.0369$), IL28B major (OR 0.3293, $p = 0.0424$) and IRRDR2a≥4 (OR 0.2684, $p = 0.0264$) were identified as predictive factors of SVR in patients infected with HCV-2a, but none was identified with HCV-2b.

**Conclusions:** The present results suggest the clinical usefulness of sequence heterogeneity of NS5A of HCV-2a (IRRDR2a≥4) in determining the viral sensitivity to PEG-IFN/RBV therapy with HCV-2a patients. The impact of the IL28B SNP and ISDR appeared to be weaker in HCV-2a and -2b infections than that seen in HCV-1b infections, and also weaker than that of the most powerful viral factor, IRRDR2a≥4, in HCV-2a infection.

### 838

**INITIATION AND DISCONTINUATION OF INTERFERON-CONTAINING THERAPY AMONG HIV/HCV CO-INFECTED PATIENTS IN A US COMMERCIALLY-INSURED POPULATION**


**E-mail:** stephanie.kirbach@abbott.com

**Background and Objectives:** Interferon alpha, a component of current HCV treatment regimens, is associated with contraindications that may impact the ability to initiate and remain on therapy. Poorer adherence to interferon-containing therapy (ICT) has been associated with certain co-morbidities, including HIV.
Approximately one-third of individuals with HIV are HCV co-infected, and typically require a longer course of ICT. Treatment patterns of ICT among HIV co-infected individuals in comparison to HCV mono-infected patients have not been fully examined. The objective of this study was to evaluate ICT initiation and discontinuation among US commercially-insured HIV co-infected versus HCV mono-infected patients.

**Methods:** Claims data from the MedStat MarketScan database (years 2005–2010) were used to identify incident HCV patients who had ≥2 years of continuous enrollment (≥1 year before and after first diagnosis). The impact of HIV co-infection (based on ICD-9 codes) in the year prior to first observed HCV diagnosis on ICT initiation and discontinuation was assessed via logistic regression and Kaplan–Meier survival analyses. Treatment was considered discontinued if ≥90 days elapsed after the last observed fill.

**Results:** 15,409 incident HCV patients were identified between 2005 and 2010; 3,282 (21%) received ICT after diagnosis. Diagnoses of HIV were present in the year prior to first observed HCV diagnosis among 246 patients; 54 (22%) of whom received ICT. HIV co-infected patients were not less likely to receive treatment, but had a longer time to ICT initiation than mono-infected individuals (10.6 versus 7.4 months, p < 0.03). HIV co-infected patients were equally likely to discontinue ICT at 12 weeks after ICT initiation, and less likely to discontinue within 24 weeks (OR = 0.572; 95% CI 0.339–0.964). Finally, of all patients discontinuing therapy early, HIV co-infected patients exhibited a marginally longer time to discontinuation (7.4 versus 5.2 months for HCV mono-infected, p = 0.05).

**Conclusions:** Among commercially-insured new HCV patients, HIV co-infection was not a barrier to initiating ICT and was associated with smaller odds of early discontinuation. However, average time to treatment discontinuation among HIV co-infected individuals far preceded the 48-week recommended course of therapy. Whether this was due to poor treatment response or non-adherence requires further evaluation.

### Table 1

<table>
<thead>
<tr>
<th>Degree</th>
<th>Relevance for decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td>2.51</td>
</tr>
<tr>
<td>Speed of progression</td>
<td>1.92</td>
</tr>
<tr>
<td>Patient’s demand for therapy</td>
<td>1.84</td>
</tr>
<tr>
<td>Expected manifestation of hepatitis C</td>
<td>2.63</td>
</tr>
<tr>
<td>Presumed tolerability</td>
<td>0.84</td>
</tr>
<tr>
<td>Presumed risk of decompensation</td>
<td>0.80</td>
</tr>
<tr>
<td>Presumed compliance</td>
<td>3.06</td>
</tr>
</tbody>
</table>

**Conclusions:** The majority of patients was recommended to receive current triple therapy. Progress in fibrosis and the patients’ demand played major roles in this decision. The main reason to defer treatment was a presumed bad tolerability. In general, differences in patient characteristics were smaller than expected. The data presented here are part of the doctoral thesis of cand. med. Nora Weiss.

### 839

**DEFER OR TREAT NOW? REASONS FOR THERAPEUTIC DECISIONS IN PATIENTS WITH CHRONIC HEPATITIS C GENOTYPE 1 IN THE EARLY ERA OF DIRECTLY ACTING ANTIVIRALS**

J.M. Kittner1, N. Weiss1, J.M. Schattenberg1, A. Gambihler1, M.F. Sprinzl1, F.R. Thieringer1, J. Wiltink2, S. Koc1, A. Weinmann1, P.R. Galle1, M. Schuchmann1, M.F. Sprinzl1, F.R. Thieringer1, J. Wiltink2, S. Koc1, A. Weinmann1, P.R. Galle1, M. Schuchmann1.

**Methods:** Fibrosis, speed of progression, patients’ demand for therapy, extrahepatic manifestations, presumed tolerability (“0” = bad), risk for hepatic decompensation induced by therapy, and the presumed compliance were rated on 0–4 point scales by the attending physician. In a second step, the relevance of each of these characteristics for the decision was assessed.

**Results:** Up to now, treatment decisions of 10 physicians in the time period from 1st September 2011 until 31st October 2012 concerning 173 patients were analysed. 104 patients (60.1%) with a mean age of 52.5 ± 12.3 years were recommended to receive triple therapy, and 69 patients with a mean age of 60.7 ± 12.4 years were recommended to wait. Available data on previous therapy show comparable rates of non-response or relapse in both groups. Mean values of the score results are shown in table 1.
most commonly reported AEs among healthy subjects: somnolence (30.8 vs. 25.0%), headache (30.8 vs. 12.5%), nausea (15.4 vs. 0%), and tremor (15% vs. 0%) among MMT subjects receiving ALV vs. placebo. PK results from Part 1 warranted confirmatory investigation in Part 2, (see table).

Conclusions: Alisporivir does not affect the in vivo disposition of R- or S-methadone suggesting there is no relevant interaction that would complicate its use in the treatment of opioid-dependent patients chronically infected with HCV.

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THE EFFECT OF MILD TO MODERATE HEPATIC IMPAIRMENT ON THE PHARMACOKINETICS OF ALISPORIVIR (ALV)
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E-mail: steven.kovacs@novartis.com

Background: ALV is a cyclophilin inhibitor with panenginotypic activity against HCV that is being developed for the treatment of chronic HCV infection. The degree of hepatic dysfunction plays an important role in the response to anti-HCV therapy as well as in the in vivo disposition of drugs generally. ALV is a CYP3A4 substrate with extensive hepatic but negligible renal clearance. The effect of mild to moderate hepatic impairment on ALV pharmacokinetics was investigated to inform dosing recommendations.

Methods: Open-label, single dose study in 16 subjects with hepatic impairment and 16 healthy subjects. Healthy subjects were matched to each group (n = 8) of subjects with hepatic impairment by age (± 10 years), weight (± 10 kg), sex, and smoking status. Degree of hepatic impairment was determined by Child-Turcotte-Pugh (CTP) scoring. Routine safety assessments were performed. Each subject received ALV 200 mg. Peripheral blood was collected for ALV PK prior to and 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after dose administration. ALV plasma concentrations were determined by LC/MS/MS; PK analyses by nonparamontal methods.

Results: All 32 subjects completed the study and tolerated ALV well. Subjects were predominantly male (93.8%) and Caucasian (87.5%). Subjects with hepatic impairment reported no AEs. Among healthy subjects, headache was most commonly reported (18.8%) and 16 healthy subjects. Healthy subjects were matched to each group (n = 8) of subjects with hepatic impairment by age (± 10 years), weight (± 10 kg), sex, and smoking status. Degree of hepatic impairment was determined by Child-Turcotte-Pugh (CTP) scoring. Routine safety assessments were performed. Each subject received ALV 200 mg. Peripheral blood was collected for ALV PK prior to and 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after dose administration. ALV plasma concentrations were determined by LC/MS/MS; PK analyses by nonparamontal methods.

Methods: Open-label, single dose study in 16 subjects with hepatic impairment and 16 healthy subjects. Healthy subjects were matched to each group (n = 8) of subjects with hepatic impairment by age (± 10 years), weight (± 10 kg), sex, and smoking status. Degree of hepatic impairment was determined by Child-Turcotte-Pugh (CTP) scoring. Routine safety assessments were performed. Each subject received ALV 200 mg. Peripheral blood was collected for ALV PK prior to and 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after dose administration. ALV plasma concentrations were determined by LC/MS/MS; PK analyses by nonparamontal methods.

Regression analyses revealed no significant relationship between numerical CTP score (range 5–9) and alisporivir AUC or Cmax.

Conclusions: Mild to moderate hepatic impairment had no significant effect on ALV pharmacokinetics suggesting dose adjustment is not necessary in patients with mild to moderate hepatic impairment (CTP 5–9).
patients developed tuberculosis within 6 months after stopping interferon therapy. Furthermore, 4 patients developed tuberculosis more than a year after completion of interferon therapy. 3 patients had diabetes and 1 also had HIV infection. 4 patient had liver decompensation as presentation of development of tuberculosis. Two patients had prior tuberculosis. Focus was pulmonary in 7 patients and abdominal in 3 patients. AKT was offered to all patients, 8 were declared cured, 1 was on treatment and 1 patient died.

**Conclusion:** Interferon treatment in HCV related CLD possibly increases susceptibility to tuberculosis which is compounded by presence of co-morbidities. Presentation of tuberculosis can be in form of liver decompensation and presentation can be delayed after completion of interferon therapy. High percentage of tuberculosis after interferon treatment occurrence was in pulmonary site as compared to CLD patients in whom extrapulmonary tuberculosis is common.

**844**

**IMPACT OF ANTIVIRAL THERAPY ON SURVIVAL IN PATIENTS WITH ADVANCED FIBROSIS – EXPERIENCE OF BEAUJON HOSPITAL 2000 TO 2010**

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**Background:** Although the benefits of successful anti-HCV treatment in patients with advanced fibrosis in the prevention of liver-related complications and patient’s survival has been shown, the impact of non-successful therapy remains debated. We retrospectively analyzed the survival in patients with advanced fibrosis admitted in Beaujon hospital between 2000 and 2010, according interferon-therapy.

**Methods:** Study population included HCV treatment naïve patients with positive HCV RNA, bridging fibrosis (n=220) or cirrhosis (n=264) assessed by a biopsy. Sustained virological response (SVR) was defined as undetectable serum HCV RNA at 24 weeks following completion of interferon therapy. Cox regression analysis and Kaplan Meier were used to determine factors associated with death or liver transplantation.

**Results:** 484 patients were included in study with a median follow up of 4.5 years; 328 (68%) were male; 354 (73%) patients were treated whereas 130 (27%) were not treated. Mean age at presentation was 49 years in treated group vs 52 in untreated group. Among treated patients, 105 (30%) had SVR. Blood parameters were not different between treated and untreated groups except for ALT (2.6 vs 2.3; p<0.01), bilirubin serum (15 vs 17 µmol/L; p=0.01) and Child Pugh A/B stage (B stage: 0% vs 22%, p≤0.0001). The median survival in treated patients without SVR and untreated patients was 133 and 64 months, respectively (log-rank p≤0.0001). Global survival rate at 5 years in patients with SVR compared with untreated patients was 100% vs 54% and was 88% vs 54% between treated patients without SVR and untreated patients (log rank p≤0.0001 and p<0.0001, respectively). The importance of treatment on mortality was confirmed using multivariate cox regression analysis (Hazard ratio (HR) = 6.8, 95%CI: [2.5; 20.5]). Other variables found significant in the multivariate analysis were low platelets account HR: 1.0, 95%CI: [0.1; 1.1], high bilirubin serum HR: 0.9, 95%CI: [0.8; 1.0] and high ferritin HR: 1.0, 95%CI: [1.2] were associated to the mortality or liver transplantation.

**Conclusion:** These results suggest that the initiation of IFN-based treatment improves the survival of patients with advanced fibrosis, even if sustained virologic response is not achieved.

**845**

**ONCE DAILY SOFOSBUVIR PLUS RIBAVIRIN FOR 12 AND 24 WEEKS IN TREATMENT-NAIVE PATIENTS WITH HCV INFECTION: THE QUANTUM STUDY**


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**Background:** QUANTUM is a Phase 2b study designed to assess the efficacy of the guanidine nucleotide analogue GS-0938 alone and in combination with the uridine nucleotide analog sofosbuvir (SOF) +/- ribavirin (RBV) in treatment-naïve HCV patients.

**Methods:** Patients were stratified by genotype, HCV RNA, and cirrhosis, and randomized equally to receive 12 or 24 weeks of GS-0938 (300 mg QD) alone, GS-0938+SOF (400 mg QD), GS-0938+SOF+RBV, SOF+RBV, or placebo for 24 weeks. The primary endpoint was SVR12.

**Results:** 235 patients were randomized and received study medication. 57% of patients had HCV genotype (GT)1a, GT1b-18%, GT2–11%, GT3–12%, and GT4–2%. Due to ALT and AST elevations >3×nadir in patients receiving GS-0938, dosing of GS-0938 arms was halted with patients having received ≤9 weeks of treatment. Patients receiving SOF+RBV continued treatment. Most ALT elevations resolved during a 12-week safety follow-up. 132 subjects initially randomized to these arms or placebo who did not achieve SVR were re-treated with SOF+RBV for 24 weeks.

**Table: Efficacy Data from the QUANTUM Study**

<table>
<thead>
<tr>
<th>Duration (wks)</th>
<th>SOF+RBV</th>
<th>SOF+RBV</th>
<th>GS-0938</th>
<th>GS-0938+SBV</th>
<th>Retreatment SOF+RBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>14/25 (56%)</td>
<td>14/25 (56%)</td>
<td>14/25 (56%)</td>
<td>14/25 (56%)</td>
<td>14/25 (56%)</td>
</tr>
<tr>
<td>24</td>
<td>14/25 (55%)</td>
<td>14/25 (55%)</td>
<td>14/25 (55%)</td>
<td>14/25 (55%)</td>
<td>14/25 (55%)</td>
</tr>
</tbody>
</table>

Three subjects from the retreatment group were excluded because SVR4 was not assessed yet at the time of this abstract. 17/24 (71%) patients who received 6–9 weeks of SOF+GS-0938 +/- RBV achieved SVR12. 8/14 (57%) cirrhotic patients who received retreatment with SOF+RBV achieved SVR4. Besides the abovementioned ALT and AST elevations, all regimens were generally safe and well tolerated: <5% of patients discontinued due to AEs; there were no treatment-related SAEs. SVR12 for all groups will be presented.

**Conclusions:** Sofosbuvir 400 mg+RBV demonstrated potent on-treatment antiviral activity and was generally well tolerated. SVR rates with 12 or 24 weeks treatment were modest with no apparent benefit from longer duration. However, the cohort of mostly GT1 patients receiving 24 weeks of SOF+RBV following cessation of the original treatment had a higher response rate. With two highly potent agents, shorter treatment durations may be possible. Development of GS-0938 is no longer being pursued.
CHARACTERIZATION OF THE EARLY VIRAL KINETICS IN COMPENSATED CIRRHTIC TREATMENT-EXPERIENCED PATIENTS TREATED WITH BOCEPREVIR AND TELAPREVI


Background and Aim: Objective of MODCUPIC study (an ancillary study of CUPIC ANRS-CO20) was to provide a precise description of the early viral kinetics during HCV protease-inhibitors-based (PI) triple therapy in compensated cirrhotic treatment-experienced patients in real-life setting.

Methods: Patients were prospectively included to receive either telaprevir (TVR)/PEG-IFN-alfa2a/RBV or boceprevir (BOC)/PEG-IFN-alfa2b/RBV (without randomization). Viral load (VL) was frequently assessed during the first week following treatment initiation at days 0, 0.33, 1, 2, 3, 7 and then at weeks 2, 3, 4, 6, 8, 12. Changes in VL levels were fitted using the standard biphasic model [Neumann et al., Science 1998]. Data fitting was performed until VL was undetectable or rebounds. In this model, the rates of the first and second phase of viral decline are roughly equal to the clearance rate of free virus and the loss rate of infected cells, respectively. The magnitude of the first phase viral decline reflects therapy's effectiveness in blocking viral production.

Results: Fifteen patients were included (6 BOC, 9 TVR), median age was 54.6 years, 13 were males and 60% had undetectable VL at week 4. Except in two patients where VL rebounded (at week 3 and 8) the biphasic model could well describe VL decline in all patients. The clearance rate of free virus and the loss rate of infected cells were not significantly different in patients treated with TVR and BOC with median values of 5.10 day⁻¹ and 0.14 day⁻¹, respectively. However the effectiveness in blocking viral production was significantly larger with TVR than with BOC (median e²TVR vs e²BOC: 0.999 vs 0.99, P = 0.04).

Conclusion: For both PIs, both phases of viral decline were about 4 times smaller than what had been observed in non-cirrhotic naive patients during TVR-based treatment [Guedj et al., Hepatology 2011; Adiwijaya et al., PLoS Computational Biology 2012] and close to typical values observed during PEG-IFN/ RBV therapy. This discrepancy could be due to impaired pharmacokinetics and lower penetration capacity of PIs in hepatocytes of cirrhotic patients. Consequently cirrhotic treatment-experienced patients may need substantially longer time to achieve viral eradication than other patients.
POSTERS

848 HIGH CONCORDANCE OF SVR4, SVR12, AND SVR24 IN PATIENTS WITH HCV INFECTION WHO HAVE RECEIVED TREATMENT WITH SOFOSBUVIR

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Background and Aims: Sofosbuvir (SOF, formerly GS-7977), a potent uridine nucleotide analog now in Phase 3 development, has demonstrated >90% end-of-therapy (EOT) response and SVR12 in interferon-containing and interferon-free regimens, and across HCV genotypes. We evaluated concordance of SVR4 with SVR12 and SVR24 in the Phase 2 program.

Methods: Sofosbuvir has been explored in more than 500 patients, with and without ribavirin (RBV), or with peginterferon (PEG)+RBV in the phase 2 studies PROTON, ELECTRON, ATOMIC, and QUANTUM. In these studies, HCVRNA was measured at least at 4, 12, and 24 weeks following the end of treatment. We assessed the concordance of sustained virologic response (SVR) at these time points.

Results: 590 patients had HCVRNA measured at 4 and 12 weeks post-treatment, 538 at 4 and 24 weeks post-treatment, and 547 at both 12 and 24 weeks post-treatment. Relapse after having achieved SVR4 was uncommon; 8 patients who had achieved SVR4 at both 12 and 24 weeks post-treatment. Relapse after having achieved SVR12 was uncommon; 8 patients who had achieved SVR12 relapsed at 24 weeks post-treatment. In two cases, patients with reported relapse at SVR4 were later found to have achieved SVR. Positive and negative predictive values are tabulated.

Table: Concordance of SVR4, SVR12, and SVR24

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<th>PPV</th>
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<td>SVR4</td>
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<tr>
<td>SVR12</td>
<td>98.5</td>
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<td>96.2</td>
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<td>SVR24</td>
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<td>SVR12</td>
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<td>SVR4</td>
<td>99.8</td>
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PPV, positive predictive value; NPV, negative predictive value.

Conclusion: High levels of concordance between SVR4 and later time points were observed. There were few relapses in patients who achieved SVR4. Positive predictive values and sensitivity of SVR4 for SVR12 and SVR24 were >98.5%. Specificity and negative predictive values were lower, reflecting the relatively higher contribution of the few discordant patients in the much smaller number of patients who relapsed. SVR12 has a 99.8% positive predictive value, demonstrating the reliability of this time point for assessing a durable response.

849 ALISPORIVIR INTERFERON-FREE TREATMENT ACHIEVED COMPARABLE EARLY HCV CLEARANCE RATES IN BOTH IL28CC AND IL28CT/IT PATIENTS INFECTED WITH GENOTYPE 2/3 HCV

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Background: Host-targeting antiviral alisporivir (ALV) inhibits cyclophilin A that is essential for HCV replication. In the phase Ib VITAL-1 study involving treatment-naïve genotype (G)2/3 patients, early HCV clearance (<LLOQ, 25 IU/mL) was achieved in 42%-49% of patients after 4–6 weeks of ALV/RBV IFN-free treatment. Here we evaluate the impact of IL28B genotype on early viral clearance following IFN-free ALV therapy.

Methods: DNA samples were obtained from 169 (65% of all patients randomized to IFN-free ALV) patients who consented to pharmacogenetic assessment and received IFN-free ALV alone or ALV/ribavirin (RBV) treatment. IL28B genotype (rs12979860) was determined by Taqman SNP genotyping assay. Logistic regression was performed to evaluate association between IL28B genotype and HCV clearance with IFN-free ALV treatment at Weeks 4 and 6. The analysis was adjusted for race, baseline viral load, body weight, ALV exposure and treatment.

Results: Patients receiving IFN-free ALV treatment with IL28B CC genotype (rs12979860) had a higher rate of HCV clearance at week 4 or 6 compared with those with non-CC genotype: 36.26% vs 28.21% and 47.25% vs 32.05%, for Weeks 4 and 6, respectively, however the difference in RVR was not significant (p = 0.12 for Week 4; p = 0.025 for Week 6). Subgroup analysis with Caucasians only (n = 95) revealed significant association between IL28B genotype and response at Week 4 (p = 0.041) and week 6 (p = 0.006). No significant impact of IL28B genotype was observed in subgroup analysis with Asians only (n = 70). Additional analysis of the effect of ALV exposure on early HCV clearance with ALV/RBV demonstrated that, following ALV/RBV therapy, patients with low ALV exposure (Cmin < 128 ng/mL), only 5% (1/20) of IL28B CC/CT patients achieved RVR compared to 35% (7/20) for patients of CC genotype. In contrast, in patients with high ALV exposure (Cmin > 423 ng/mL), those with IL28B CC and non-CC achieved the same rate of RVR (50%, 12/24, and 50%, 7/14 of non-CC and CC patients, respectively).

Conclusions: In treatment-naïve HCV G2/3 patients receiving Alisporivir+RBV, interferon-free treatment, high alisporivir exposure is more important than IL-28 genotype in determining early HCV clearance.
EVR rate analysis according to virus genotype showed that in patients with genotype 2 or 3 HCV EVR was achieved in 100% of patients of the celPEG-INF-α-2b 1.5 μg/kg group, in 95.7% of patients of celPEG-INF-α-2b 2.0 μg/kg group, and in 95.5% of patients receiving PEG-INF-α-2b. In patients with HCV genotype 1, EVR was observed in 88.5% and 92.6% of patients receiving celPEG-INF-α-2b 1.5 and 2.0 μg/kg vs. 82.1% in PEG-INF-α-2b group. There were no statistically differences between groups where patients received celPEG-INF-α-2b in different doses and the reference group.

Adverse events (AE) occurring during the treatment with celPEG-INF-α-2b are dose-dependent; however, their frequency is no more than in patients receiving standard doses of PEG-INF-α-2b. Based on the absence of differences in efficacy and more favorable safety profile of a lower dose of the study drug, the therapeutic dose of celPEG-INF-α-2b was selected to be 1.5 μg/kg/week.

**Conclusions:** CelPEG-INF-α-2b is at least no-inferior to PEG-INF-α-2b in regard to frequency of EVR in treatment-naïve patients with CHC. Analysis of occurrence rate of AE demonstrated overall similarity of safety profiles of different doses of celPEG-INF-α-2b and the reference drug PEG-INF-α-2b.

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**B ACTIVATING FACTOR (BAFF) AND IFN-γ OR IP10 FOR PREDICTION OF RAPID VIROLOGICAL RESPONSE IN PATIENTS WITH CHRONIC HEPATITIS C**


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**Background:** B-activating factor (BAFF), a member of the tumor necrosis family of ligands, which play an important role in the process of maturity and differentiation of B cells. BAFF levels are up-regulated by interleukin-10 (IL-10) and interferon gamma (IFN-γ). The predictive value of IL10 and Interferon-gamma-gamma protein 10 (IP10) on treatment outcome has been described in patients with chronic hepatitis C. Data on combining BAFF, IFN-γ with these predictors in CHC patients are not available.

**Methods:** 72 CHC patients were enrolled with PEG-INF-α-2a (180 μg, qw)/RBV (800–1200 mg/d) for at least 12w. HCV genotype (G) were detected at the baseline, while serum HCV RNA loads and BAFF, IL10, IFN-γ, IP10 levels were assessed on 25 health blood donors (HBD). Patients with higher BAFF levels (84.2% & 77.1%, P=0.037; 74.2% & 62.3%, P=0.028). Patients with higher BAFF levels and lower IFN-γ or lower IP10 levels achieved the highest RVR rate than other patients (92.1%, P<0.05).

**Conclusion:** The combination of BAFF and IFN-γ or IP10 levels may represent a predictive model of RVR in both G6a and G1b patients with CHC.

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**B-LYMPHOCYTE STIMULATOR (BLys) AND HCV GENOTYPE OR IL28B SNPS FOR PREDICTION OF RAPID VIROLOGIC RESPONSE IN PATIENTS WITH CHRONIC HEPATITIS C**


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**Background:** B-lymphocyte stimulator (BLys), a member of the tumor necrosis factor family of ligands, which play an important role in the process of maturity and differentiation of B cells. Hepatitis C virus (HCV) genotype and single nucleotide polymorphisms (SNPs) around the IL28B gene have been shown to associated with response to antiviral treatment in patients with chronic hepatitis C (CHC). The predictive value of combination of BLys with HCV genotype and IL28B SNPs on treatment outcome in CHC patients are not available.

**Methods:** 80 CHC patients were enrolled with PEG-INF-α-2a (180 μg, qw)/RBV (800–1200 mg/d) for at least 12w. HCV genotype and IL28B SNPs rs12979860 and rs8099917 were detected at the baseline, while serum HCV RNA loads and BLys levels (assessed by enzyme-linked immunosorbent assay) were detected before and 4w, 12w after treatment. BLys levels were assessed on 20 health blood donors (HBD).

**Results:** 32 patients (40%) were G6a, while 48 (60%) were G1b in all 80 patient. 62 patients (77.5%) were IL28B SNP rs12979860 C/C, while 18 (22.5%) were non-C/C; 52 (65.0%) were SNP rs8099917 T/T, while 28 (35.0%) were non-T/T. 54 patients (67.5%) achieved rapid virologic response (RVR), while 26 (32.5%) achieved earlier virologic response (EVR). BLys levels were higher in CHC patients (2024.8±656.4pg/ml) than in HBD (1123.7±348.4pg/ml), P=0.027. BLys levels were up-regulated (4058.4±1125.3pg/ml) after treatment than before (P=0.018). Patients with BLys levels increasing twice times on 4w than before achieved higher RVR rates than patients less than twice times (78.2% & 57.2%, P=0.024). Patients with higher BLys levels achieved higher RVR rates than patients with lower BLys levels in both G6a and G1b (88.5% & 80.1%, P=0.041; 73.5% & 60.1%, P=0.033). Patients with higher BLys levels in G6a achieved higher RVR rates than patients with the same BLys levels in G1b (P=0.008). IL28B C/C (SNP rs12979860) or T/T (SNP rs8099917) and higher BLys levels achieved higher RVR rates than patients with lower BLys levels (83.1% & 71.0%, P=0.000; 79.7% & 61.6%, P=0.000).

**Conclusion:** The combination of BLys and HCV genotype or IL28B SNPS represents a predictive model of RVR in HCV patients.

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**ANEMIA IN A COHORT OF PATIENTS WITH CHRONIC HEPATITIS C ON TRIPLE THERAPY**

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**Introduction:** Anemia is the most frequent adverse effect observed in patients with hepatitis C (HCV) on triple therapy. The aim of our study was to evaluate its incidence, characteristics and factors related with its presence.

**Methods:** From October 2011 to October 2012 basal and follow up data from HCV patients, genotype1, treated with peginterferon, ribavirin and telaprevir (TEL) or boceprevir (BOC) (PI) were collected prospectively.

**Results:** 140 patients were included in two tertiary hospitals. Mean age 55 (SD9), 54.3% male. Median follow up 12 weeks (8–48). Mean liver stiffness (LS) 16.7 kPa (SD12.2) (<F2: 19.3%; F3: 25%; F4: 55.7).
BIOC was used in 60 (42.9%) and TEL in 80 (57.1%), with 'lead-in’ in 69 (49.3%).

Anemia (Hemoglobin (Hb) (gr/dL) <12) was proved in 103 (73.6%) (75% BOC; 72.5% TEL). It appeared in week ≤4 in 50.2% weeks 4–8 in 14.0% weeks 8–12 in 14.6% and weeks 12–24 in 8.5%. Grade 3 anemia (Hb <10) was observed in 55 patients (39.3%), it was set up in week ≤4 in 25.5%, weeks 4–8 in 40%, weeks 8–12 in 18.2% and weeks 12–24 in 7.3%. None of the patients showed <9 before week 4. Grade 4 (Hb <8.5) anemia was observed in 14 (10%). Mean drop of hemoglobin in week 2 (PI treatment) was 2.5 (SD 1.8) and in week 4 2.7 (SD 2.0). Drop was significantly higher in week 4 vs week 2 (PI treatment) was 2.5 (SD 1.8) and in week 4, 2.7 (SD 2.0). Drop was significantly higher in week 4 vs week 2 (PI treatment) was 2.5 (SD 1.8) and in week 4, 2.7 (SD 2.0).

Treatment of anemia was ribavirin dose reduction (RDR) in 28 (40%), RDR and use of erythropoetin (EPO) in 10 (18.2%) and RDR, EPO and blood transfusion in 18 (32.7%). None of the patients had to stop treatment because of anemia.

Conclusions: Anemia is present in 73.6% of patients treated with PI. Factors related with anemia were age, basal hemoglobin and drop in week 4, and F4. First monitoring of hemoglobin can be made safely at week 4.

**854 POLYMORPHISMS IN HISTONE DEACETYLASES IMPROVE THE PREDICTIVE VALUE OF IL-28B FOR CHRONIC HEPATITIS C THERAPY**

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**Introduction:** Classification of chronic hepatitis C (CHC) patients by IL-28B genotyping improves sustained virological response (SVR) prediction to antiviral combination therapy. However, an important percentage of patients still manifest uncertain therapy outcome. Histone deacetylases (HDACs) influence a broad repertoire of physiological processes, which interestingly include the differential modulation of STAT activity in response to IFN, signifying them as attractive targets for further study. Therefore, we evaluated the influence of selected single nucleotide polymorphisms (SNPs) within HDAC1–11 genes in order to identify additional predictive host genetic factors which may favor current prognosis of CHC treatment outcome.

**Methods:** 27 SNPs in HDAC1–11 genes were genotyped using the Illumina GoldenGate® Genotyping Assay. Their association with SVR was analyzed by logistic regression and adjusted by other covariates in 285 CHC patients.

**Results:** HDAC2 (rs3778216), HDAC3 (rs976552) and HDAC5 (rs368328) SNPs were significantly associated with SVR. CHC patients simultaneously bearing favorable C/C genotypes of HDAC2 and IL-28B SNPs reached a 90% of SVR; meanwhile, additional genotyping of HDAC5 rs368328 allowed the identification of a subgroup of good responder patients despite bearing T allele of IL-28B (SVR=75%). The performance of IL-28B genotype (AUC=0.630) was notably improved by the addition of these 3 HDAC’s SNPs (AUC=0.747, p=0.021) and resulted further enhanced once other significant clinical covariates were included (AUC=0.776). Interestingly, HDAC2 rs3778216 also remained predictive of SVR for HCV non-1 patients (AUC=0.733).

**Conclusions:** Genotyping of herein described HDAC polymorphisms, combined to IL-28B, might benefit the classification of CHC patients in accordance to their likelihood of attain SVR.

**855 A CLINICAL TRIAL EVALUATING LOW HCV RNA VIREMIA IN PATIENTS TREATED WITH TRIPLE THERAPY REGIMENS: IMPLICATIONS FOR PATIENT MANAGEMENT USING DIFFERENT ASSAYS IN CLINICAL PRACTICE**

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**Background and Aims:** Treatment duration (24 vs 48 weeks) with HCV protease inhibitors (PI) plus pegylated-interferon/ribavirin for genotype 1 patients is determined using response-guided therapy (RGT) based on HCV RNA results at 4 and 12 weeks of PI therapy. To shorten treatment duration an HCV RNA “undetectable” (target not detected, TND) result is required. In this study we compared HCV RNA viral load results using different assays at RGT time-points with clinical outcome and in low viremic samples.

**Methods:** We studied retrospective samples of 42 patients with chronic HCV infection treated with triple therapy including telaprevir initially tested with the COBAS AmpliPrep/COBAS®_TaqMan HCV Test-v1.0 (TAQMAN® HCV Test-v1.0). Samples were collected at baseline, week 4 and 12 and, re-tested with the COBAS AmpliPrep/COBAS®_TaqMan HCV Test-v2.0 (TAQMAN® HCV Test-v2.0) and the COBAS®_TaqMan® HCV Test-v2.0 (HPS/TAQMAN® Test-v2.0). Low viremic samples (n=141) were also collected (previous result, <50 IU/mL) and re-tested, comparing concordance between assays by overall percent agreement. So far 95% of the patients ended treatment and for 48% final treatment outcome was available.

**Results:** In 48% HCV RNA was “undetectable” at week 4 and 12 according to the CAP/CTM-v1.0. Only 31% had HCV RNA results that were “TND” by all three methods. None of these patients experienced a virological breakthrough or relapse. In contrast, of those having a “detectable” result by at least one method at 4 weeks and/or 12 weeks 55% experienced a virological failure including 9 patients with “undetectable” HCV RNA results at both time-points in one or two of the assays. Samples with an “undetectable” HCV RNA result showed good agreement between the CAP/CTM-v2.0 and the HPS/CTM-v2.0 when a 25 IU/mL cutoff was used (83%) and to a lesser extent (77%) with a lower cutoff (15 IU/mL).

**Conclusions:** RGT-based treatment decisions may significantly vary when different assays are used. Here we show a greater likelihood of treatment failure when an HCV RNA detectable result is found at any time-point and assay. Likewise, two undetectable results (4, 12 weeks) are not always associated with achieving an SVR if a single method was used. Additional time-points or testing
may improve the assessment of early treatment responses and SVR predictions.

856 CLINICAL SIGNIFICANCE OF DRUG–DRUG-INTERACTIONS IN THE ERA OF DIRECT ACTING ANTIVIRAL AGENTS AGAINST HEPATITIS C – A REAL-WORLD EXPERIENCE

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Background and Aims: With the introduction of direct-acting antivirals (DAAs) for the treatment of chronic HCV GT1 infection hepatologists have to face the new challenge of drug–drug interactions (DDIs). The two approved PIs boceprevir and telaprevir are both inhibitors and substrates of cytochrome P450 3A4 (CYP3A4). Data are limited to what extend patients with chronic HCV infection are treated with other drugs metabolized via CYP3A4. We here investigated the clinical significance of potential DDIs in a real-world cohort of patients receiving PIs for chronic HCV GT 1 infection.

Patients and Methods: Between June and November 2011 86 patients with chronic HCV GT 1 were selected for a PI-based antiviral treatment at our center. Full drug chart was assessed of all patients prior to therapy. All drugs were checked for potential DDIs with the approved PIs mainly using different DDI websites and the prescribing information. Risk for DDIs was graded as (1) no significant interaction, (2) potential interaction or (3) should not be coadministered.

Results: The 86 patients (mean age 54y) took 74 different co-medications. The median number of drugs/per patient was 2 (max. 11) and 19% reported to take at least 5 different drugs. On the other hand 30% of the patients did not regularly take any medication. Most common medications were levothryoxine (14%), metformin (12%) and propranolol (12%). Overall, no significant DDIs were expected based on DDI-websites for 65% of the drugs. For 32% DDIs with a PI were considered but were estimated to be tolerable if closely monitored or managed by dose modifications at some point. Only 3% of the drugs were strictly not recommended for coadministration. Consequently, DDIs were considerable in 36% of the patients but in only five patients current medication was strictly not suitable for a PI-based treatment.

Conclusions: Many patients with chronic HCV GT1 infection were affected by potential DDIs if treated with a PI. Still, expected significance of most DDIs was moderate, although requiring a closer monitoring or may lead to dose modifications. Overall, challenge of DDIs appears to be well manageable by a careful review of the patient’s drug chart.

857 TRIPLE THERAPY FOR HEPATITIS C VIRUS (HCV) INFECTION IN PATIENTS WITH COMPENSATED LIVER CIRRHOSIS: LESSONS LEARNED FROM THE FIRST REAL-WORLD EXPERIENCE

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Background: In phase-III studies addition of HCV protease inhibitors (PIs) Boceprevir and Telaprevir to PEG-Interferon-alfa and Ribavirin (P/R) was accompanied by a remarkable increase in SVR rates and an overall moderate safety profile. Still, patients with liver cirrhosis were underrepresented in these trials. We here aimed to analyse the safety and efficacy of P/R/PI in patients with liver cirrhosis in a real-world setting of a tertiary referral and transplant center.

Methods: Between June and November 2011 48 cirrhotic patients with chronic HCV GT1 infection were selected for an antiviral treatment with P/R/PI. Liver cirrhosis was determined by FibroScan (≥14.5 kPa), liver biopsy or obvious clinical signs. Here we show on-treatment data for safety and efficacy until week 24/28 of therapy (according to label).

Results: The 48 patients were at a mean age of 57 years and only 31% were treatment-naive. At baseline mean platelet count was 124/μl (29% <90/μl), mean Fibroscan result was 29kPa and 15% had a MELD-Score of >10. Only two out of 28 patients that remained on treatment until week 24/28 had detectable HCV/RNA at this stage. However, 20 (42%) patients had already discontinued treatment at this time. Most common reason was treatment intolerance or adverse events affecting 12 patients. Treatment was accompanied by a high number of haematological side effects. 50% of the patients developed anemia (<10g/dl) and in 27% of the patients Hb-level dropped below 8.5g/dl. Dose reductions of P/R were necessary in 50% and 44%, respectively. During this first 24/28 weeks of treatment 27 SAEs in 17 (35%) patients were documented. Most prevalent reasons for hospitalization were severe anemia (44%), infections (15%) and hepatic decompensation (11%); 3 patients died, 2 due to severe infections, for which a relation to treatment was suspected. Platelet count of <110/μl and a Child–Pugh-Score of >5 were associated with hospitalization as well as treatment failure.

Conclusions: Treatment with P/R/PI offers a high efficacy in patients with compensated liver cirrhosis. However, safety is limited especially in patients with advanced liver disease. We here identified platelet count as well as Child–Pugh-Score as valuable predictors for therapy-associated risk and treatment failure.
transfusion requirement. Median ferritin was 564 ng/ml at start and 1193 ng/ml at end-of-treatment and 921 one after treatment (6 patients available). No clinically relevant hematologic problems have been observed.

**Conclusions:** P+R is a safe and effective therapy for CHC in thalassemics. Considering the small number of patients, it is not possible to confirm the role of IL28B as outcome-predictor in this setting.

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POSSIBLE TOXIC HEPATITIS DUE TO POLYETHYLENE GLYCOL DURING TREATMENT FOR CHRONIC HEPATITIS C

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**Background and Aims:** Serum transaminases rose significantly in 7 patients with chronic hepatitis C who were treated with pegylated interferon α2a and ribavirin.

**Methods:** 219 patients with chronic hepatitis C, all with genotype 2/3, were treated at Gartnavel hepatitis clinics in Glasgow between 2005 and 2011, following the same protocol. For the 7 (3.2%) patients a full liver screen revealed chronic hepatitis C infection only. The same liver screen was repeated following the transaminase rise during treatment and failed to reveal additional comorbidity.

**Results:** 5 male and 2 female patients (3 Asian, 3 cirrhosis) with chronic hepatitis C experienced a significant rise (1.5–14 × baseline) in serum transaminases within weeks after commencement on treatment with pegylated interferon α2a and ribavirin. They all achieved rapid and end of treatment virological responses. 3 of the patients achieved sustained virological response, 3 relapsed and for 1 the 6 month post treatment test is pending.

There was no evidence to suggest that steatosis, autoimmunity, reactivation of hepatitis B or intercurrent illness was the cause of the liver injury. In 1 patient subsequent substitution of pegylated interferon α2b produced the same reaction and for 3 patients the level of transaminases recovered after pegylated interferon was changed to non-pegylated interferon. Additionally, it is evident that in those patients whose treatment was temporarily or permanently aborted, their transaminases rapidly improved and returned to baseline.

**Conclusion:** Our experience suggests the possibility of a toxic reaction to polyethylene glycol in a small number of patients being treated with pegylated interferon resulting in an acute hepatic response, which resolved when therapy was stopped or switched to non-pegylated interferon. Interestingly, this reaction only occurred in patients with non-genotype 1 infection. No alternative explanation for the acute hepatic reaction was elicited despite extensive investigation of these patients.

**860 REVISING PREDICTORS OF VIROLOGIC RESPONSE TO PEGIFN+RBV THERAPY IN HIV/HCV CONJUGATED PATIENTS: THE ROLE OF METABOLIC FACTORS AND ELEVATED GGT LEVELS**

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**Introduction:** Baseline predictors of sustained virologic response (SVR) in HIV/HCV coinfected patients (HIV/HCV) have increasingly become of scientific interest. One major limitation of previous has to be considered: No study included all well-established predictors of SVR. The aim of this study was to evaluate metabolic factors and elevated γ-glutamyltransferase (GGT) levels as independent predictors of treatment failure in a thoroughly documented cohort of HIV/HCV.

**Methods:** Sixty-four HIV/HCV treated with pegylated interferon-α-2a plus ribavirin (PEGIFN+RBV) at the Medical University of Vienna within a prospective trial were included in this study. In addition, 124 HIV/HCV from the EuroSIDA and the AHIVCOS cohorts were included as a validation cohort. Advanced liver fibrosis, GGT elevation, insulin resistance (IR), and low CD4+ T-lymphocyte nadir were defined as METAVIR F3/F4, GGT levels >1.5 × U/L, homeostasis model assessment of insulin resistance >2 mg/(mL × h) and CD4+ T-lymphocyte nadir <350 cells/µL, respectively.

**Results:** Patient characteristics of the derivation cohort: male: 70%, age: 38 ± 15.2, HCV-GT 1/4: 66%, advanced liver fibrosis: 36%, IL28B non-C/C: 64%, and CD4+ T-lymphocyte count: 529 ± 239. In the derivation cohort, lower rates of SVR were observed in patients with HCV-GT 1/4 (55% vs. 77%; p = 0.077), advanced liver fibrosis (39% vs. 76%; p = 0.004), IL28B non-C/C (51% vs. 83%, p = 0.013), GGT elevation (49% vs. 77%, p = 0.017), insulin resistance (40% vs. 67%; p = 0.039), and low CD4+ T-lymphocyte nadir (53% vs. 83%; p = 0.026) (Figure 1). HCV-GT 1/4 (OR26.3; p = 0.006), advanced liver fibrosis (OR20.2; p = 0.009), IL28B non-C/C SNP (OR8.27; p = 0.02), and GGT elevation (OR7.97; p = 0.012) were independent predictors of treatment failure in the derivation cohort, while both IR (OR3.51; p = 0.106), and low CD4+ T-lymphocyte nadir (OR2.64; p = 0.263) were not independently associated with treatment failure. A statistically significant correlation between GGT elevation and prior alcohol abuse (r = 0.259; p = 0.039), liver steatosis (r = 0.301; p = 0.034), and LDL-cholesterol (r = −0.256; p = 0.041) was observed. The importance of GGT elevation as an independent predictor of treatment failure was confirmed in the validation cohort (OR2.76; p = 0.026).
Conclusions: While GGT elevation emerged as an independent predictor of treatment failure in both the derivation and the validation cohort, no independent associations between metabolic factors and treatment failure were observed. Thus, our findings suggest that GGT elevation is an independent predictor of treatment failure in HIV/HCV that can easily be incorporated into predictive algorithms.

861 THE IMPACT OF IL28B rs12979860 SNP AND ADVANCED LIVER FIBROSIS ON RESPONSE-GUIDED THERAPY IN HIV/HCV COINFECTED PATIENTS OF PARTICULAR CLINICAL INTEREST

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Introduction: According to current European AIDS Clinical Society (EACS) guidelines for treatment of chronic hepatitis C, HCV-Genotype (GT) and rapid virologic response (RVR) exclusively determine chronic hepatitis C therapy duration in HIV/HCV coinfected patients (HIV/HCV). The aim of our study was to investigate the impact of interleukin 28B rs12979860 SNP (IL28B) and advanced liver fibrosis on the association between treatment duration and sustained virologic response (SVR) in groups of HIV/HCV in which either shortening or extension of treatment duration is recommended.

Methods: 430 HIV/HCV who received antiviral therapy with pegylated interferon plus ribavirin (PEGIFN+RBV) were included in this multinational, retrospective analysis. Patients with either GT2/3 and RVR (GT2/3-RVR) or GT1/4 without RVR (HCV1/4-noRVR) were divided into groups according to their treatment duration (12–36 vs. 36–60 and 60–84 vs. 60–84 weeks, respectively). Information on advanced liver fibrosis, defined as either METAVIR F3/F4 or liver stiffness >9.5 kPa was available in 341 patients.

Results: Patient characteristics: male: 81%, age: 41.5 ± 7.3 years, comitant antiretroviral therapy: 82%, baseline CD4+ T-lymphocyte count: 530 ± 246 cells/μl, treatment duration: 41.6 ± 16.4 weeks, GT1/4: 63%, IL28B non-C/C: 66%, and advanced liver fibrosis: 42%. Mean treatment duration was 41.6 ± 16.4 weeks, with RVR and SVR rates of 33% and 53%, respectively. In HCVGT1/4/noRVR patients, higher SVR rates in patients with extended treatment duration (60–84 weeks) compared to patients treated for 36–60 weeks were observed (35% vs. 60%; p = 0.008). While the difference did not attain statistical significance in patients with IL28B C/C (69% vs. 48%; p = 0.207), a statistically significant difference was observed among patients with IL28B non-C/C (56% vs. 28%; p = 0.011). SVR rates were comparable between HCV1/4/noRVR patients without advanced liver fibrosis treated for 36–60 and 60–84 weeks (46% vs. 61%; p = 0.176). In contrast, significantly lower SVR rates were observed in patients with advanced liver fibrosis (11% vs. 46%; p = 0.031). HCV2/3-RVR patients with shortened treatment duration (12–36 vs. 72 weeks) displayed high rates of SVR ranging from 75% to 100%, regardless of IL28B and advanced liver fibrosis.

Conclusions: Our study supports the extension of therapy duration to 72 weeks for HIV/HCV with GT1/4-noRVR as recommended by current EACS guidelines. Especially GT1/4-noRVR patients with IL28B non-C/C and advanced liver fibrosis benefit from 72 weeks of treatment. In patients with GT2/3-RVR, the shortening of treatment duration to 24 weeks is associated with excellent SVR rates, regardless of IL28B and advanced liver fibrosis.

862 THE IMPACT OF IL28B rs12979860 SNP ON THE ASSOCIATION BETWEEN TREATMENT DURATION AND SUSTAINED VIROLOGIC RESPONSE IN HIV/HCV COINFECTED PATIENTS

M. Mandorfer1, T. Reiberger1, B.A. Payer1, K. Neukam2, A. Rivero1, M. Puoti3, D. Ernst4, C. Boesecke5, A. Baumgarten6, J. Jaroszewicz7, A. Grzeszczuk8, R. Zangerle9, D. Meyer-Olson5, J.K. Rockstroh10, P. Ferenci1, J.A. Pineda2, M. Peck-Radosavljevic1, Vienna HIV & Liver Study Group. 1Department of Internal Medicine III, Division of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria; 2Unit of Infectious Diseases and Microbiology, Hospital Universitario de Valme, Seville, Spain; 3Department of Infectious Diseases, AO Ospedale Niguarda Ca’ Granda, Milano, Italy; 4Department of Clinical Immunology and Rheumatology, Medical University Hannover, Hannover, Germany; 5Department of Infectious Diseases, Medical University of Bialystok, Bialystok, Poland; 6Department of Dermatology and Venerology, Innsbruck Medical University, Innsbruck, Austria; 10Department of Dermatology and Venerology, Medical University of Vienna, Vienna, Austria

Introduction: Interleukin 28B rs12979860 single nucleotide polymorphism (IL28B) is a major predictor of virologic response in both HCV monoinfected and HIV/HCV coinfeected patients (HIV/HCV) treated with pegylated interferon plus ribavirin (PEGIFN+RBV). However, the impact of IL28B on the association between treatment duration and sustained virologic response (SVR) in HIV/HCV has yet to be examined.

Methods: 430 HIV/HCV who received antiviral therapy with PEGIFN+RBV were included in this multinational, retrospective analysis and divided into groups according to their treatment duration (12–36 vs. 36–60 vs. 60–84 weeks). Extended treatment duration was defined as a treatment of at least 12 weeks longer than recommended by the 2011 European AIDS Clinical Society (EACS) guidelines. The following treatment durations are recommended: HCV-genotype (HCV-GT) 2/3 with rapid virologic response (RVR) 24, without RVR 48 weeks; HCV-GT 1/4 with RVR 48, without RVR 72 weeks.

Results: Patient characteristics: male: 81%, age: 41.5 ± 7.3 years, comitant antiretroviral therapy: 82%, baseline CD4+ T-lymphocyte count: 530 ± 246 cells/μl, treatment duration: 41.6 ± 16.4 weeks, GT1/4: 63%, IL28B non-C/C: 66%, and advanced liver fibrosis: 42%. Mean treatment duration was 41.6 ± 16.4 weeks, with RVR and SVR rates of 33% and 53%, respectively. In HCVGT1/4/noRVR patients, higher SVR rates in patients with extended treatment duration (60–84 weeks) compared to patients treated for 36–60 weeks were observed (35% vs. 60%; p = 0.008). While the difference did not attain statistical significance in patients with IL28B C/C (69% vs. 48%; p = 0.207), a statistically significant difference was observed among patients with IL28B non-C/C (56% vs. 28%; p = 0.011). SVR rates were comparable between HCV1/4/noRVR patients without advanced liver fibrosis treated for 36–60 and 60–84 weeks (46% vs. 61%; p = 0.176). In contrast, significantly lower SVR rates were observed in patients with advanced liver fibrosis (11% vs. 46%; p = 0.031). HCV2/3-RVR patients with shortened treatment duration (12–36 vs. 72 weeks) displayed high rates of SVR ranging from 75% to 100%, regardless of IL28B and advanced liver fibrosis.

Conclusions: Our study supports the extension of therapy duration to 72 weeks for HIV/HCV with GT1/4-noRVR as recommended by current EACS guidelines. Especially GT1/4-noRVR patients with IL28B non-C/C and advanced liver fibrosis benefit from 72 weeks of treatment. In patients with GT2/3-RVR, the shortening of treatment duration to 24 weeks is associated with excellent SVR rates, regardless of IL28B and advanced liver fibrosis.
Conclusions: The association between treatment duration and SVR is influenced by IL28B. Nevertheless, decisions on treatment duration should primarily be based on HCV-GT and RVR, whereas IL28B may be considered as an additional factor for guiding individual treatment durations.

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**CORRELATION OF IL28B POLYMORPHISM WITH DEGREE OF FIBROSIS: ANALYSIS OF TREATMENT-NAIVE AND TREATMENT-EXPERIENCED CAUCASIAN PATIENTS INFECTED WITH HCV GENOTYPE 1 IN THE GEN-C STUDY**

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E-mail: a.mangia@tin.it

**Introduction:** The recently described single nucleotide polymorphism (SNP rs12979860) 3 kb upstream of the host IL28B gene is the strongest baseline factor associated with treatment response in G1 patients chronically infected with HCV. The association between this SNP and fibrosis progression has been the subject of debate. The current analysis evaluates the correlation of IL28B polymorphism with a diagnosis of cirrhosis or transition to cirrhosis among chronic hepatitis C (CHC) Caucasian patients infected with HCV G1 from the Gen-C study.

**Methods:** Gen-C is a large, prospective, international, multicentre, ongoing study in patients with CHC. At the time of this interim analysis, data from 1695 patients with IL28B data were available from patients of any race, HCV genotype and treatment status. The current analysis is restricted to treatment-naive or -experienced Caucasian patients infected with HCV G1 from the Gen-C study.

**Results:** Among the 564 treatment-naive patients, 27.8%, 58.2% and 14.0% respectively had IL28B CC, CT and TT polymorphism. Among the 340 patients with cirrhosis or transition to cirrhosis (CC=16.9%; CT=30.8%; TT=27.3%), 30.8% had IL28B CC, CT and TT polymorphism. Unlike treatment-naive patients the proportion of treatment-experienced patients with cirrhosis or transition to cirrhosis was not significantly different across polymorphisms (CC=36.3%; CT=30.8%; TT=27.3% [CA trend test p=0.2118]).

**Conclusion:** This interim analysis suggests that among G1 treatment-naive Caucasian patients the T allele (SNP rs12979860) is associated with a higher likelihood of cirrhosis or transition to cirrhosis. In G1 treatment-experienced patients there is no significant association between IL28B polymorphism and degree of fibrosis, which might be caused by a selection effect increasing the proportion of cirrhotic patients with IL28B CC and CT treatment-experienced patients.

Acknowledgement: Funded by F. Hoffmann-La Roche Ltd

<table>
<thead>
<tr>
<th>Table: Baseline characteristics of treatment-naive G1 patients</th>
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<tbody>
<tr>
<td>Mean parameter</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Age, years ±SEM</td>
</tr>
<tr>
<td>Male, %</td>
</tr>
<tr>
<td>Mode of infection, %</td>
</tr>
<tr>
<td>Injection drug use</td>
</tr>
<tr>
<td>Transfusion</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Time since infection, years ±SEM</td>
</tr>
<tr>
<td>Body mass index, kg/m² ±SEM</td>
</tr>
<tr>
<td>HCV RNA, log{10} IU/mL ±SEM</td>
</tr>
<tr>
<td>Cirrhosis/transition to cirrhosis, %</td>
</tr>
<tr>
<td>ALT &gt;3×ULN, %</td>
</tr>
<tr>
<td>Platelets, 10¹²/L ±SEM</td>
</tr>
</tbody>
</table>

*Upper limit of normal; †includes one patient with missing information.

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**ITPA ACTIVITY PREDICTS HB DECLINE AFTER FOUR WEEKS OF PI TRIPLE THERAPY, REGARDLESS OF PREVIOUS TREATMENT OR NAÏVE STATUS AND FIBROSIS: A MULTICENTER REAL-LIFE EXPERIENCE**

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**Background:** Anemia is one of the major morbidities associated with protease inhibitor (PI) therapy for HCV, and may require blood transfusions or RBV dose reduction/discontinuation which may compromise efficacy of treatment. Identification of patients at greatest risk for severe anemia would be clinically useful. ITPA polymorphisms causing IT(P)ase deficiency have been associated with protection from ribavirin-induced hemolytic anemia. We evaluated the association between ITPA polymorphisms and anemia during PI therapy in a real-world cohort.

**Methods:** 147 patients from Australia, Europe and USA who have received at least 4 weeks of PI triple treatment (50% boceprevir). Weight-based RBV (1000/1200 mg if < or >75Kg) in combination with Peg/IFN and PI was given according to label. Hb was evaluated at baseline and 4 weeks after the introduction of PI. Activity of ITPA was estimated (Fellay 2009) and correlation with week 4 Hb decline evaluated. Statistical analysis was performed by Pearson ρ2, Mann–Whitney test. IL28B (rs12979860) and ITPA polymorphisms (rs1127354 and rs7270101) genotyped with Taqman PCR. A MVR model including ITPA genotype, gender, age, fibrosis stage, and RBV dose (mg/kg) was used. RBV dose reduction and blood transfusions were organized according to product information and clinician discretion.

**Results:** Patients median age was 58 (28–75); 92% had advanced liver fibrosis/cirrhosis. 33% of patients were ITPA deficient. ITPA activity was associated with >3 g/dl Hb drop at week 4 with frequency of 64%, 22% and 9% among patients having 100, 60% and 30% activity (p=0.004). No association between week 4 Hb decline and gender, age <45yrs, fibrosis stage, treatment history or IL28B genotype was observed. ITPA deficient patients required transfusions during the first 12 weeks of treatment at rates lower than patients with 100% ITPA activity (15% vs 85%)
(p = 0.034). Female received more transfusions than male (59% vs 41%) (p = 0.016). At MVR, both gender and ITPA were predictive of transfusions OR 3.3 (95% CI 1.3 – 8) and OR 3.7 (95% CI 1.2 – 12), respectively. Rates of RBV dose reductions through the end-of-treatment by ITPA on the complete cohort will be presented.

**Conclusions:** In patients receiving PI triple treatment, pre-treatment ITPA evaluation predicts early anemia development. It also identifies patients who will require blood transfusions during treatment.

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**12 WEEK RESPONSE-GUIDED TREATMENT WITH THE NSSA INHIBITOR, GS-5885, THE NS3 PROTEASE INHIBITOR, GS-9451, PLUS Pegylated INTERFERON/RIBAVIRIN IN TREATMENT NAIVE GENOTYPE 1 HEPATITIS C INFECTED PATIENTS**

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**Background and Aims:** The efficacy of 2 direct acting antivirals (DAA); GS-5885 (NS5A inhibitor) and GS-9451 (NS3 protease inhibitor) with Peg + RBV, or combining GS-5885 with Peg + RBV, for a shortened treatment duration in early viral responders was assessed in treatment naïve chronic HCV genotype 1 infected patients.

**Methods:** 351 non-cirrhotic genotype 1 infected HCV patients were randomized 2:1 into two arms. **Arm 1** subjects received the four-drug combination of: GS-5885 (30 mg QD) + GS-9451 (200 mg QD) + Peg (180μG/week) + RBV (1000–1200 mg/day). Patients in **Arm 2** received the three drug combination of GS-5885 + Peg + RBV. Subjects in Arm 1 who achieved an extended vRVR (defined as HCVRNA concentrations below the limit of quantitation (LLOQ) at Weeks 2 and 4 that remained undetectable through week 8) were randomized to stop treatment at either Week 12 or Week 24.

**Results:** Analysis of available SVR4 rates are shown below based on sub-genotype, IL28B status, and treatment duration. Very rapid viral responders (76% of Arm 1) demonstrated high SVR4 rates in with both 24 weeks (95%) and a shortened 12 week (92%) course of therapy with all four drugs. The regimen was well tolerated with both 24 weeks (95%) and a shortened 12 week (92%) course of therapy. The most common adverse events were diarrhea and nausea. No clinically significant changes were seen in vital signs or ECGs; 2 and 1 patient(s) had transient elevations of triglycerides and bilirubin, respectively. Median reduction in HCVRNA concentrations following 7-days of ALS-2200 200mg or placebo (PLB) for 7 days. HCVRNA samples were analyzed using COBAS® Taqman® HCV Test (Version 2.0, Roche).

**Conclusions:** Additional data for the remaining patients will be presented.

<table>
<thead>
<tr>
<th>Table: HCVRNA reduction after 7 days</th>
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<tbody>
<tr>
<td><strong>HCV RNA (Log10 IU/mL)</strong></td>
</tr>
<tr>
<td><strong>Compensated cirrhosis ALS-2200 200 mg (N=3)</strong></td>
</tr>
<tr>
<td><strong>Placebo (N=1)</strong></td>
</tr>
<tr>
<td><strong>GT2-4 ALS-2200 200 mg (N=5)</strong></td>
</tr>
<tr>
<td><strong>Placebo (N=1)</strong></td>
</tr>
</tbody>
</table>

**Conclusions:** ALS-2200 200 mg given for 7 days was well tolerated and showed strong antiviral activity in treatment-naive patients with compensated cirrhosis or GT2–4 infection.
ARE CURRENT TREATMENT RATES SUFFICIENT TO REDUCE HCV PREVALENCE AMONG PEOPLE WHO INJECT DRUGS? MODEL PROJECTIONS IN SEVEN UK SETTINGS

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PROJECTIONS IN SEVEN UK SETTINGS

HCV PREVALENCE AMONG PEOPLE WHO INJECT DRUGS? MODEL ARE CURRENT TREATMENT RATES SUFFICIENT TO REDUCE

POSTERS

Edinburgh; reductions of: Manchester, 11.5% (95%CrI 6.9–16.6%) in Nottingham, 11.9% (95%CrI 7.1–17.6%); (a) current levels of treatment (b) 2-fold increase in venues and relative prevalence reductions of 2.7% (95%CrI 1.4–4%) in Bristol, 6.1% (95%CrI 3.0–10.4%) in East London, 7.0% (95%CrI 3.1–11.4%) in Manchester, 7.9% (95%CrI 5.6–10.3%) in Plymouth, 21.0% (95%CrI 14.0–29.3%) in Dundee, and 14.2% (95%CrI 10.0–19.4%) in Edinburgh. A 2-fold increase in treatment rates could result in 10-year relative prevalence reductions of >30% in Nottingham, Plymouth, Dundee, and Edinburgh; >15% in East London and Manchester; and >5% in Bristol. Less impact is achieved with higher baseline prevalence, lower baseline treatment rates, or if the average injecting duration is below 11 years. Uncertainty in baseline prevalence of chronic HCV and baseline treatment rates (because of uncertainty in PWID prevalence) contributed 37% (95% CI 22–56%) and 31% (95% CI 15–47%), respectively, of the variability in 10-year impact.

Conclusion: Current levels of treatment may result in modest reductions in prevalence over 10 years in some settings, but in most cases may go undetected given uncertainty in key parameters such as chronic HCV prevalence and PWID prevalence. However, in four settings a doubling of treatment rates could lead to >30% reduction in prevalence in 10 years.

TREATMENT WITH TELAPREvir-BASEd THERAPY AFTER EXPOSURE TO PEG-INF/RBV IN THE REALIZE STUDY: RESULTS FROM THE PHASE IIIB C219 ROLLOVER STUDY

P. Mathurin1, C. Sarrazin2, H.W. Reesink3, O. Weiland4, M. Diago5, G. Dusheiko6, A. Ascione7, Z. Ben-Ari8, L.G. Lyra9, W. Sievert10, M. Gschwantler11, T.L. Hassani12, E. Janzewska13, K. Janssen14, D. Luo15, A. Ghy16, S. De Meyer17, G. Picchio18, K. de Backer19, J. Witek15, Service Maladie de l'Appareil Digestif and INSERM U995, Université Lille 2, Lille, France; 1Johann Wolfgang Goethe University Medical Center, Frankfurt am Main, Germany; 2Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; 3Karolinska Institute, Karolinska University Hospital, Huddinge Stockholm, Sweden; 4Hospital General de Valencia, Valencia, Spain; 5Royal Free Hospital, London, UK; 6Centre for Liver Disease, Fatebenefratelli Hospital, Napoli, Italy; 7Sheba Medical Center, Tel Hashomer, Israel; 8Hospital Sao Rafael, Gastro-Hepatology, Brazil; 9Monash Medical Centre and Monash University, Melbourne, VIC, Australia; 10Wilhelminenspital, Vienna, Austria; 11University of California, School of Medicine, San Diego, La Jolla, CA, USA; 12Outpatients Clinic for Hepatology, Myslowicz, Poland; 13Janssen Infectious Diseases, Beerse, Belgium; 14Janssen Research & Development LLC, Titusville, NJ, USA

Background and Aims: C219 (NCT01054573) was an open-label, single-arm, rollover study that examined telaprevir (TVR)-based therapy in patients with chronic HCV genotype 1 infection who failed to achieve a sustained virologic response (SVR) following peginterferon/ribavirin (PR) alone in the REALIZE study, or with ≥1 dose of TVR in Phase I studies. We present data from patients previously treated with PR alone in the REALIZE study.

Methods: Patients received TVR 750 mg q8h plus PR at standard doses (180 µg once-weekly and 1000 or 1200 mg/day, respectively) for 12 weeks, followed by PR alone for 36 weeks. HCV RNA levels were evaluated using the COBAS TaqMan assay v2.0. The primary endpoint was SVR24, defined as having HCV RNA <<25 IU/mL target not detected< 24 weeks after last medication intake.

Table: Treatment outcome by prior response

<table>
<thead>
<tr>
<th>p (%)</th>
<th>Prior relaper</th>
<th>Prior partial</th>
<th>Prior null</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 27)</td>
<td>(N = 22)</td>
<td>(N = 32)</td>
<td>(N = 81)</td>
</tr>
<tr>
<td>SVR</td>
<td>22 (81)</td>
<td>16 (73)</td>
<td>11 (34)</td>
<td>49 (60)</td>
</tr>
<tr>
<td>On-treatment virologic failure1</td>
<td>1 (4)</td>
<td>3 (14)</td>
<td>8 (25)</td>
<td>22 (27)</td>
</tr>
<tr>
<td>Relapse2</td>
<td>1/25 (4)</td>
<td>1/19 (5)</td>
<td>1/34 (21)</td>
<td>5/58 (9)</td>
</tr>
<tr>
<td>Other3</td>
<td>3 (11)</td>
<td>2 (9)</td>
<td>0</td>
<td>5 (6)</td>
</tr>
</tbody>
</table>

1Based on entry to C219.
2Meeting a virologic stopping rule or having viral breakthrough.
3Denominator = number of patients with HCV RNA <<25 IU/mL target not detected< at end of treatment.
4Patients with detectable HCV RNA at end of treatment without viral breakthrough or patients with undetectable HCV RNA at end of treatment, but who discontinued study before SVR assessment.

Results: Of the 81 patients who did not achieve SVR in the PR arm of the REALIZE study, 27 (33%) were relapers. 22 (27%) partial responders, and 32 (40%) null responders. The study included 70% males, mostly Caucasian (93%) and median age 53 years. At baseline, an equal proportion of patients had genotype 1a/1b (50%/50%); 25% had cirrhosis. Median log10 HCV RNA was 6.62 IU/mL, with HCV RNA ≥800,000 IU/mL in 83% of cases. 90% of patients completed TVR treatment, and 67% completed PR treatment. Overall, 81%, 73%, and 34% of prior relapers, partial responders and null responders, respectively, achieved SVR (Table). Among patients with virologic failure, observed resistant variants were consistent with those previously described for TVR. The most frequent (>30%) adverse events (AEs) occurring during TVR treatment phase were pruritus (42%), fatigue (41%), rash (36%), and anemia (32%), most of which

were Grade 1/2. Serious AEs occurred in 5 (6%) patients in the TVR phase: anemia (4%), biliary colic and pyelonephritis (each 1%). TVR was permanently discontinued in 4 (5%) patients due to AEs.

**Conclusions:** In this treatment-experienced population, the efficacy, safety and tolerability of TVR-based therapy were consistent with previous studies. A similar safety profile was observed in the overall treatment phase as in the TVR phase.

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**ADDITION OF FLUVASTATIN TO STANDARD OF CARE TREATMENT DOES NOT IMPROVE SUSTAINED VIROLOGICAL RESPONSE IN CHRONIC HEPATITIS C GENOTYPE 3 PATIENTS**


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**Background:** A twenty-four week combination of pegylated interferon and ribavirin presents standard of care (SOC) treatment for chronic hepatitis C (CHC) genotype 3 patients with sustained virological response (SVR) being achieved in 76 to 82%. In vitro studies showed inhibitory effect of statins to HCV replication. In some clinical trials combination of statins and SOC improved SVR in CHC genotype 1. The aim of the study was to investigate the efficacy of SOC with added fluvastatin on SVR in a cohort of Slovene patients infected with HCV genotype 3.

**Methods:** The prospective study enrolled all over 98 naïve CHC genotype 3 Caucasian patients, 45 in the study group that were treated with the combination of fluvastatin 80mg per day and SOC, and 53 historical controls matching the study group in genotype, age and gender, treated with SOC alone.

**Results:** In the fluvastatin group there were 73 males, 93% aged ≤40 years, 96% with body mass index (BMI) ≤30, 38% presenting viral load ≤600,000 IU/ml and 49% having METAVIR stage <2 on liver biopsy. In the control group there were 62 males, 85% aged ≤40 years old, 98% with BMI ≤30, 55% with low viral load and 55% presenting METAVIR stage <2. The differences in baseline characteristics between the two groups did not reach statistical significance, except for the higher number of younger patients with low viral load in the fluvastatin group (p=0.026). SVR was 94.7% in the fluvastatin group vs. 92.5% in the control group. Rapid (RVR), early (EVR) and end of treatment (ETR) viral responses as well as SVR did not differ significantly between the two groups. Univariate analysis identified no significant differences regarding the influence of BMI, METAVIR stage, baseline LDL and triglyceride levels, RVR and EVR on SVR between the groups. Seven patients in the fluvastatin group discontinued treatment prematurely, one patient discontinued fluvastatin due to side effect.

**Conclusions:** Addition of fluvastatin to SOC did not improve SVR in patients with CHC genotype 3. High SVR in both groups was most probably due to some favourable pre-treatment factors (age, BMI, METAVIR stage).

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**REAL-WORLD EFFICACY AND SAFETY OF TELAPREVIR IN COMBINATION WITH PEGINTERFERON alfa-2a AND RIBAVIRIN: INTERIM ANALYSIS FROM THE GERMAN NON-INTERVENTIONAL PAN STUDY**

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**Introduction:** Until recently experience with telaprevir (TVR) was based almost exclusively on the results of controlled randomized clinical trials in highly selected patients. In October 2011 TVR was approved in Germany in combination with peginterferon alfa-2a or alfa-2b plus ribavirin in chronic hepatitis C patients infected with HCV genotype 1.

In particular, a greater risk of anaemia occurs when RBV concentrations exceed 3 mg/L in the first 4 weeks of therapy, that is, before steady state occurs. Currently dose reduction of RBV only after anaemia develops is advocated.

**Aim:** To construct and validate a model to predict Day 28 (approaching steady-state) plasma concentrations of RBV using either Day 7 or Day 14 plasma concentrations.

**Methods:** A population pharmacokinetic model was constructed (Kinetica) using pharmacokinetic data from Caucasian (n=48) and Japanese (n=28) populations. Blood samples were collected (Days 0, 7, 14 and 28 of therapy) from HCV patients (n=10) treated with RBV and pegylated-interferon, to validate the model. Plasma RBV concentrations were measured by HPLC. These data and the model parameters derived from the Caucasian and Japanese populations were examined using Bayesian forecasting (Abbotbase). The Day 28 AUC0–12.5 h (‘observed’) was then calculated using trapezoidal analysis. Trough and 2 h post-dose concentrations on Day 7 or Day 14 were used to estimate the AUC on Day 28 (‘predicted’) for each patient. Bias and precision were calculated for ‘observed’ versus ‘predicted’ AUC0–12.5 h.

**Results:** A two-compartment population pharmacokinetic model provided the best fit to the RBV concentrations. The observed versus predicted AUC0–12.5 h on Day 28 derived from plasma concentrations on Day 7 gave a bias and precision of ±21.9% and 32.2%, respectively. Using the plasma concentrations on Day 14, bias and precision improved, ±8.4% and 19.6%, respectively, and were within clinically acceptable limits (±20%).

**Conclusions:** The model was sufficiently robust to predict RBV plasma concentrations on Day 28 using Day 14 measurements. Application of this approach could result in earlier dose adjustment reducing the risk of early onset of anaemia, whilst maintaining concentrations for optimal efficacy.

Acknowledgements: SydPath, St. Vincent’s Hospital, for assistance with experimental setup and supplying materials and apparatuses for analytical experiment. Dr Akihito Tsubota and Prof Paul Glue, for providing plasma ribavirin concentration-time data from respective clinical studies sponsored by Merck Sharpe & Dohme.
**POSTERS**

**Methods:** The PAN study is a non-interventional study conducted by the Association of German Gastroenterologists in Private Practice (bng) in collaboration with Roche. Patients are eligible if they are prescribed TVR or boceprevir plus peginterferon alfa-2a/ribavirin. Here we restrict the analysis to treatment naive patients receiving TVR plus peginterferon alfa-2a/ribavirin who have, or had the potential to, complete 12 weeks of treatment.

**Results:** Overall 239 patients, including 24 (10.0%) with cirrhosis were included in the present analysis. Among patients with evaluable data at each time point the proportion with undetectable HCV RNA at week 4 and 12 was 100/155 (64.5%) and 135/166 (81.3%), respectively. A subset of 73 patients (30.5%) of patients did not have an evaluable week 12 value. The positive predictive value for week 4 undetectable HCV RNA for week 12 HCV RNA <1,000 IU/mL (futility threshold) was 94.3%. Among the 124 patients with adequate data to determine eRVR status (undetectable HCV RNA at week 4 and 12) 80 (64.5%) achieved an eRVR and would therefore be potentially eligible for shortened treatment duration. Over the first 12 weeks 15.7% and 5.0% of patients dose modified ribavirin or peginterferon alfa-2a, respectively. Up to week 12 a total of 21 (8.9%) and 62 (26.3%) patients had haemoglobin <8.5 g/dL or ≥8.5 but <10 g/dL respectively. Adverse events reported at ≥15% included fatigue (49.8%), skin disorder (31.0%), pruritus (25.1%), nausea (24.7%), anaemia (23.0%) and headache (15.9%).

**Mean parameter**

| Age >40 years, n (%) | 163 (68.2) |
| Male, n (%)          | 137 (57.3) |
| Caucasian race, n (%)| 234 (97.9) |
| Body mass index, kg/m² ±SD | 25.6 ±4.0 |
| Diagnosis of cirrhosis, n (%) | 24 (10.0) |
| Platelets, x10^9/L ±SD | 211 ±73 |
| ALT >3 × ULN*, n (%) | 50 (23.1) |
| HCV RNA, log_{10} IU/mL ±SD | 6.0 (0.9) |
| HCV RNA >400,000 IU/mL, n (%) | 174 (75.7) |
| Genotype, n (%) |  |
| 1a | 63 (26.4) |
| 1b | 131 (54.8) |
| 1, subtype: other/unknown | 45 (18.8) |
| 1L28B genotype |  |
| CC | 15 (6.3) |
| CT | 36 (15.1) |
| TT | 14 (5.9) |
| Unknown | 174 (72.8) |

1 >1 result concluding cirrhosis. biopsy, clinical appearance, sonography, elastography.

**Conclusion:** Real world experience with telaprevir plus peginterferon alfa-2a/ribavirin are generally consistent with the results of the published phase 3 trials. Of concern a high proportion of patient did not have a week 12 value which is essential to determine treatment duration.

Acknowledgement: Funded by Roche

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**Substantial Renal Impairment is Not Infrequent in HCV Patients Under Triple Therapy with Telaprevir or Boceprevir**


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E-mail: stefan.mauss@center-duesseldorf.de

**Introduction:** Until recently experience with telaprevir (TVR) and boceprevir (BOC) was based exclusively on clinical trials in selected patients. In late summer 2011 TVR and BOC were approved in Germany and made available for treatment in clinical practice. In late summer 2011 TVR and BOC were approved in Germany and made available for treatment in clinical practice.

**Methods:** The PAN study is a non-interventional study conducted by the Association of German Gastroenterologists in Private Practice (bng) in collaboration with Roche. Patients are eligible treated with peginterferon alfa-2a/ribavirin (PEG/RBV) or triple therapy with TVR or BOC. Here we restrict the analysis to patients having completed at least 12 weeks of treatment. For estimation of glomerular filtration rate (GFR) the recently presented CKD-EPI formula was chosen as it may be best suited to reflect changes of eGFR in patients with normal or mildly impaired renal function.

**Results:** Overall 907 patients were included, 582 on TVR, 214 on BOC and 111 on dual therapy. At week 12 a decrease to <60 ml/min (=renal insufficiency stage 3) in patients with an eGFR >60 ml/min at baseline was observed in 49/907 (5.4%). Patients on TVR 38/583 (6.5%) and BOC 10/214 (4.7%) experienced more frequently a decrease in renal function to <60 ml/min compared to patients on PEG/RBV 1/111 (0.9%) (p < 0.05). Risk factors associated with renal insufficiency stage 3 were age (p < 0.001), arterial hypertension (p < 0.001), Diabetes mellitus (p < 0.05) and being on triple therapy with TVR or BOC (p < 0.05). There was no association with anemia, treatment history or sex.

**Conclusion:** Renal impairment has not been reported as safety signal in clinical trials with TVR or BOC. However in this large cohort including older patients and patients with risk factors for renal impairment a marked decline in renal function was observed in about 5% of patients on triple therapy. In addition to being a safety concern, substantial ribavirin dose reductions have to be considered in these patients.

**A Scoring Model for Prediction of Relapse Among Chronic HCV Genotype 4 Patients Treated with Peginterferon and Ribavirin**

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Chronic hepatitis C is a major health problem in Egypt, treatment is costly and has multiple side effects, identifying the predictors of relapse of the disease after treatment will help in better selection of patients and avoidance of unnecessary side effects.

**Materials and Methods:** This retrospective study was done on 1081 Egyptian chronic HCV patients, who were treated in Shebein El-
Kom teaching hospital, Egypt, with pegylated IFN and Ribavirin for one year. Follow up for patients for 24 weeks after (EOT) end of treatment. Demographic, laboratory and liver histopathology data were analyzed against relapse by univariate and logistic regression analysis. Values of β coefficient in the regression model was used to build a scoring model.

**Results:** Out of 1081 patients, 661 achieved End of Treatment (EOT) Response (57%), 570 of them showed Sustained virological response (92.5%) and 46 patients relapsed after EOT response (7.5%). Logistic regression analysis showed that independent predictors of relapse after EOT response were male gender (OR 5.68, 95% CI 2.23–14.45, P < 0.001), age (OR 1.08 95% CI 1.04–1.13, P < 0.001), BMI (OR 1.18 95% CI 1.06–1.30, P < 0.001), stage of fibrosis (OR 2.32 95% CI 1.41–3.82, P < 0.001), development of anemia (OR 2.69 95% CI 1.72–4.22, P < 0.001), and development of thrombocytopenia (OR 3.07 95% CI 1.72–5.48, P < 0.001). A scoring model for prediction of relapse was calculated based on the β value of each predictor. The lower limit of the score was 6.77 and the upper limit was 19.87. The ROC curve for prediction of relapse by the score showed that the area under the curve (AUC) is 95.3 (95% CI 92.8–97.8, P < 0.001). A cutoff value of 12.15 had 93.5% sensitivity, 82.1% specificity, 99.4% negative predictive value and 29.7% positive predictive value.

**Conclusion:** Rate of relapse among Egyptian chronic HCV patients (genotype 4) treated with peg-IFN and ribavirin is low (7.5%). A scoring model using age, BMI, stage of fibrosis, and development of anemia or thrombocytopenia during therapy can efficiently predict relapse.

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**RAPID VIROLOGICAL RESPONSE IN HIV/HCV-COINFECTED PATIENTS WITH GENOTYPE 1 RECEIVING TELAPREVIR OR BOCEPREVIR OUTSIDE CLINICAL TRIALS: RESULTS FROM HEPAVIR-THERAPY COHORT**


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**Background and Aims:** According to data reported in clinical trials, the efficacy of pegylated interferon (peg-IFN) and ribavirin (RBV) along with boceprevir (BOC) or telaprevir (TVR) is superior to the combination of peg-IFN and RBV for the treatment of chronic hepatitis C with genotype 1 in HIV-infected patients. However, there is no information about the response to this triple therapy used in real conditions in these patients. Since rapid virological response (RVR) accurately predicts sustained vireological response, we have determined the rate of RVR among HIV/HCV-coinfected Spanish patients receiving BOC or TVR outside clinical trials.

**Methods:** HIV/HCV-coinfected individuals who started peg-IFN/RBV plus BOC or TVR during 2012 at four hospitals in Spain who had reached week 4 or week 8, in the case of TVR or BOC therapy, respectively, were included in this study. Patients receiving peg-IFN alpha-2a (180 μg weekly) or peg-IFN alpha-2b (1.5 μg/kg weekly) plus RBV (800–1200 mg/day) along with BOC (800 mg 3 times daily) or TVR (750 mg or 1125 mg 3 times daily, if efavirenz was given concomitantly). All subjects treated with BOC had a 4-week lead-in of peg-IFN and RBV. RVR was defined as undetectable HCV RNA in serum 4 weeks after starting BOC or TVR.

**Results:** At the time of abstract submission, 31 patients had been included in this study. Their baseline characteristics were: male: 77%; genotype 1b subtype: 45%; pretreated: 90% (null-responders 39%, partial-responders 30%, relapsers 26%), discontinuation due to adverse events 3%; cirrhosis: 52%; IL28B CC: 30%; HCV viral load >600,000 UI/L: 90%, undetectable HIV viral load: 90%; mean CD4 cell count: 602/mm³. Antiretrovirals most used during HCV therapy were tenofovir (71%), emtricitabine (61%) and raltegravir (61%). Eighteen (58%) patients received TVR and 13 (42%) BOC. RVR was observed in 27 (87%) individuals. Eleven (85%) subjects who received BOC and 16 (89%) patients treated with TVR achieved RVR. Only one patient discontinued HCV therapy due to adverse events during this period study.

**Conclusion:** According to this study, the rate of RVR in HIV/HCV-coinfected patients receiving BOC or TVR based-therapy outside trials is very high and comparable to that showed in previous clinical trials.
**Conclusion:** Real world experience with BOC plus peginterferon alfa-2a/ribavirin in Germany show similar virological outcomes and side effects to the phase 3 trials. Week 8 HCV RNA values essential to determine suitability for reduced treatment duration were not collected in a significant proportion of patients.

**Acknowledgement:** Funded by Roche

<table>
<thead>
<tr>
<th>Mean parameter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;40 years, n (%)</td>
<td>52 (76.5)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>36 (52.9)</td>
</tr>
<tr>
<td>Caucasian race, n (%)</td>
<td>68 (100)</td>
</tr>
<tr>
<td>Body mass index, kg/m² ± SD</td>
<td>270±5.2</td>
</tr>
<tr>
<td>Diagnosis of cirrhosis, n (%)</td>
<td>8 (11.8)</td>
</tr>
<tr>
<td>Platelets, ×10³/L ± SD</td>
<td>220±74</td>
</tr>
<tr>
<td>ALT &gt;3×ULN, n (%)</td>
<td>12 (20.0)</td>
</tr>
<tr>
<td>HCV RNA, log₁₀ IU/mL ± SD</td>
<td>6.0 (0.9)</td>
</tr>
<tr>
<td>HCV RNA &gt;400,000 IU/mL, n (%)</td>
<td>46 (71.9)</td>
</tr>
</tbody>
</table>

1a 21 (30.9)
1b 32 (47.1)
1, subtype: unknown 15 (22.1)

**IL28B genotype**
- CC CT TT Unknown 9 (13.2)
- CT 9 (13.2)
- TT 2 (2.9)
- Unknown 48 (70.6)

‡ ≥1 result concluding cirrhosis. biopsy, clinical appearance, sonography, elastography.

**876**

**ACH-3102, A SECOND GENERATION NS5A INHIBITOR, DEMONSTRATES POTENT ANTIVIRAL ACTIVITY IN PATIENTS WITH GENOTYPE 1a HCV INFECTION DESPITE THE PRESENCE OF BASELINE NS5A-RESISTANT VARIANTS**


1Duke Clinical Research Institute, Durham, NC, 2Avail Clinical Research, LLC, DeLand, FL, 3Alamo Medical Research, Laboratory, Los Alamos, NM, USA

**Methods:** In vitro, ACH-3102 demonstrates potent activity against HCV genotype 1 (GT1); furthermore, it retains activity against multiple viral variants. Presented here are phase I study results in non-cirrhotic chronic HCV GT1a patients.

**Results:** ACH-3102 was safe and well-tolerated in patients with chronic HCV GT1a infection at all tested doses. Robust, rapid and sustained suppression of plasma HCV RNA levels was observed after all tested single doses of ACH-3102. Viral kinetics parameters estimated from modeling of ACH-3102 monotherapy demonstrated high drug efficiency. These data provide evidence that ACH-3102 is a safe and well-tolerated second-generation NS5A inhibitor with potent anti-viral activity against both wild-type HCV virus and NS5A-resistant viral variants, making it a promising candidate for future use in the treatment of chronic HCV infection.

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**THE EFFICACY AND SAFETY OF TREATING HEPATITIS C IN PATIENTS WITH A DIAGNOSIS OF SCHIZOPHRENIA**


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**Background:** Treating hepatitis C with pegylated interferon alpha may induce or exacerbate psychiatric illness including depression, mania and aggressive behavior. There is limited data regarding treatment in the context of chronic schizophrenia. We sought to establish the safety and efficacy of treatment of patients with a diagnosis of schizophrenia amongst patients attending treatment centres in Greater Glasgow.

**Methods:** Patient and treatment data collected on the Scottish hepatitis C database were retrospectively analysed according to the presence or absence of a diagnosis of schizophrenia. Combination antiviral therapy was defined as Interferon (pegylated or standard) and Ribavirin. Treatment outcomes including sustained viral response (SVR) rates, reasons for treatment termination and adverse events were documented.

**Results:** 5497 patients were recorded on the database, of whom 64 (1.2%) had a diagnosis of schizophrenia. Patients with and without schizophrenia were of similar age at diagnosis [median 34 (IQR 31–40) vs 36 (IQR 29–41) years, p=0.85]. Patients with schizophrenia had higher rates of current or previous intravenous drug use [50/64 (78.1%) vs 3015/5433 (55.5%), p<0.01] and prior alcohol excess (>21 units/week) [25/64 (39%) vs 1211/5433 (22.2%), p=0.02]. More patients with schizophrenia had a diagnosis of cirrhosis [13/64 (20.3%) vs 589/5419 (10.86%), p=0.02]. Of those patients who had attended at least one clinic appointment 1639/4415 (37.1%) of patients without schizophrenia commenced treatment versus 26/61 (42.6%) of patients with schizophrenia (p=0.21). Patients with schizophrenia took almost three times as long to commence treatment after initial referral [median 1123 (IQR 531–2130) vs 421 (IQR 209–1086) days, p<0.01], despite similar times from referral to first attendance [median 65 (IQR 36–141) vs 62 (IQR 35–130) days, p=0.92]. The treatment outcomes are presented in the table.

**Table:**

<table>
<thead>
<tr>
<th>Discontinuation</th>
<th>Schizophrenia (n=22)</th>
<th>Patients without schizophrenia (n=1453)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>due to side effects</td>
<td>4.5% (1)</td>
<td>13% (189)</td>
<td>0.34</td>
</tr>
<tr>
<td>due to non-compliance</td>
<td>0% (0)</td>
<td>4.9% (72)</td>
<td>0.62</td>
</tr>
<tr>
<td>Overall sustained viral response (SVR)</td>
<td>81.8% (18)</td>
<td>54.2% (788)</td>
<td>0.09</td>
</tr>
<tr>
<td>SVR in Genotype 1</td>
<td>33.3% (2/6)</td>
<td>32.8% (199/555)</td>
<td>0.25</td>
</tr>
<tr>
<td>SVR in Genotype 2&amp;3</td>
<td>100% (16/16)</td>
<td>82% (589/714)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Conclusions: Patients with stable schizophrenia are good candidates for hepatitis C treatment.

878 RENAL DYSFUNCTION DURING PEG-IFN + RBV + TVR TREATMENT
M. Nakamuta1, M. Kohjima1, T. Yoshimoto1, M. Kurokawa1, T. Nakamura1, M. Iwata1, N. Fukushima1, K. Fukuizumi1, N. Fujimori1, K. Kawabe1, K. Haraguchi1, Y. Sumida1, N. Harada1, H. Nomura2, M. Enjoji3, 1Gastroenterology, Clinical Research Center, Kyushu Medical Center, Fukuoka, Japan
2Gastroenterology, Shin-Kokura Hospital, Kitakyushu, Japan
3Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan

Background: Telaprevir (TVR) is an NS3/4A protease inhibitor that is used for the treatment for chronic HCV patients in combination with peg-IFN and ribavirin (RBV). Although TVR were usually used 2250 mg/day tid, we sometimes need to reduce the dose of TVR for undersized patients or aged women. Furthermore, break of the therapy is required because of a number of adverse events like renal dysfunction as well as serious anemia or rash. In this study, we have examined the factor involved in renal dysfunction during the treatment, and compared viral kinetics during the therapy with respect to the dose of TVR.

Methods: The patients with HCV genotype 1b have been treated with peg-IFNα2b + RBV + TVR from 2011 (n = 96). We have used the reduced-dose of TVR (1500 mg/day bid) for patient aged 65 or over. We examined the factor involved in renal dysfunction during the treatment, and compared viral kinetics with the dose of TVR.

Results: eGFR was dropped off for every patient from early course of the treatment (day 3). The degree of exacerbation for renal function was milder in patients with reduced TVR despite of their lower eGFR at the baseline. On viral kinetics during early course of the therapy (day 0 – day 56), the response of HCV was similar between the treatment, and compared viral kinetics with the dose of TVR.

Conclusion: Renal dysfunction during peg-IFN + RBV + TVR treatment might come from pre-renal dysfunction as well as failure of renal tubule. The reduction of TVR could prevent renal dysfunction during the therapy, while the reduction will not affect HCV clearance during early course of the therapy.

879 AGING AND ANTI HCV TREATMENT OUTCOME IN HCV INFECTED WOMEN WITH AND WITHOUT HIV INFECTION
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Background: Little data is available about HCV treatment outcome in women treated with Peg-IFNα2b plus Ribavirin (PegIFN/RBV), particularly if co-infected with HIV.

Methods: Variables associated with the sustained virological response (SVR) in a cohort of women with chronic hepatitis C (CHC) who started PegIFN/RBV, followed in a single centre of Infectious Diseases in Brescia, Italy, have been retrospectively analysed. Chi-Square or Fisher’s Exact test and t-test for unpaired samples were used for statistical analysis. A p value <0.05 was considered to be significant. Multivariate logistic analysis has been adjusted for use of MVC, age, sex, fibrosis stage.

Results: 57 patients were included in the analysis: 26 in control-arm and in 31 in MVC-arm, 22% female, median age 46 (IQR43–48) years, CD4 506 (405–685) cell/mm3, AST42 (IQR31–59) IU/ml, ALT66 (IQR45–96) IU/ml, HCV-RNA 5.7 (5.3–6.2) log10 IU/ml. At 48W analysis has been assessed. Chi-Square or Fisher’s Exact test and t-test for unpaired samples were used for statistical analysis. A p value <0.05 was considered to be significant. Multivariate logistic analysis has been adjusted for use of MVC, age, sex, fibrosis stage.

Results: 57 patients were included in the analysis: 26 in control-arm and in 31 in MVC-arm, 22% female, median age 46 (IQR43–48) years, CD4 506 (405–685) cell/mm3, AST42 (IQR31–59) IU/ml, ALT66 (IQR45–96) IU/ml, HCV-RNA 5.7 (5.3–6.2) log10 IU/ml. At 48W analysis has been assessed. Chi-Square or Fisher’s Exact test and t-test for unpaired samples were used for statistical analysis. A p value <0.05 was considered to be significant. Multivariate logistic analysis has been adjusted for use of MVC, age, sex, fibrosis stage.

Conclusions: Our data suggest that aging is related to poor treatment outcomes in HCV infected women as well as the presence of cirrhosis, HIV infection and HCV genotypes difficult to treat. Therefore, an early treatment of HCV infected women should be strongly recommended, particularly before menopause, in order to achieve higher rates of SVR.
INTERIM ANALYSIS
PRIOR RELAPSERS WITH G1 CHRONIC HEPATITIS C: CONCISE

Conclusion: Maraviroc based HAART may contribute to slow the inflammation and liver stiffness worsening in HIV/HCV coinfected persons.

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HIGH SVR RATES (SVR4) FOR 12-WEEK TOTAL TELAPREVIR COMBINATION THERAPY IN IL28B CC TREATMENT-NAÏVES AND PRIOR RELAPSEs WITH G1 CHRONIC HEPATITIS C: CONCISE INTERIM ANALYSIS

PREENTIONAL TREATMENT D.R. Nelson1, F. Poordad2, J.J. Feld3, M.W. Fried4, J.M. Jacobson5, I.M. Jacobson5, K. Neukam1, A. Caruz2, A. Rivero-Juarez3, P. Labarga4, M. Marquez5, L.M. Real1, R. Herrero2, A. Rivero2, V. Soriano3, J.A. Pineda1. 1Unit of Infectious Diseases and Microbiology, Hospital Universitario de Valme, Seville, 2Faculty of Sciences, University of Jaen, Jaen, 3Unit of Infectious Diseases, Hospital Universitario Reina Sofia, Cordoba, 4Department of Infectious Diseases, Hospital Carlos III, Madrid, 5Hospital Virgen de la Victoria, Malaga, Spain

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Introduction: The rates of sustained virological response (SVR) to pegylated interferon (Peg-IFN) plus ribavirin (RBV) highly depend on viral and host-related factors, as well as viral kinetics during therapy. The latter define treatment duration. However, in the case of HCV genotype 3 (HCV-3), predictive tools for treatment response are scarce. A correlation between plasmatic levels of low-density lipoprotein (LDL) and SVR has been described. Variations in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene are known to interfere with the LDL/LDL receptor system and could thus impact on SVR.

Objective: To evaluate the impact of variations in the PCSK9 gene on response to therapy against HCV-3 infection with Peg-IFN plus RBV.

Patients and Methods: 132 HCV-3-infected patients who fulfilled a course of Peg-IFN plus RBV were prospectively followed. Individuals were genotyped for 16 single nucleotide polymorphisms (SNPs) that covered the genomic region of PCSK9 gene. Those SNPs associated with SVR with a p<0.025 in a standard case/control allelic associations (1 degree freedom) analysis were selected. Univariate and multivariate analyses were performed to identify those SNPs associated either with SVR or with rapid virological response (RVR).

Results: The SNP rs2479409 fulfilled the criteria for further analyses. The genotype frequencies were: 57 (43%) AA, 60 (45%) AG and 15 (11%) GG. 108 (82%) individuals of the overall population presented SVR. Forty-five (79%) patients with genotype AA, 55 (92%) with genotype AG and 8 (53%) with genotype GG showed SVR (p=0.002). RVR was achieved by 72 (71%) subjects with genotype AA/AG versus 6 (43%) with genotype GG (p=0.033). In an analysis adjusted for sex, age, baseline HCV-RNA, ALT and presence of advanced fibrosis, rs2479409 genotype AA/AG was independently associated with SVR [adjusted odds ratio: 6.89 (95% confidence interval=1.65–28.8), p=0.008].

Conclusion: The genotype of rs2479409 at the PCSK9 gene independently predicts SVR to Peg-IFN plus RBV in HCV-3-infected. Thus, in spite of high overall rate of SVR in HCV-3 infections, the inclusion of PCSK9 genotype as a predictor of viral response during treatment could be a useful tool to individualize the duration of therapy.

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GENETIC VARIATIONS IN proprotein convertase subtilisin/kexin type 9 (PCSK9) GENE ARE ASSOCIATED WITH RESPONSE TO PEGYLATED INTERFERON/RIBAVIRIN IN HEPATITIS C VIRUS GENOTYPE 3-INFECTED PATIENTS
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Background and Aims: Genotype 1 chronic hepatitis C-infected treatment-naïve or prior relapse patients without cirrhosis who receive telaprevir, peginterferon alfa/ribavirin (T/PR) and have undetectable HCV RNA at Weeks 4 and 12 are eligible to receive 24-weeks of total therapy. Retrospective analyses from PROVE2 suggested that T/PR treatment duration in patients with the IL28B CC genotype could be further reduced to 12 weeks. We report an interim analysis of the CONCISE study.

Methods: Treatment-naïve or prior relapse non-cirrhotic patients with IL28B CC genotype received T/PR for 12 weeks (T 1125mg bid, P 180mg/week, weight-based R 1000 or 1200mg/day). Patients with Rapid Virologic Response (RVR: Week 4 HCV RNA <25 IU/mL, target not detected) and who continued all study drugs through the first 12 weeks of therapy were eligible for randomization (2:1) at Week 12 to receive no further treatment (T12/PR12) or 12 additional weeks of PR (T12/PR24). SVR rates were assessed 4 (SVR4) or 12 (SVR12) weeks after end of planned treatment.

Results: 128 patients were followed 16 or more weeks: 75 (59%) male, mean age 47 years, and 91 (71%) are genotype 1a. Race was Caucasian (86.7%), Black (1.6%), Asian (7.0%) and Other (4.7%). Randomization occurred in 85 (66%) of whom 70 (82%) were treatment naïve; 57 in T12/PR12 and 28 in T12/PR24 arms respectively. SVR rates were assessed 4 (SVR4) or 12 (SVR12) weeks after end of planned treatment.

Conclusions: High SVR rates from the CONCISE study interim analyses suggest the potential for the defined IL28B CC patients with RVR to shorten duration of telaprevir plus peginterferon/ribavirin to 12 weeks. The safety profile is similar to that seen in previous clinical trials with telaprevir combination treatment.

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PREDECTION OF RESPONSE TO PEGYLATED INTERFERON/RIBAVIRIN BY INTERLEUKIN 28B GENOTYPE IS MODIFIED BY SNPS AT MULTIPLE GENES IN HIV/HEPATITIS C VIRUS GENOTYPE 1/4-COINFECTED PATIENTS

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Introduction: The SNP rs12979860 near the interleukin 28B (IL28B) gene is a potent predictor for sustained virologic response (SVR) to pegylated interferon (PEG-IFN)/ribavirin (RBV) in HIV/hepatitis C virus (HCV)-coinfected patients. Likewise, it has a lower, but evident, impact on the outcome of naive patients on protease inhibitors-based therapy. Its predictive capacity can be modulated by viral and genetic factors, such as LDLR genotype.

Aim: To identify predictors of therapy response that enhance the predictive value of IL28B genotype.

Methods: Genotyping of 144 SNPs at genes involved in cholesterol metabolism, fibrogenesis or HCV immune response was conducted in 205 HCV genotype 1/4-infected patients who received a complete course of Peg-IFN/RBV at five Spanish hospitals. SNPs associated with SVR with a p < 0.05 in an analysis using the Plink software were included in this study.

Results: SVR was achieved by 73 (36%) patients. SNPs at eight different genes were associated with SVR (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Protein</th>
<th>SNP</th>
<th>GT(+)</th>
<th>GT(−)</th>
<th>SVR in GT(+), n/N (%)</th>
<th>SVR in GT(−), n/N (%)</th>
<th>P univariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL28B</td>
<td>rs12949860 CT/TT</td>
<td>44/79 (56)</td>
<td>29/126 (23)</td>
<td>2×10−6</td>
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<td></td>
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<tr>
<td>LDLR</td>
<td>rs2116898 CG AA</td>
<td>45/112 (40)</td>
<td>2/9 (22)</td>
<td>0.284</td>
<td></td>
<td></td>
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<tr>
<td>TGF-β</td>
<td>rs1800469 GG AG</td>
<td>68/174 (39)</td>
<td>2/25 (8)</td>
<td>0.002</td>
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<td></td>
</tr>
<tr>
<td>AQP-2</td>
<td>rs287871 CC GC</td>
<td>37/73 (51)</td>
<td>36/132 (27)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDLR</td>
<td>rs7032549 AA GG</td>
<td>23/48 (48)</td>
<td>50/157 (32)</td>
<td>0.042</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTLA-4</td>
<td>rs231775 GG AA</td>
<td>5/11 (46)</td>
<td>65/187 (35)</td>
<td>0.471</td>
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<tr>
<td>TLR-4</td>
<td>rs4986791 AA GG</td>
<td>13/23 (57)</td>
<td>60/181 (33)</td>
<td>0.028</td>
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<td></td>
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<tr>
<td>APO-E</td>
<td>rs405599 AA CC</td>
<td>18/47 (38)</td>
<td>55/157 (35)</td>
<td>0.682</td>
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</tr>
</tbody>
</table>

SNP: single nucleotide polymorphism; GT(+): favorable genotype; GT(−): unfavorable genotype; SVR: sustained virologic response; IL28B: interleukin 28B; LDLR: low-density lipoprotein receptor; TGF-β: transforming growth factor β; AQP-2: aquaporine 2; VLDLR: very-low-density lipoprotein receptor; CTLA-4: cytotoxic T-lymphocyte antigen 4; TLR-4: toll-like receptor 4; APO-E: apolipoprotein E.

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LOW TREATMENT RATES AND SUBOPTIMAL TREATMENT COMPLETION RATES TO HEPATITIS C VIRUS (HCV) THERAPY: A REAL-WORLD ANALYSIS OF A LARGE US COHORT

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Background and Aims: Successful treatment completion is essential to optimize outcomes in hepatitis C virus (HCV) infection. Measurement and understanding of treatment completion rates in the real-world is critical in ensuring the most effective use of anti-HCV therapies.

Methods: We analyzed the use of the dual regimen (peginterferon+ribavirin, P+R) from January 2009-May 2011 (pre-protease inhibitors [PI]) and the PI in their triple regimen combinations from June 2011-June 2012 (post-PI) using data from a large cohort of commercial and Managed Medicare US insured patients. Patients were selected for study inclusion based on HCV diagnostic criteria and initiation of anti-HCV therapy.

Results: Treatment rates were low among HCV patients overall. Pre-PI treatment rates varied by line of business, with commercially-insured patients having a higher rate (11.7%) compared to Medicare patients (8.4%). The introduction of PIs did not substantially change the treatment rates (10.3% commercial and 8.6% Medicare). In patients on P+R in the pre-PI period, 91.7% remained on treatment by week 12 but dropped by 31.6 percentage points between weeks 12 and 24. During the post-PI period, drop-out rates were 14.0% by week 12 but dropped by 31.6 percentage points between weeks 8 and 12. The number continuing on B+P+R regimen dropped sharply through week 24 with only 25% remaining on the same therapy. Analysis of demographic characteristics for the P+P+R regimen patients showed that male patients were more likely to remain on therapy (33.6%), age was inversely related to completion rates, and Hispanic (35.7%) and African-American (33.9%) were only slightly more likely to continue on therapy than Caucasians (32.9%).

Conclusions: The current analysis indicates that treatment completion to both dual and triple treatment regimens is suboptimal in the real-world clinical setting with approximately half or more patients on triple therapy not completing the recommended length of therapy, especially female and older patients.
Background: Egypt has made important efforts regarding access to HCV treatment. However, because of economic constraints and logistical issues, not all patients can be treated. Decision makers should determine in whom treatment should be prioritized. We assessed the cost-effectiveness of different HCV treatment strategies according to the fibrosis stage at presentation to care in Egypt.

Methods: We developed a Markov model to compare lifetime costs, life expectancy, quality-adjusted life years (QALYS), and incremental cost-effectiveness ratio (ICER) of different treatment strategies, according to fibrosis stage at diagnosis in a population with a median age of 40 years (F1 to F4, F4 currently not included in Egyptian treatment guidelines).

Table (abstract 885)

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Cost* ($)</th>
<th>Life expectancy* (years)</th>
<th>QALY* (years)</th>
<th>ICER ($/QALY)†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline analysis:</strong> Treatment with dual-therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 Treat immediately if elevated ALT, wait F2 if normal ALT**</td>
<td>6,770</td>
<td>19.11</td>
<td>16.95</td>
<td>−</td>
</tr>
<tr>
<td>Wait F2</td>
<td>7,640</td>
<td>19.11</td>
<td>16.87</td>
<td>Dominated1</td>
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<tr>
<td>No treatment</td>
<td>8,440</td>
<td>18.55</td>
<td>15.88</td>
<td>−</td>
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<tr>
<td>Wait F3</td>
<td>8,745</td>
<td>18.88</td>
<td>16.08</td>
<td>Dominated1</td>
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<tr>
<td>F2 Treat immediately</td>
<td>6,230</td>
<td>18.67</td>
<td>16.21</td>
<td>−</td>
</tr>
<tr>
<td>Wait F3</td>
<td>8,415</td>
<td>18.28</td>
<td>14.85</td>
<td>Dominated1</td>
</tr>
<tr>
<td>No treatment</td>
<td>8,510</td>
<td>17.55</td>
<td>14.27</td>
<td>Dominated1</td>
</tr>
<tr>
<td>F3 Treatment</td>
<td>8,100</td>
<td>17.26</td>
<td>12.89</td>
<td>−</td>
</tr>
<tr>
<td>No treatment</td>
<td>8,490</td>
<td>15.94</td>
<td>11.84</td>
<td>Dominated1</td>
</tr>
<tr>
<td>F4 No treatment</td>
<td>7,890</td>
<td>12.26</td>
<td>9.18</td>
<td>−</td>
</tr>
<tr>
<td>Treatment</td>
<td>10,800</td>
<td>14.26</td>
<td>10.7</td>
<td>1,914†</td>
</tr>
<tr>
<td><strong>Sensitivity analysis:</strong> Treatment with dual-therapy &lt;2016 and with more efficacious treatments ≥2016, and perfect QoL (=1) for F1/F2 patients before treatment and after SVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 Treat immediately if elevated ALT, wait F2 if normal ALT**</td>
<td>7,070</td>
<td>19.12</td>
<td>18.69</td>
<td>−</td>
</tr>
<tr>
<td>No treatment</td>
<td>8,440</td>
<td>18.55</td>
<td>15.83</td>
<td>Dominated1</td>
</tr>
<tr>
<td>Wait F2</td>
<td>8,790</td>
<td>19.17</td>
<td>19.06</td>
<td>4,649†</td>
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<tr>
<td>Wait F3</td>
<td>9,390</td>
<td>19.05</td>
<td>18.08</td>
<td>Dominated1</td>
</tr>
<tr>
<td>F2 Treat immediately</td>
<td>6,240</td>
<td>18.67</td>
<td>17.52</td>
<td>−</td>
</tr>
<tr>
<td>No treatment</td>
<td>8,510</td>
<td>17.55</td>
<td>14.27</td>
<td>Dominated1</td>
</tr>
<tr>
<td>Wait F3</td>
<td>9,320</td>
<td>18.55</td>
<td>16.18</td>
<td>Dominated1</td>
</tr>
</tbody>
</table>

*Discounted 3% per year. **2008 Egyptian guidelines.
†-dominated = more expensive and less effective; †strategy cost-effective if ICER ≤three times Egyptian GDP = 8500$.
‡Discounted 3% per year. **2008 Egyptian guidelines.
†-dominated = more expensive and less effective; †strategy cost-effective if ICER ≤three times Egyptian GDP = 8500$.

Results: Immediate treatment of patients in F1/F2 stages but also F3 patients was less expensive and more effective than delaying treatment at more severe stages or not treating (Table). Treatment of F4 patients was cost-effective (ICER=1,914$/QALY, ≤three times Egyptian GDP = 8500$). When considering the availability of more efficacious treatments, delaying treatment in F1 patients until F2 stage became more effective but was not cost-effective. When in addition we considered a higher QoL associated to F1/F2 stages, it became cost-effective (ICER=4,649$/QALY). For F2 patients, immediate treatment remained the less expensive and more effective strategy.

Conclusion: In Egypt, treating F4 patients is cost-effective. Immediate treatment of F1/F2/F3 patients is less expensive and more effective than delaying treatment but immediate treatment in F1 is sensitive to the next disposal of new and better tolerated treatments, suggesting that decision makers should prioritize treatment for F4 patients and delay treatment for F1 patients.
IMPACT OF THE VIRAL KINETICS OF CHRONIC HEPATITIS C PATIENTS TREATED WITH TELAPREVIR IN COMBINATION WITH PEGYLATED INTERFERON α2b AND RIBAVIRIN

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Background and Aims: The prediction of SVR estimated on the basis of viral kinetics has been shown to be clinically reliable for the standard antiviral treatment for chronic hepatitis C virus (HCV) infection. However, it is unclear if or how the viral kinetics of chronic hepatitis C patients treated with telaprevir in combination with pegylated interferon (PEG-IFN) α2b plus ribavirin (RBV) affect the treatment outcome.

Methods: This prospective, multicenter study consisted of 406 Japanese patients with chronic HCV genotype 1 infection who were enrolled at 21 hospitals. All patients received telaprevir, PEG-IFNα2b, and RBV for 12 weeks, followed by PEG-IFNα2b and RBV alone for 12 more weeks. HCV RNA was tested at baseline, on day 3, and at weeks 1–12 and 16, 20, and 24 by the Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV assay.

Results: The overall rate of sustained virological response (SVR) 12 was 80.0%. The rates of HCV negativity in the SVR12 group were significantly higher than those in non-SVR12 group at each point (all P < 0.01). In multivariable logistic regression analysis, significant independent pretreatment predictors, including background and treatment factors, of SVR12 were IL28B TT (rs8099917) genotype (OR 5.93, P < 0.01) and rapid virological response (RVR; undetectable at week 4) (OR 5.00, P < 0.01). However, a significant difference in the rate of HCV negativity was found between the IL28B TT and non-TT groups at week 4 only (P < 0.05). Receiver operating characteristic analyses to determine optimal threshold values for the HCVRNA level at day 3 for predicting RVR showed that the areas under the curve (AUC) was high (AUC 0.78, HCV RNA cutoff value: 3.0 LogU/mL).

Conclusions: HCV viral kinetics was reliable for predicting the outcome of patients treated with telaprevir in combination with PEG-IFNα2b and RBV. Based on the viral kinetics, SVR12 can be predicted by measurements of HCV RNA on day 3 and at week 4.

PHARMACOKINETICS OF SIMEPREVIR (TMC435) IN VOLUNTEERS WITH MODERATE OR SEVERE HEPATIC IMPAIRMENT

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Background and Aims: Simeprevir (TMC435) is an oral, once-daily (QD), HCV NS3/4A protease inhibitor in Phase III development for treatment of chronic infection with HCV genotypes 1 and 4. In Phase IIb trials, simeprevir with peginterferon/ribavirin improved SVR rates compared with placebo/peginterferon/ribavirin, including in HCV-infected patients with compensated cirrhosis. In HCV-infected patients with compensated liver disease (mild hepatic impairment), simeprevir exposure was ~2-fold greater versus healthy volunteers (historical data). This Phase I, open-label study (TM435-C113; NCT01046058) investigated simeprevir pharmacokinetics (PK) and safety in HCV-negative volunteers with moderate or severe hepatic impairment.

Methods: Panel A included volunteers with moderate (Child–Pugh B) hepatic impairment and healthy matched controls (age, sex, race, ethnicity, body mass index, smoking status). Panel B included volunteers with severe (Child–Pugh C) hepatic impairment. Simeprevir 150 mg QD was administered for 7 days under fed conditions to Panels A and B (Panel B dose and enrollment were determined following evaluation of Panel A PK/safety data). Simeprevir PK profile was determined on Day 7 up to 48 hours post-dose. Clinical laboratory parameters, cardiovascular safety and adverse events (AEs) were monitored. PK data from heptatically-impaired volunteers in both panels were compared with Panel A controls, and with historical data from HCV-infected OPERA-1 patients with compensated liver disease (simeprevir 150 mg QD cohort).

Results: All patients completed the study. Table 1 shows LS means ratios (90% CI) for comparisons. One serious AE (pneumonia, unrelated to study drug) occurred in Panel A. There were no permanent discontinuations due to AEs.

Conclusion: Simeprevir exposure was ~2-fold higher in volunteers with moderate hepatic impairment versus matched healthy controls. Exposure in volunteers with severe impairment was higher than in both those with moderate impairment and HCV-infected patients with compensated liver disease (~2-fold and ~3-fold, respectively). Simeprevir was generally well tolerated in this study. Dosing recommendations based upon results of this and other studies will be presented.

SAFETY AND TOLERABILITY OF TELAPREVIR-BASED TRIPLE THERAPY IN HIV/HCV COINFECTED VERSUS HCV MONOINFECTED PATIENTS IN “REAL LIFE”: A MATCHED-COHORT STUDY

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Background: Clinical trials have suggested relatively good tolerance of protease inhibitor-based triple therapy in HIV/HCV-coinfected patients, however data on treatment effectiveness is lacking. This retrospective cohort study aimed to compare the safety of telaprevir (TVR)-based triple therapy in HIV/HCV-coinfected versus HCV-monoinfected patients during the first 12 weeks of treatment.

Methods: Among those initiating TVR-based triple therapy, 23 HIV/HCV patients were consecutively recruited and matched 1:1 with 23 HCV-monoinfected patients according to METAIVIR fibrosis score (17.4% F0–F2, 82.6% F3–F4) and prior exposure to anti-HCV treatment (17.4% treatment-naïve, 82.6% treatment-experienced). Hematological events and rash were graded according to the modified World Health Organization grading system. Proportions
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of patients presenting with any grade adverse event (AE) were compared between infection groups.

Results: At baseline, HIV/HCV and HCV-infection groups, respectively, had similar gender distributions (87% vs 73.9% males), HCV viral load (median 6.14 vs 5.99 log_{10} IU/mL), ALT (75 vs 75 IU/mL), hemoglobin level (14.8 vs 14.2 g/dl), neutrophil count (2094 vs 2575/mm^3) and platelet count (134,000 vs 147,000/mm^3). Among co-infected patients, median CD4+ cell count was 428/mm^3 (282–558) and 78.3% had undetectable HIV viral load. Prior to week 12, treatment was stopped in HIV/HCV and HCV-infection groups due to serious-AE (n=1 vs n=2) or according to stopping rules (n=3 vs n=2). At week 4 of treatment, there were no significant differences between infection groups in the proportion of patients presenting with anemia (21.7 vs 27.3%, p=0.4), neutropenia (52.2 vs 36.4%; p=0.3) or thrombocytopenia (56.5 vs 40.9%; p=0.3). At week 12, anemia occurred significantly less often in HIV/HCV co-infected patients (28.6 vs HCV-monoinfected: 57.9%, p<0.05). Accordingly, EPO-use was higher in HIV/HCV-co-infected patients (60.9% vs HCV-monoinfected: 34.8%, p=0.08). No difference was observed between infection groups in proportion of patients developing rashes (26.1% vs 28.6 vs HCV-monoinfected: 57.9%, p<0.05). Accordingly, EPO-use was higher in HIV/HCV-co-infected patients (60.9% vs HCV-monoinfected: 34.8%, p=0.08). No difference was observed between infection groups in proportion of patients developing rashes (26.1% vs 31.8%, p=0.8). Rates of RVR and eRVR were respectively lower in HIV/HCV-co-infected (43.5% and 65.2%) than HCV-monoinfected patients (78.3 and 95.7%) (p=0.02).

Conclusion: TVR-based triple therapy is well tolerated in HIV/HCV patients, with no major difference in hematological and dermatological toxicity compared to HCV-monoinfected patients. The lower frequency of anemia could be explained by different management strategies within the two populations.

889 EFFICACY AND SAFETY OF PEGYLATED-INTERFERON α-2a PLUS RIBAVIRIN THERAPY IN NAÏVE HCV CIRRHOSIS. PREDICTIVE RESPONSE FACTORS


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Aims: To evaluate sustained virological response (SVR) and safety of antiviral therapy in naïve HCV cirrhosis and the factors associated to response.

Methods: Retrospective study of the cohort of cirrhotic patients naïve to therapy who received Pegylated-interferon α2a (PEG2a) plus ribavirin (RBV) therapy between January-05 and June-12. They were treated with full doses from the beginning for 48 [genotypes (G) 1–4] or 24 weeks (G 2–3). In the absence of rapid virological response (RVR), they were offered therapy to be prolonged to 72 (G 1–4) or 48 weeks.

Results: 73 patients (75% men), of 51.3±8.6 years and MELD score=9±2.7, 18% have had some decomposition and 52% esophageal varices (EV). 10 (all G1) received prolonged treatment. 20% (G1, 10%; G-Non 1, 47%; p=0.003), 52% (G1, 41%; G-Non 1, 79%; p=0.007), 68.5% (G1, 67%; G-Non 1, 71%) and 37% (G1, 32%; G-Non 1, 52%) achieved RNA-HCV negative at 4 weeks, 12 weeks, end-of-treatment and SVR, respectively. Overall SVR were associated to the absence of EV (54% vs 22%; p=0.007) and tended to be higher in compensated patients (41% vs 25%). SVR were also associated to RVR (75% vs 25%; p=0.002) and HCV-RNA negative at 12 weeks (67% vs 13%; p=0.0001). In G1 patients, SVR were associated to RVR (75% vs 28%; p=0.089) and RNA-HVC negative at 12 weeks (61% vs 15%; p=0.003) and tended to be higher in patients without RVR who had received prolonged therapy (60% vs 31%). Anemia, neutropenia and thrombocytopenia were observed in 41%, 34% and 36% of patients, respectively. There were 2 death, 14% had decomposition during the treatment and 20% of patients had to discontinue treatment due adverse events.

Conclusions: 1. A third of G1 and a half of G-Non 1 cirrhotics patients achieved SVR starting with full doses of PEG2a and RBV and prolonging therapy in patients without RVR.
2. To have had decompenation and especially the presence of EV reduced SVR significantly.
3. RVR is also a potent predictive SVR factor in cirrhosis and could be used as a criteria to assess the therapy with proteas inhibitors.
4. To prolong therapy in G1 without RVR tended to enhance SVR.
5. Severe adverse events are frequent.

890 HIGH PREVALENCE OF SVR TO STANDARD OF CARE THERAPY IN INJECTION DRUG USERS (IDUS): AN ITALIAN MONOCENTRIC STUDY

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Background: Hepatitis C is one of the most common infectious disease among IDUs and still debated is the tag of treating them since they are considered difficult to treat.

Aim: To examine the response to antiviral treatment in IDUs compared to non-IDUs population.

Patients and Methods: We analyzed all the 181 (122 IDUs, 67.4%) consecutive adult patients with chronic hepatitis C who were treated with a PEG-IFNα and ribavirin in a tertiary center for IDUs of Southern Italy from 2008 to 2011. Age, sex, genotype, clinical parameters, response to previous therapy, rapid (RVR), early (EVR), end-of-treatment (EOT) and sustained virological response (SVR) were evaluated in all patients.

Results: The IDUs were mainly male (89.3% vs. 57.6%, p<0.001), young (mean±SD age: 41±8.8 vs. 62±14.9, p<0.001), naïve (73.8% vs. 50.8%, p<0.005) and had less frequently cirrhosis (8.2% vs. 45.8%, p<0.001) compared to non-IDUs group. No significant difference was found regarding steatosis (66.4% vs. 76.3%), viral load (>600,000 IU/ml 63.1% vs. 61%) and apparent duration of disease (>5 years: 71.3% vs. 74.5%). Genotype 3 was significantly higher in the IDUs group (42.6% vs. 6.7%, p<0.001), while genotype 1 and 2 in IDUs group (genotype 1: 48.4% vs. 64.4%, p=0.043; genotype 2: 6.5% vs. 28.8%, p=0.001). At the Intent-to-treat analysis higher rate of overall RVR was found in IDUs group (59.8% vs. 44%, p=0.046), while no differences were found regarding EVR, EOT, SVR. Since there were 24 dropouts in IDUs group and only one in non-IDUs group, the Per-protocol analysis showed in IDUs higher rate of RVR, higher rate of EOT (95.9% vs. 84.5%, p=0.013) and SVR (84.7% vs. 58.6%, p<0.001). At the multivariate analysis independent predictors of SVR were age (O.R.: 1.046, C.I. 95%: 1.007–1.086, p=0.021), RVR (O.R.: 4.244, C.I. 95%: 1.844–9.766, p<0.001) and cirrhosis (O.R.: 0.108, C.I. 95%: 0.032–0.368, p<0.001).

Conclusions: Our data seem to suggest that, although the IDUs are considered difficult to treat, they had a high rate of SVR, probably because young, with genotype 3 and without cirrhosis.
Background and Aim: Newly approved HCV GT1 triple regimens are complex and associated with additional toxicities. Besides RCT results, only limited data are available for BOC und TVR under real life conditions, especially for difficult to treat patients with non-response and advanced liver disease.

Methods: Data from 143 patients who initiated triple therapy apart from clinical studies or Early-Access-Programs with BOC (n=69) or TVR (n=74) at the IFI Institute were obtained from medical record review and analysed on an intention to treat basis. Hemoglobin level <9 g/dl or a >4.5 g/dl decrease from baseline defined severe anemia, fibrosis scores of >3 by Fibroscan or ARFI defined advanced fibrosis.

Results: Median age of the study group was 55yrs with 29% of more than 60yrs, 67% males, 90% Caucasian, GT1a 39%, GT1b 60%, IL28 C/C 11%, non C/C 88%. 42% of patients showed advanced fibrosis, 23% of patients presenting with complete cirrhosis and platelets of <120.000/ul. 26% were treatment-naive, of 74% of patients with prior failure to therapy 50% were non-responders. In this ongoing study, severe adverse events (SAEs) were reported in 64% and 53% of patients during BOC and TVR therapy, respectively. All patients with cirrhosis and all patients above 60yrs of age developed SAEs. Severe anemia occurred in 65% of patients, 38% of patients received blood transfusions, all patients above 60yrs and all cirrhotic patients received blood transfusions. Discontinuation rate because of SAEs or virological failure was 33% (BOC) and 31% (TVR). Interestingly, virological failure was more often related to virological break throughs compared to failure in reaching primary stopping rules at week 4 (TVR) or 12 (BOC). The interim data suggest that SVR rates will be significantly lower compared to RCTs.

Conclusions: Despite improved efficacy with DAA triple therapies, real world data reflect the treatment complexity and concerns about side effects and safety even at experienced centres. Especially in patients of older age and with liver cirrhosis reflected by low platelets the safety profile is poor for both drugs. Patients should be clearly treated but very cautiously and difficult to treat patients need to be monitored very carefully.

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EARLY VIROLOGICAL RESPONSE RATES IN MONOINFECTED PATIENTS WITH HCV AND COINFECTED WITH HCV/HIV, GENOTYPES 1&4 TREATED WITH PEGINTERFERON alpha 2a AND RIBAVIRIN. OPTIM STUDY


Background and Aims: Anemia occurs in approximately one-third of patients treated with peginterferon/RBV. Adding a protease inhibitor improves efficacy, but also increases anemia. It has previously been reported that a greater than 3 g/dl decline in hemoglobin (vs. ≤3) is associated with increased sustained virologic response (SVR) rates (43.7% vs. 29.9%) in patients treated with peginterferon/RBV, and similarly in those treated with a triple regimen. However, it is unknown if this relationship is valid in patients with cirrhosis. The current analysis explores whether anemia predicts SVR in patients with cirrhosis treated with peginterferon alfa-2a/RBV.

Methods: Post-hoc analysis was conducted based on treatment-naïve HCV mono-infected G1/4 subjects who received peginterferon alfa-2a/RBV from four Roche international HCV clinical trials. Anemia was defined as either a decline in hemoglobin to ≤10 g/dl or a decrease of ≥3 g/dl from baseline.

Results: 374 cirrhotic subjects were compared to 1838 non-cirrhotic subjects. Those without cirrhosis were younger and had higher platelet counts, but were otherwise comparable at baseline, including hemoglobin levels. Rates of anemia, by either definition, were similar in both cohorts with 17.5% of cirrhotics vs. 18.5% of non-cirrhotics experiencing a decline in hemoglobin to <10 g/dl, while 74.3% of cirrhotics vs. 70.6% of non-cirrhotics experienced a ≥3 g/dl decline from baseline in hemoglobin. SVR rates were higher in patients with cirrhosis who experienced anemia compared to those that did not experience anemia (29.9% vs. 26.7% with hemoglobin levels <10 g/dl [p=0.0610] and 30.6 vs. 17.7% with ≥3 g/dl hemoglobin decline [p=0.0147]).

Conclusions: Anemia rates in peginterferon alfa-2a/RBV-treated subjects were the same in those with and without cirrhosis. A decrease in hemoglobin of ≥3 g/dl predicts SVR in those with cirrhosis. It will be important to verify this relationship in cirrhotic patients who are treated with a DAA-containing triple regimen given the increased rates of anemia observed with triple therapy. As the toxicity of DAs improve, understanding the limitations imposed by peginterferon/RBV is imperative, especially in patients with cirrhosis.

Acknowledgement: Funded by Roche/Genentech.

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REAL WORLD EXPERIENCE WITH TRIPLE THERAPY FOR HCV GT1 PATIENTS IN DIFFICULT TO TREAT PATIENTS: SEVERE ADVERSE EVENTS AND HIGH RATES OF VIROLOGICAL BREAK-THROUGH


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Background and Aim: Newly approved HCV GT1 triple regimens are complex and associated with additional toxicities. Besides RCT results, only limited data are available for BOC und TVR under real life conditions, especially for difficult to treat patients with non-response and advanced liver disease.

Methods: Data from 143 patients who initiated triple therapy apart from clinical studies or Early-Access-Programs with BOC (n=69) or TVR (n=74) at the IFI Institute were obtained from medical record review and analysed on an intention to treat basis. Hemoglobin level <9 g/dl or a >4.5 g/dl decrease from baseline defined severe anemia, fibrosis scores of >3 by Fibroscan or ARFI defined advanced fibrosis.

Results: Median age of the study group was 55yrs with 29% of more than 60yrs, 67% males, 90% Caucasian, GT1a 39%, GT1b 60%, IL28 C/C 11%, non C/C 88%. 42% of patients showed advanced fibrosis, 23% of patients presenting with complete cirrhosis and platelets of <120.000/ul. 26% were treatment-naive, of 74% of patients with prior failure to therapy 50% were non-responders. In this ongoing study, severe adverse events (SAEs) were reported in 64% and 53% of patients during BOC and TVR therapy, respectively. All patients with cirrhosis and all patients above 60yrs of age developed SAEs. Severe anemia occurred in 65% of patients, 38% of patients received blood transfusions, all patients above 60yrs and all cirrhotic patients received blood transfusions. Discontinuation rate because of SAEs or virological failure was 33% (BOC) and 31% (TVR). Interestingly, virological failure was more often related to virological break throughs compared to failure in reaching primary stopping rules at week 4 (TVR) or 12 (BOC). The interim data suggest that SVR rates will be significantly lower compared to RCTs.

Conclusions: Despite improved efficacy with DAA triple therapies, real world data reflect the treatment complexity and concerns about side effects and safety even at experienced centres. Especially in patients of older age and with liver cirrhosis reflected by low platelets the safety profile is poor for both drugs. Patients should be clearly treated but very cautiously and difficult to treat patients need to be monitored very carefully.

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ANEMIA PREDICTS SUSTAINED VIROLOGIC RESPONSE IN CIRRHOTIC AND NON-CIRRHOTIC CHRONIC HEPATITIS C (CHC) PATIENTS TREATED WITH PEGINTERFERON alfa-2a AND RIBAVIRIN IN FOUR CLINICAL TRIALS

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Background and Aims: Anemia occurs in approximately one-third of subjects treated with peginterferon/RBV. Adding a protease inhibitor improves efficacy, but also increases anemia. It has previously been reported that a greater than 3 g/dl decline in hemoglobin (vs. ≤3) is associated with increased sustained virologic response (SVR) rates (43.7% vs. 29.9%) in patients treated with peginterferon/RBV, and similarly in those treated with a triple regimen. However, it is unknown if this relationship is valid in patients with cirrhosis. The current analysis explores whether anemia predicts SVR in patients with cirrhosis treated with peginterferon alfa-2a/RBV.

Methods: Post-hoc analysis was conducted based on treatment-naïve HCV mono-infected G1/4 subjects who received peginterferon alfa-2a/RBV from four Roche international HCV clinical trials. Anemia was defined as either a decline in hemoglobin to ≤10 g/dl or a decrease of ≥3 g/dl from baseline.

Results: 374 cirrhotic subjects were compared to 1838 non-cirrhotic subjects. Those without cirrhosis were younger and had higher platelet counts, but were otherwise comparable at baseline, including hemoglobin levels. Rates of anemia, by either definition, were similar in both cohorts with 17.5% of cirrhotics vs. 18.5% of non-cirrhotics experiencing a decline in hemoglobin to <10 g/dl, while 74.3% of cirrhotics vs. 70.6% of non-cirrhotics experienced a ≥3 g/dl decline from baseline in hemoglobin. SVR rates were higher in patients with cirrhosis who experienced anemia compared to those that did not experience anemia (29.9% vs. 26.7% with hemoglobin levels <10 g/dl [p=0.0610] and 30.6 vs. 17.7% with ≥3 g/dl hemoglobin decline [p=0.0147]).

Conclusions: Anemia rates in peginterferon alfa-2a/RBV-treated subjects were the same in those with and without cirrhosis. A decrease in hemoglobin of ≥3 g/dl predicts SVR in those with cirrhosis. It will be important to verify this relationship in cirrhotic patients who are treated with a DAA-containing triple regimen given the increased rates of anemia observed with triple therapy. As the toxicity of DAs improve, understanding the limitations imposed by peginterferon/RBV is imperative, especially in patients with cirrhosis.

Acknowledgement: Funded by Roche/Genentech.
weeks of P+R therapy seems to be crucial to correctly classify patients according to interferon sensitivity: patients showing low sensitivity to IFN (decline <1Log HCV-RNA at week 4) and high chance of achieving SVR (when HCV-RNA was negative at week 4).

Patients and Methods: Prospective and observational study including naïve patients aged ≥18, CHC genotype 1&4; excluding other liver diseases. Patients were treated with peginterferon-alfa 2a plus ribavirin. This analysis shows the differences between both populations in terms of baseline characteristics and first 4 weeks virological responses.

Results: Seven-hundred and twenty-four patients were analyzed prospectively: 538 monoinfected/186 coinfected.

Baseline characteristics coinfected vs. monoinfected: Prospectively: 538 monoinfected/186 coinfected.

The combination of genetic (IL28B) and viral genotype with the new DAA in order to improve response rates in HIV-coinfected patients. Therefore, it is of particular interest to have as soon as possible combined treatments between 0 and 12 weeks after treatment, 50% (5/10) between weeks 0 and 12 (SD: 8.9); Male (66.4%), BMI: 26 (4.4); Forns Index: 5.47 (2.0); Forns <4.2: 182/699 (26%); HVL (>800.000 UI/ml): 59%; Genotype-1: 81%; IL28B-CC 38%. Decline ≥1Log at week 4 of HCV-RNA: 509 (80.2%) and RVR 148 (22.5%).

Decline ≥1 Log HCV-RNA prediction. Estimation cohort, related factors: HIV-coinfection (OR: 0.439; 95%CI: 0.240–0.801; p = 0.007); Forns index (OR: 0.73; 95%CI: 0.52–0.87; p = 0.005); LVL (OR: 1.84; 95%CI: 0.992–3.315; p = 0.0053); IL28B-CC (OR: 7.830; 95%CI: 3.34–18.31, p = 0.0001); Genotype-1 (OR: 1.95; 95%CI: 0.99–3.83 p = 0.053).

AUROC was 0.81 (95%CI: 0.76–0.86) in the Estimation cohort and 0.71 (95%CI: 0.62–0.79) in the Validation cohort. Sensitivity 78%; Specificity 65%; PPV 89.9%; NPV 42.4%.

RVR prediction. Estimation cohort, related factors: HIV-coinfection (OR: 0.367; 95%CI: 0.170–0.793; p = 0.011); Forns index (OR: 0.72; 95%CI: 0.61–0.85; p = 0.001); LVL (OR: 4.445; 95%CI: 2.33–8.46; p = 0.0001); IL28B-CC (OR: 7.21; 95%CI: 3.8–13.71; p = 0.0001); genotype-1 (OR: 0.48; 95%CI: 0.24–0.98; p = 0.044).

AUROC was 0.83 (95%CI: 0.78–0.88) in the estimation cohort and 0.82 (95%CI: 0.76–0.88) in the validation cohort. Sensitivity 75.9%; Specificity 74.8%; PPV 46.9%; NPV 91.3%.

Conclusions: The combination of genetic (IL28B) and viral genotype together with low VL, HIV-coinfection and fibrosis stage defined a tool able to predict RVR and ≥1 Log HCV-RNA decline in week 4 in patients with CHC genotype 1&4 treated with peginterferon-alpha-2a plus ribavirin. The implementation of this tool could be very useful in clinical practice.
100% of patients with RV in week 48 maintained undetectable HCV RNA levels at follow-up at 2 and 3 years. 5 of the 6 patients, who relapsed after a week, had received short courses of oral antiviral.

**Conclusions:** The highest percentage of relapses in patients who have received treatment with direct antivirals occurs between week 12 and week 24 of follow-up (50%). The sustained viral response at week 48 (one year post-treatment) remained in 100% of patients during follow-up. The presence of late recurrences may require redefining of the SVR.

896 SUCCESSFUL HCV ERADICATION DUE TO ANTIVIRAL THERAPY IS ASSOCIATED WITH IMPROVED LONG TERM OUTCOME OF PATIENTS WITH CHRONIC HEPATITIS C

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**Background:** Antiviral therapy of patients with chronic hepatitis C (CHC) is aimed to eradicate HCV in order to reduce long-term sequelae like liver cirrhosis, hepatocellular carcinoma and to increase survival. Aim of the study was to evaluate long term outcome of CHC patients who underwent antiviral therapy with emphasizes on long-term complications, durability of HCV eradication and overall survival.

**Methods:** The long-term outcome of 703 patients completing antiviral therapy (mean age: 49.7±11.8 years, F:269, GT-1: 463, GT-2: 18, GT-3: 145; GT-4: 77) was studied. Most patients participated in a follow up program and were seen in 6–12 months intervals. The survival status of those without follow up (N=122) was obtained from Statistics Austria. Patients received IFN-α monotherapy (N=19), IFN-α/ RBV (N=132) or pegylated-IFN/RBV therapy (N=552). A pre-treatment liver biopsy was available in 523 (74.4%) patients. 196/523 (37.5%) had an advanced stage of fibrosis (F3/F4).

**Results:** 531 patients had a SVR (75.5%) and 172 were nonresponders (NR). Duration of clinical follow up in the SVR group was 3.3[1–19.0] years. One late relapse was observed 12 months after the end of treatment with PegIFN/RBV (1/531, 0.2%). 3 patients were reinfected with another HCV genotype 22, 36 and 67 months after achieving a SVR. 87.8% of SVR patients had normal transaminases at last follow up. HCC was detected in 8 (1.5%) SVR patients. 8 SVR patients (1.5%) developed hepatic decompensation during follow up. One, 5 and 10 year mortality rates in SVR group were 0%, 1.2% (4/331) and 11.2% (10/89), respectively. Corresponding mortality rates in NR were 1.7% (3/172), 15.5% (19/123) and 52.8% (28/53), respectively (p<0.01). In SVR patients with pretreatment cirrhosis overall mortality (6.3% versus 1.5%; p<0.025) and HCC incidence was higher than in those without cirrhosis (7.1% versus 0.7%; p<0.01).

**Conclusion:** SVR is durable after long term follow up and is associated with improved clinical outcome and is thus a useful parameter to assess the efficacy of antiviral therapy in chronic hepatitis C. However, the risk of HCC development and of progressive liver disease is not prevented completely and is mainly determined by the presence of cirrhosis prior to treatment.

897 INTERLEUKIN-28B CC GENOTYPE PREDICTS TREATMENT EARLY RESPONSE AND CT/TT GENOTYPES PREDICT NON RESPONSE IN HCV GENOTYPE 3 INDIAN PATIENTS

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**Background and Aim:** The response rate to antiviral therapies in HCV genotype 3 patients is about 70%. There is limited data on the predictive value of IL28b (rs12979860 genotypes) for treatment response. We studied the clinical relevance of IL28b (rs12979860) in predicting the response to peg-interferon and ribavirin therapy in HCV genotype 3 patients.

**Patients and Methods:** Genomic DNA was isolated from HCV patients (n=401) and healthy controls (n=356). PCR specific to IL28b followed by DNA sequencing and genotyping were performed to identify the CC, CT, or TT at the polymorphic site, rs12979860. Treatment response was defined as HCV RNA negativity at day 7, day 28 (rapid viral response, RVR), at the end of therapy and 6 months after completion of therapy (SVR). Non-response was defined as HCV RNA positivity at the end of therapy or relapsed within 6 months after stopping therapy.

**Results:** In responders, frequencies of IL28b genotypes CC, CT, TT were 60.9%; 33.8%; and 5.3% respectively and which were comparable to that observed in healthy controls (CC=64.6%; CT=31.2%; and TT=4.2%). In responders (including early response at day 7) frequencies of IL28b genotypes CC (60.9%) was higher than in non responders CC (25%). In non responders, frequency of CT genotype was higher than responders (59.3% vs. 33.8%; p=0.001). More importantly, the CT and TT genotypes taken together were found to be significantly higher in non-responders than in responders (75.0% vs. 39.1%, p=0.001). TT genotype was significantly more often associated with non-responders than responders (15.7% vs. 5.3%, p=0.01). Further, the CC genotype was associated with early reduction of more than Log2 of HCV RNA at day 7 or at day 28 (p=0.001) in comparison to CT and TT genotypes.

**Conclusion:** Our data confirms that IL28b (CT/TT) genotype strongly correlates to treatment non response in HCV genotype 3 infected cohort of Indian patients. The CC genotype is associated with higher and rapidier HCV RNA reduction at day 7 and 28.

898 TREATMENT WITH TELAPREVIR/PEG-IFN/RBV AFTER 14-DAY TELAPREVIR EXPOSURE IN PHASE I STUDIES: RESULTS FROM THE PHASE IIIB C219 ROLLOVER STUDY

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**Background and Aims:** C219 (NCT01054573) was an open-label, single-arm, rollover study that examined telaprevir (TVR)-based therapy in patients infected with chronic genotype 1 HCV who failed to achieve a sustained virologic response (SVR) following peginterferon/ribavirin (PR) alone in the REALIZE study, or with ≥1 dose of TVR (alone or in combination with PR) in Phase I studies (101 and 103). Here we present the primary endpoint, SVR24, as of patients who had prior exposure to TVR only from the Phase I studies.

**Results:** 898 TREATMENT WITH TELAPREVIR/PEG-IFN/RBV AFTER 14-DAY TELAPREVIR EXPOSURE IN PHASE I STUDIES: RESULTS FROM THE PHASE IIIB C219 ROLLOVER STUDY

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Methods: In studies 101/103, all patients received 14 days TVR monotherapy. TVR-resistant variants had been detected by clonal sequence analysis after dosing in studies 101/103 for all patients. In the C219 study, patients received TVR 750 mg q8h plus PR at standard doses (180µg once-weekly and 1000 or 1200 mg/day, respectively) for 12 weeks, followed by 36 weeks of PR. HCV RNA levels were determined using COBAS TaqMan assay v2.0. SVR24actual was defined as HCV RNA <25 IU/mL target not detected at least 24 weeks after the last medication intake.

Results: Nine patients from trial 101 (n = 8) or 103 (n = 1): six subtype 1a; three subtype 1b, were enrolled. Baseline HCV RNA was ≥800,000 IU/mL for all patients; four patients had cirrhosis. Median time since last TVR exposure was 5.7 years (range 4.9–6.0). No TVR-resistant variants were detected by population sequencing or deep sequencing (Illumina assay) before entering study C219. Five patients achieved SVR (Table); two patients had on-treatment virologic failure; two patients relapsed. In three non-SVR patients with available sequence data at the time of failure, the most common known TVR-resistant variants V36M + R155K were detected, similar to those found in the 101/103 studies. Observed adverse events were consistent with those reported for Phase III studies.

Conclusions: Among patients who had previously received 14 days of TVR monotherapy, 5/9 achieved SVR with TVR/PR. For treatment failures, ongoing deep sequencing analysis will further explore a possible phylogenetic relationship of isolates harbouring resistant variants observed during first and second TVR exposure.

Table: Treatment outcome by prior response

<table>
<thead>
<tr>
<th>Prior PR treatment response</th>
<th>Patients achieving SVR, n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment naïve</td>
<td>2/2</td>
</tr>
<tr>
<td>Treatment experienced</td>
<td>3/7</td>
</tr>
<tr>
<td>Relapsers</td>
<td>2/3</td>
</tr>
<tr>
<td>Non-responders¹</td>
<td>1/4</td>
</tr>
</tbody>
</table>

¹Non-responders = prior null responders, prior partial responders and prior breakthrough.
²Prior partial responder.

899 INFLUENCE OF GENDER, AGE AND ITPA POLYMORPHISM (rs6051702, rs1127354, rs7270101) ON THROMBOCYTOPENIA IN HCV GENOTYPE 1 AND 4 PATIENTS ON ANTIVIRAL THERAPY


Background: Recently we have shown that besides ITPA polymorphism ribavirin-induced anemia in HCV-gt 1 patients is influenced by gender. This study analyzed the influence of gender, age and ITPA polymorphism on interferon-induced thrombocytopenia in HCV genotype 1 and 4 patients.

Patients and Methods: 671 [HCV-gt 1:586, HCV-gt 4:85; age: 42.8 ± 10.6 (mean ± SD), BMI: 25.5 ± 4.1] noncirrhotic patients who participated in 3 multicenter-studies were investigated. All patients received 180mg peginterferon alpha-2a/week (PEG) and ribavirin (RBV; <75kg: 1000 mg; ≥75kg: 1200 mg), 38 patients received additional treatment with 200 mg amantadine/day. In 360 (HCV-gt 1:295, HCV-gt 4:65) patients analysis of rs6051702, rs1127354 and rs7270101 was available.

Results: Baseline thrombocyte count was higher in female compared to male patients [244.5G/l (CI95% 236.7–252.2) vs 217.5 (212.2–222.8), p < 0.001; HCV-gt1:245.1 (237.0–253.2) vs 219.8 (213.8–225.8), p < 0.001; HCV-gt4:234.8 (55.8–2.5) vs 205.6 (195.0–216.3), p < 0.001]. Overall, the drop in platelet count (Δthr) at week 4 was 63.1G/l (59.4–66.8) [HCV-gt1:65.7 (61.7–69.6), HCV-gt4: 45.1 (35.8–54.3)]. Δthr was higher in female compared to male patients [74.7 (68.0–81.3) vs 56.7 (52.4–61.0); p < 0.001]. This difference was preserved analyzing the relative thrombocyte drop [30.0% (27.5–32.5) vs 25.9 (24.1–27.7), p = 0.006]. Δthr was higher in patients aged <50 years (n = 495) compared to patients >50 years [n = 176; 67.3 (63.0–71.6) vs 51.3 (44.3–58.2); p < 0.001], which was still the case when data was separated into male and female patients [50 f: 81.4 (73.0–89.8), m: 60.6 (55.8–65.4), p < 0.001; >50 f: 61.1 (50.5–71.7), m: 43.2 (34.3–52.2), p < 0.019]. PEG/kg doses were lowest in male patients [f: 2.7 (2.7–2.8), m: 2.3 µg/kg (2.3–2.3)] and were highest in female patients <50 years [f: 2.7 (2.7–2.9), m: 2.3 µg/kg (2.3–2.4), p < 0.001]; >50 f: 2.6 (2.5–2.7), m: 2.2 (2.2–2.3), p < 0.001]. Influence of ITPA mutation and gender on Δthr in HCV-gt 1 and 4 patients is shown in the table.

Conclusion: Like RBV-induced anemia thrombocytopenia is influenced by ITPA mutations, gender and age. However, most of the differences may reflect differences in body weight corrected PEGIN2 doses.

900 TELAPREVIR OR BOCEPREVIR THERAPY IN CHRONIC HEPATITIS C PATIENTS WITH END STAGE RENAL DISEASE ON HEMODIALYSIS IS SAFE, TOLERABLE AND FEASIBLE

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Introduction: Limited data exists regarding the use of telaprevir or boceprevir to treat hepatitis C (HCV) in end stage renal disease (ESRD) patients on hemodialysis.

Aims: To assess and compare the safety and tolerability of telaprevir (T) and boceprevir (B) in this population.

Methods: We analyzed 14 HCV genotype 1 patients with ESRD, of which 8 were being evaluated for kidney- and 6 for combined liver- kidney transplantation. All patients received pegylated interferon alpha 2a (P), ribavirin (R) and either boceprevir (B) 800mg thrice daily or telaprevir (T) 750 mg thrice daily. All patients treated with BPR received 1 month lead-in, P 180 micrograms weekly and R 200 mg thrice weekly whereas those treated with TPR received no lead-in, P 135micrograms weekly and R 200 mg – daily (12.5%), twice weekly (50%) and thrice weekly (37.5%) at the discretion of the clinician.

Results: Cohort characteristics include mean age of 56 years, 71% male, 57% African-Americans, 57% genotype 1a, 71% advanced fibrosis and 100% IL28B non CC. Both groups had mean baseline hemoglobin of 12.3 g/dL. Twelve patients completed 12 weeks of therapy, 1-completed 8 weeks and 1-discontinued due to non-compliance. Among 6 patients receiving BPR, none had discontinued therapy, 2 were hospitalized (anemia, pneumonia). Among 8 patients
receiving TPR, 3 required hospitalization (anemia, infection and ascites). Anemia occurred in 50% in both groups but more aggressive management was required in the TPR group.

Table: AE profile of BPR & TPR

<table>
<thead>
<tr>
<th></th>
<th>BPR (N=6)</th>
<th>TPR (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-Americans</td>
<td>50%</td>
<td>62%</td>
</tr>
<tr>
<td>Advanced fibrosis</td>
<td>83%</td>
<td>62%</td>
</tr>
<tr>
<td>Anemia &gt;2 g/dl</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>PRBC transfusion</td>
<td>17%</td>
<td>25%</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>50%</td>
<td>75%</td>
</tr>
<tr>
<td>Ribavirin dose reduction</td>
<td>33%</td>
<td>50%</td>
</tr>
</tbody>
</table>

Discussion: Among this difficult to treat HCV and ESRD patients, telaprevir or boceprevir resulted in 50% rate of anemia which could be safely managed. More than 85% completed 12 weeks therapy and are still undergoing response guided therapy. No SAE, discontinuations due to treatment or deaths were noted in our cohort.

901 ADDING SIMEPREVIR (TMC435) TO PEGYLATED INTERFERON/ RIBAVIRIN DOES NOT INCREASE PATIENT REPORTED FATIGUE IN TREATMENT-EXPERIENCED PATIENTS WITH CHRONIC HCV INFECTION: RESULTS FROM THE ASPIRE TRIAL

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Background and Aims: Patients receiving peginterferon-α/ribavirin (PR) treatment for chronic HCV infection often experience debilitating fatigue throughout treatment. In ASPIRE (NCT009980330), a Phase IIb trial of simeprevir (an investigational, oral, once daily [QD] HCV protease inhibitor in Phase III trials) or placebo, plus PR, in 462 treatment-experienced, HCV genotype 1-infected patients, SVR rates significantly increased compared with placebo/PR (Zeuzem et al. EASL 2012). To understand the impact of treatment on fatigue, patient-reported fatigue and perceived health status were assessed using the Fatigue Severity Scale (FSS) and EQ-5D questionnaire throughout the trial.

Methods: Patients were randomised to receive simeprevir (100 or 150 mg QD) for 12, 24 or 48 weeks or placebo, plus PR (Zeuzem et al. EASL 2012). Patients self-completed the FSS and EQ-5D questionnaires at baseline and throughout the study. Mean total FSS scores (range 1–7; high score=high fatigue) were compared using a piecewise linear model. Mean EQ-5D visual analog scale (EQ-5D-VAS) scores (0–100; lower score=worse perceived health status) were plotted over time. Psychometric analyses to evaluate reliability and validity of the FSS were conducted using data pooled across treatments.

Results: Mean (± standard error) total FSS score at baseline was 3.37±0.08 (simeprevir; n=385) and 3.16±0.22 (control; n=64), increased similarly in all groups from baseline to Week 12, and then remained stable through Week 48. As expected, FSS scores were inversely related to EQ-5D-VAS scores during treatment. By end of study, FSS scores decreased and EQ-5D-VAS scores increased to values comparable to or better than those at baseline in all groups. There were no statistically or clinically significant differences in FSS between groups over the 72-week period (AUIC=268.9 [control], 288.9 [simeprevir 100 mg], 285.6 [simeprevir 150 mg]; p > 0.025). FSS validation analyses demonstrated high internal consistency (Cronbach’s α=0.96), test-retest reliability (intraclass correlation 0.86), and concurrent validity (correlation between FSS total score and EQ-5D-VAS −0.66).

Conclusions: Adding simeprevir to PR increased the likelihood of treatment response without increasing patient-reported fatigue or reducing perceived health status beyond that seen in patients receiving PR alone.

902 IMPROVED SVR WITH SIMEPREVIR (TMC435) ASSOCIATED WITH REDUCED TIME WITH PATIENT-REPORTED FATIGUE IN TREATMENT-NAIVE, HCV-INFECTED PATIENTS IN THE PILLAR PHASE IIb TRIAL

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Background and Aims: Increased fatigue is associated with HCV infection and worsens with peginterferon-α/ribavirin (PR) treatment. The Phase IIb PILLAR trial (NCT00882908) evaluated PR plus simeprevir (a once-daily [QD] oral, investigational HCV protease inhibitor in Phase III clinical trials) or placebo in treatment-naive, HCV genotype 1-infected patients. The majority of simeprevir-treated patients completed treatment after 24 weeks and experienced SVR (Fried et al. AASLD 2011). This analysis evaluated the impact of treatment on patient-reported fatigue using the Fatigue Severity Scale (FSS) questionnaire, and validated FSS use for patients with chronic HCV infection.

Methods: 386 patients received simeprevir 75 or 150 mg QD or placebo, plus PR (Fried et al. AASLD 2011). Total PR treatment duration was 24/48 weeks with simeprevir (response-guided therapy) and 48 weeks with control. Patients self-completed FSS and EQ5D Health Status questionnaires at baseline and throughout the study. Mean FSS total scores (1–7; high score=high fatigue) were compared using a piecewise linear model. Mean EQ5D visual analog scale (EQ5D-VAS) scores (0–100; lower score=worse perceived health status) were plotted over time. Data were pooled across treatments for psychometric analyses.

Results: Mean (± standard error) total FSS scores were 3.31±0.12 (simeprevir; n=195) and 3.20±0.21 (control; n=50), and increased by Week 24 in all groups comparably. In simeprevir-treated patients, mean FSS scores returned to baseline by Week 36, and were significantly lower than control at Weeks 36 (3.15±0.10 vs 4.26±0.17) and 48 (2.93±0.10 vs 4.23±0.20). Control scores
did not return baseline until Week 60. Over the entire study, fatigue was lower for simprevir-treated patients than controls \((p<0.001)\). EQ5D-VAS plots showed an expected inverse trend to FSS. FSS demonstrated high internal consistency (Cronbach’s \(\alpha = 0.95\)), reasonable test-retest reliability (intra-class correlation 0.74) and concurrent validity (correlation between FSS total score and EQ5D-VAS at Week 24; −0.63).

**Conclusions:** Addition of simprevir to PR significantly increased SVR rates without significantly increasing fatigue beyond that seen with PR alone. In simprevir-treated patients, shorter treatment durations with higher SVR rates led to less time with elevated FSS scores or depressed EQ5D VAS scores, compared with control.

**903 OMEGA-3 FATTY ACIDS AND/OR FLUVASTATIN IN HEPATITIS C PRIOR NON-RESPONDERS TO COMBINATION ANTI-VIRAL THERAPY – A PILOT RANDOMISED CLINICAL TRIAL**

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**Background and Aims:** Hepatitis C virus (HCV) utilises cholesterol and lipoprotein metabolism for replication and infectivity. Statins and omega-3 (n-3) polyunsaturated fatty-acids (PUFA) have been shown to have antiviral properties in vitro. This open label pilot study evaluated the effect of fluvastatin (Lescol® 40–80mg) and n-3 PUFA (Omactor® 1g and 2–4g) on HCV-RNA and lipoviral particles (LVP) in difficult to treat prior non-responders.

**Methods:** Patients \((n = 60)\) were randomly allocated in a factorial design to: no active drug; low-dose n-3 PUFA; high-dose n-3 PUFA; fluvastatin; low dose n-3 PUFA + fluvastatin; or high dose n-3 PUFA + fluvastatin. 50/60 completed study drugs for 12-weeks and followed up to week-24. Comparison was made between fluvastatin \((n = 24)\) vs no-fluvastatin \((n = 26)\) and n-3 PUFA high-dose \((n = 17)\) vs low-dose \((n = 17)\) vs none \((n = 16)\). The primary outcomes were change in total HCV-RNA, LVP and ALT at week-12 compared to baseline. Secondary outcome was change in interferon-gamma-inducible-protein-10 (IP10) as a measure of interferon activation.

**Results:** The study included 35% with compensated cirrhosis and 45% prior null-responders. There was no significant change in total HCV-RNA, LVP, non-LVP or LVP ratio in patients receiving fluvastatin or n-3 PUFA in low or high-dose. Fluvastatin was associated with increased ALT. Low dose n-3 PUFA were associated with decreased in IP10 concentration by week-12 \((−97.7 pg/mL, P = 0.019)\).

**Conclusions:** Fluvastatin and n-3 PUFA have no effect on plasma HCV-RNA or LVP. Low dose n-3 PUFA decreased IP10 and therefore warrants further prospective evaluation as a supplemental therapy to enhance interferon sensitivity. EudraCT 2006–004335–29

**904 HEALTH-RELATED QUALITY-OF-LIFE AMONG GENOTYPE 1 TREATMENT-EXPERIENCED CHRONIC HEPATITIS C PATIENTS: POST-HOC ANALYSES FROM THE REALIZE STUDY**

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**Background and Aims:** The REALIZE study assessed telaprevir (T) in combination with peginterferon/ribavirin (PR) versus PR in genotype 1 chronic hepatitis C infected patients (including patients with compensated cirrhosis) with prior PR failure (prior null, prior partial response, and prior relapse). In this post-hoc analysis, health-related quality of life (HRQL) was examined among REALIZE patients who received 12-week T with a total of 48-week PR (T12PR48) versus 48-week PR (PR48) alone.

**Methods:** Patients completed the EQ-5D (EuroQoL Group) questionnaire (range: 0, 1) at baseline and at Weeks 4, 12, 24, 48, and 72. Patients’ responses on their health state on five dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression) were used to calculate the EQ-5D index. Mean values (95% CIs) and percentage of patients reporting problems on each dimension were calculated over time by treatment group, liver fibrosis stage, and prior response to PR.

**Results:** Data from 396 patients (T12PR48: 265; PR48: 131) were included in the analysis. Among these patients, 26% had evidence of cirrhosis at treatment initiation. At baseline, for the pooled treatment groups the EQ-5D index (mean, 95% CI) was 0.90 (0.88, 0.92) (bridging fibrosis/cirrhosis) and 0.93 (0.91, 0.94) (no, minimal, or portal fibrosis). At Week 4, overall estimates were 0.81 (0.79, 0.83) for the T12PR48 group and 0.85 (0.83, 0.88) for the PR48 group; the corresponding values at Week 12 were 0.79 (0.77, 0.81) for T12PR48 and 0.82 (0.79, 0.85) for PR48. Mean values returned to baseline levels by Week 72 in both treatment groups. Liver fibrosis stage and prior response to PR treatment did not have a statistically significant impact on the EQ-5D index across study visits.

**Conclusion:** Post-hoc analyses of data from REALIZE, which included patients with compensated cirrhosis, suggested that HRQL worsened during the first 12 weeks of treatment in patients receiving T/PR or PR; HRQL returned to baseline by Week 72 across both treatment groups. Overall, HRQL was not statistically significantly different in patients who received T12PR48 and PR48 and for patients with early and advanced fibrosis.
ADHERENCE WITH TELAPREVIR BID vs. q8h DOSING IN TREATMENT-NAIVE HCV-INFECTED PATIENTS: RESULTS FROM THE PHASE III OPTIMIZE STUDY


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Background and Aims: The OPTIMIZE study was a randomized, open-label, multicenter, Phase III study in treatment-naïve patients with chronic genotype 1 HCV infection (NCT01241760). OPTIMIZE established the non-inferior efficacy of telaprevir (TVR, T) twice daily (bid) in combination with peginterferon/ribavirin (PR) compared with every 8 hours (q8h). In this retrospective analysis, TVR adherence and its association with virologic outcomes were explored.

Methods: 740 patients were randomized to either: TVR 1125 mg bid or TVR 750 mg q8h in combination with PR. The primary endpoint was SVR12 (HCV RNA <25 IU/mL after 12 weeks of follow-up). TVR adherence was measured using a patient-completed electronic diary (e-diary) that captured the amount and timing of TVR dosing relative to the prescribed regimen. Additionally, adherence to TVR and PR was measured by dispensed versus returned medications (pill count). Adherence was expressed as the percentage of prescribed doses during the treatment period and categorized by thresholds established from previous literature and from the distribution of adherence results from the current study. The e-diary analysis was performed using the intent-to-treat (ITT) population, where missing entries were considered as 0% adherent. Observed data analyses were also performed.

Results: E-diary and pill count adherence data were available for 700 (95%) patients. Adherence results by treatment arm are provided in the table. Self-reported (e-diary) adherence rates were lower than those based on pill count data: this difference was more marked for ITT compared with observed analyses. Mean adherence was significantly greater by all three analysis methods for bid compared with q8h dosing. In a multivariate analysis, higher adherence was associated with greater odds of SVR12, irrespective of adherence measure (for example, <95% vs ≥95% pill count: SVR12=69% vs 79%, OR=1.86; 95%CI: 1.29, 2.69).

Conclusions: Adherence based on patient electronic medication diaries was lower than that based on pill count; but this difference was smaller when observed analyses were used. Irrespective of adherence measure used, greater mean adherence was observed with bid versus q8h TVR dosing.

Table (abstract 906)

<table>
<thead>
<tr>
<th>Adherence method</th>
<th>Statistic</th>
<th>T12(bid)/PR (N=369)*</th>
<th>T12(q8h)/PR (N=371)*</th>
<th>P-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pill count (ITT, M=F)</td>
<td>Mean (SE)</td>
<td>99 (0.2)</td>
<td>98 (0.3)</td>
<td>0.0202</td>
</tr>
<tr>
<td>Range</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥85% adherent (%)</td>
<td>91</td>
<td>91</td>
<td>0.2868</td>
<td></td>
</tr>
<tr>
<td>≥95% adherent (%)</td>
<td>97</td>
<td>97</td>
<td>0.7868</td>
<td></td>
</tr>
<tr>
<td>e-diary (ITT, M=F)</td>
<td>Mean (SE)</td>
<td>87 (1.1)</td>
<td>85 (1.2)</td>
<td>0.0080</td>
</tr>
<tr>
<td>Median</td>
<td>95</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–100</td>
<td>0–100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥85% adherent (%)</td>
<td>56</td>
<td>48</td>
<td>0.0336</td>
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<td>≥95% adherent (%)</td>
<td>74</td>
<td>71</td>
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</tr>
<tr>
<td>e-diary (Observed)</td>
<td>Mean (SE)</td>
<td>95 (0.5)</td>
<td>92 (0.7)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Median</td>
<td>99</td>
<td>97</td>
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<tr>
<td>Range</td>
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<td>≥85% adherent (%)</td>
<td>74</td>
<td>64</td>
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</tr>
<tr>
<td>≥95% adherent (%)</td>
<td>90</td>
<td>87</td>
<td>0.1789</td>
<td></td>
</tr>
</tbody>
</table>

M=F: missing = failure; SE: standard error.

906 RIMOPLOSTIM’S EFFECT TO OPTIMIZE SVR WITH TELAPREVIR, RIBAVIRIN, AND PEG INFERNER-alfa 2a IN THROMBOCTOPENIC CIRRHOTICS WITH CHRONIC HEPATITIS C. RESTRAINT C- PLACEBO RCT


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Objectives: Treating HCV cirrhotic patients with thrombocytopenia is challenging, often requiring dose reduction/discontinuation to avoid complications. Significant dose reduction affects response guided therapy (RGT); affecting outcomes. Thrombopoietin (TPO) agonists are used to avoid disruption or therapeutic failure to optimize SVR. This study evaluated the use of TPO agonist in thrombocytopenia in cirrhotics with CHC.

Methods: Forty-five (n=45) cirrhotic treatment experienced CHC-GT1 patients were recruited with mean MELD 16, mean platelet count 95. Group A (n=15) placebo plus reduced dose of p-IFN with Ribavirin and Telaprevir. Group B (n=15) Romiplostim 500mcg lead in 1 month prior to initiation of therapy and SOC with Telaprevir. Group C (n=15) Ethisubose 50 mg orally daily lead in prior 15 days and SOC with Telaprevir for 12 weeks. RGT was analyzed with serial platelet counts, hemoglobin/hematocrit, absolute neutrophils count and platelet antibodies. HCV RNA 1st, 2nd, 4th, 12th, 24th, 36th and 60th weeks for SVR.

Results: See the table.

Table (abstract 906)

<table>
<thead>
<tr>
<th>AVR 1 wk</th>
<th>VRVR 2 wks</th>
<th>RVR 4 wks</th>
<th>EVR 12 wks</th>
<th>MTVR 24 wks</th>
<th>ETVR 36 wks</th>
<th>ETVR 48 wks</th>
<th>SVR 60 wks</th>
<th>SVR 72 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>R7 PR=8 BT=0 5/15 (33%)</td>
<td>7/15 (47%)</td>
<td>9/15 (60%)</td>
<td>112K</td>
<td>101K</td>
<td>101K</td>
<td>93K</td>
<td>98K</td>
</tr>
<tr>
<td>Platelet Count 90K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>R8 PR=6 BT=1 9/15 (60%)</td>
<td>10/15 (66%)</td>
<td>11/15 (77%)</td>
<td>12/15 (80%)</td>
<td>210K</td>
<td>90K</td>
<td>96K</td>
<td>R1/15 (7%)</td>
</tr>
<tr>
<td>Platelet Count 68K</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>R7 PR=6 BT=2 7/15 (47%)</td>
<td>8/15 (53%)</td>
<td>9/15 (60%)</td>
<td>101K</td>
<td>102K</td>
<td>90K</td>
<td>80K</td>
<td>R1/15 (7%)</td>
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<tr>
<td>Platelet Count 128K</td>
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</table>
Conclusions: This study demonstrates the efficacy of Romiplostim in thrombocytopenic cirrhotics in optimizing SVR (Group A – 53%, Group B – 67% and Group C – 60%). A larger trial is needed to validate.

907
IS SHORT TIME COMBINED THERAPY WITH PEGYLATED INTERFERON PLUS RIBAVIRIN EFFECTIVE IN PATIENTS WITH ACUTE HEPATITIS C?

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Background and Aims: Standardized therapeutic approaches in acute hepatitis C virus (HCV) infection are difficult to develop, because severe clinical, diagnostic and social reasons are leading to under-reporting and limited epidemiological data. We aimed to evaluate the efficacy and safety of pegylated interferon plus ribavirin combined antiviral regimen versus mono therapy of pegylated interferon for the treatment of acute HCV infection.

Methods: In a prospective, non-randomized study, 13 patients (F/M: 8/5, mean age 34.2 years) with acute hepatitis C were included. Genotype was 1b and 1a, in 6 and 7 patients, respectively. All patients remained HCV-RNA positive at week 12 after an initial, well documented, acute episode of HCV infection. At week 12, patients received pegylated interferon alpha-2b (1.5 mg/kg/weekly) for 24 weeks (Group A, n = 6) or pegylated interferon alpha-2b at the same dose plus ribavirin (1000–1200 mg/daily) for 12 weeks (Group B, n = 7). Sustained virological response investigated at 24 weeks after ending of the therapy.

Results: Overall, SVR was achieved in all patients. Distribution of genotype IL-28B did not differ between the two groups. No severe adverse events or need blood transfusion were detected in both groups, and no treatment discontinuations were observed.

Conclusion: In patients with acute hepatitis C, short time combined therapy with pegylated interferon alpha-2b plus ribavirin (for 12 weeks) was effective and safe at least as monotherapy with pegylated interferon alpha-2b given for 24 weeks. These results deserve to be investigated further prospective, large-scale studies.

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REAL-WORLD EFFICACY OF BOCEPREVIR IN COMBINATION WITH PEGINTERFERON alpha-2a AND RIBAVIRIN IN TREATMENT-EXPERIENCED PATIENTS: INTERIM ANALYSIS FROM THE GERMAN NON-INTERVENTIONAL PAN STUDY

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Introduction: Boceprevir (BOC) in combination with peginterferon alpha-2a or alpha-2b plus ribavirin is approved in Germany for treatment naive and experienced patients with chronic hepatitis C infected with HCV genotype 1. The PAN study is a non-interventional study conducted by the Association of German Gastroenterologists in Private Practice (bng) in collaboration with Roche.

Methods: Treatment naive or experienced patients are eligible for inclusion in the PAN study if they are prescribed BOC or telaprevir plus peginterferon alpha-2a/ribavirin. Here we restrict the analysis to treatment experienced patients receiving BOC plus peginterferon alpha-2a/ribavirin who completed 12 weeks of treatment including the 4 week lead-in phase with peginterferon alpha-2a/ribavirin.

Results: Overall 108 patients were included in the present analysis. Patients had a mean age of 53.0 years and a mean BMI of 27.2 kg/m², 58 (53.7%) were male, 105 (97.2%) were Caucasian, 15 (13.9%) had one diagnostic measure consistent with cirrhosis, and the mean HCV RNA was 6.0 log_{10} IU/mL. Twenty five (23.1%) and 67 (62.0%) of the patients were infected with HCV G1a and G1b, respectively (14; 13.0% unknown subtype). The proportion of patients with evaluable data with undetectable HCV RNA at week 4, 8 and 12 was 29%, 41.4% and 64.1%, respectively (Table). However, at week 8 a total of 50 patients (46.3%) had no data on HCV RNA.

<table>
<thead>
<tr>
<th>Week 4. n/N (%)</th>
<th>Non-response</th>
<th>Partial-response</th>
<th>Relapse</th>
<th>All patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/31 (0)</td>
<td>0/9 (0)</td>
<td>2/37 (5.4)</td>
<td>2/70 (2.9)</td>
</tr>
<tr>
<td>Week 8. n (%)</td>
<td>Non-response</td>
<td>Partial-response</td>
<td>Relapse</td>
<td>All patients*</td>
</tr>
<tr>
<td></td>
<td>7/25 (28.0)</td>
<td>4/5 (8.0)</td>
<td>12/34 (35.3)</td>
<td>13/36 (36.1)</td>
</tr>
<tr>
<td>Week 12. n (%)</td>
<td>Non-response</td>
<td>Partial-response</td>
<td>Relapse</td>
<td>All patients*</td>
</tr>
<tr>
<td></td>
<td>22/44 (50.0)</td>
<td>6/11 (54.5)</td>
<td>35/45 (77.8)</td>
<td>59/92 (64.1)</td>
</tr>
</tbody>
</table>

*Among the 108 patients included in the analysis 49, 3, 13, 56, 5 and 1 reported prior non-response, null-response, partial-response, relapse, lack of tolerability, and personal reasons for the failure of their previous treatment regimen (multiple responses were possible).

Conclusion: Early virological responses among treatment experienced patients treated with BOC plus peginterferon alpha-2a/ribavirin in Germany are generally consistent with the results of published phase 3 trials. A high proportion of patient did not have a week 8 value for HCV RNA which may reflect the BOC European prescribing information for patients with previous treatment failure who are required to undergo a full 48 weeks course of re-treatment irrespective of the HCV RNA result at week 8.

Acknowledgement: Funded by Roche

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IMPACT OF DEMOGRAPHIC CHARACTERISTICS ON RESPONSE TO PEGYLATED INTERFERON/RIBAVIRIN TREATMENT IN GENOTYPE 2 AND 3 CHC PATIENTS

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Background and Aims: Data from clinical trials indicate that genotype-3 (G3) CHC patients do not respond as well as genotype-2 (G2) to PegIFN/RBV therapy. However, some studies have not consistently reported a different SVR between the two genotypes. This discrepancy could be explained by the different distribution of some epidemiological and demographic characteristics, usually associated to SVR, between the genotypes. We conducted a retrospective case–control study to assess the influence of clinical
and demographic characteristics on the SVR rate in 301 G2 and G3 CHC patients from north-Italy.

**Material and Methods:** We compared the rates of SVR and the clinical and demographic characteristics of 192 (63.8%) G2 and 109 (36.2%) G3 patients consecutively treated with PegIFN/RBV in 7 non-tertiary centres. The SVR rates were also compared in the two subgroups of G2 and G3 patients matched according to sex, age and severity of liver disease.

**Results:** In the entire cohort, no statistically significant differences in SVR-rate was observed between G3 and G2 patients (79.4 vs 86.9%; odds ratio [OR]: 1.7, 95% confidence interval [CI]: 0.9–3.1, P=0.11). Compared with G2 patients, G3 patients showed a lower mean age (39.3±0.7 vs 53.5±0.9 years; p<0.001), a lower prevalence of female gender (26.6 vs 57.3; p<0.001) and a higher prevalence of history of injection drug use (46.8% vs 21%; p<0.001).

**Conclusions:** Significant differences in demographic characteristics may account for the similar outcome of therapy observed between patients infected with G3 and G2 in the setting of day-to-day clinical practice.

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**GENOTYPING OF TA DINUCLEOTIDE REPEAT, rs72258881, COULD IMPROVE THE PREDICTION OF INTERFERON-BASED CHRONIC HEPATITIS C THERAPY AND SPONTANEOUS CLEARANCE FOLLOWING rs8099917 GENOTYPING**

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**Background and Aims:** Polymorphisms around IL-28B gene, rs8099917 and rs12979860, are strongly associated with the interferon-based therapy and the spontaneous clearance (SC) of chronic hepatitis C (CHC). Our recent study revealed one of the functional SNPs consisting TA dinucleotide repeat, in regulatory region of IL-28B gene. To determine the effect of the TA dinucleotide repeat, the TA repeat polymorphism was applied to CHC and SC to validate the effect on the clinical prediction.

**Methods:** Specific primers conjugating fluorescent molecule were designed to directly amplify TA repeat of IL-28B from genome extracted from PBMC. Of 132 healthy volunteer (HV), 65 CHC and 50 SC individuals for validation group were enrolled designed to directly amplify TA repeat of IL-28B from genome extracted from PBMC. Of 132 healthy volunteer (HV), 65 CHC and 50 SC individuals for validation group were enrolled in this study. All individuals are Japanese from an area with similar socioeconomic living conditions. The (TA)n number is defined as the range from 10 to 20 repeats

**Results:** The (TA)n number showed the range from 10 to 20 number in overall samples. Age and gender were matched among three groups in all samples. In test samples, ALT (average HV:CHC:SC = 20.7±49.6:19.9 IU/mL), PLT (20.8±18.1:17.9±10^4/mm^3), rs8099917 (TT/TG, 107/25:9/53/23), and TA repeat (S/M/L, 0/98/34:7/37/21:0/16/18) showed statistical significance (p<0.01, 0.01 and 0.03, respectively).

For validation analysis, independent samples were also reached statistical significance containing TA repeat variation among three groups (each p<0.05). On treatment response of CHC patients, the (TA)n genotype, S, M, or L was observed in 10%, 83%, and 7% of the patients and corresponded to NVR rate of 80%, 40%, and 0%, respectively (p=0.074). However, the TA repeat was applied as a second predictor of response to HCV therapy following the classification of rs8099917 genotype. The (TA)n genotype, S, M, or L was observed in 18%, 68%, and 14% of the patients with rs8099917 heterozygote and corresponded to NVR rate of 100%, 80%, and 0%, respectively (p<0.05).

**Conclusion:** The TA repeat genotype could be a second factor and improve the prediction of treatment response in CHC patients and SC population.
LEUKOCYTE DECLINE PREDICTS SUSTAINED VIROLOGIC RESPONSE IN PATIENTS UNDERGOING PEGINTERFERON alfa-2b/RIBVIRIN TREATMENT OF CHRONIC HCV GENOTYPE 1 INFECTION

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BACKGROUND AND AIM: Recently, a strong association between hemoglobin decline during peginterferon alfa-2b (Peg2b)/ribavirin (RBV) treatment and increasing SVR rates of patients with HCV genotype 1 (G1) infection has been shown which most likely reflects pharmacodynamic effects of RBV. In contrast, leukocyte decline during antiviral treatment may reflect pharmacodynamic effects of pegylated interferons. We therefore investigated the possible association between leukocyte decline and SVR in patients treated with Peg2b/RBV for chronic HCV G1 infection.

METHODS: Data from the German Peg2b/RBV observational study were retrospectively analyzed. This real-life cohort study assessed the safety and efficacy of treatment of G1 infection with Peg2b 1.5 μg/kg/week+weight-based RBV (800–1200mg/day) for up to 48 weeks at 285 sites. Patients who discontinued for non-response or other reasons were analyzed. SVR was defined as undetectable serum HCV-RNA 24 weeks after EOT response.

RESULTS: 1890 patients with G1 infection (44.1±12.3 years, female 43%, BMI 25.3±4.7, 52.2% with high baseline viral load ≥600,000 IU/ml) baseline had and at least one leukocyte measurement during therapy. Overall sustained virologic response (SVR) following Peg2b/RBV treatment was 42.3%. When leukocytes declined during treatment by <1000/μl or by 1000–2000/μl from baseline, poor SVR rates of only 23.2% and 33.9% were observed (Table 1). In contrast, higher SVR rates up to 48.6% were achieved when leukocyte declined by 2000/μl or more. A similar but inverse correlation was observed between maximal leukocyte decline and non-response rates while relapse rates did not show any association with the decline in leukocyte counts.

Table 1.

<table>
<thead>
<tr>
<th>Leukocyte decline (n/μl)</th>
<th>SVR % (n/N)</th>
<th>Non-response % (n/N)</th>
<th>Relapse % (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1000</td>
<td>23.2 (16/69)</td>
<td>40.6 (28/69)</td>
<td>11.1 (2/18)</td>
</tr>
<tr>
<td>≥1000 &lt;2000</td>
<td>33.9 (57/168)</td>
<td>38.1 (64/168)</td>
<td>25.0 (19/76)</td>
</tr>
<tr>
<td>≥2000 &lt;3000</td>
<td>38.8 (140/361)</td>
<td>39.3 (142/361)</td>
<td>23.1 (42/182)</td>
</tr>
<tr>
<td>≥3000 &lt;4000</td>
<td>45.3 (212/468)</td>
<td>31.2 (146/468)</td>
<td>23.2 (64/276)</td>
</tr>
<tr>
<td>≥4000 &lt;5000</td>
<td>44.1 (161/365)</td>
<td>35.1 (128/365)</td>
<td>23.3 (49/210)</td>
</tr>
<tr>
<td>≥5000</td>
<td>48.6 (203/418)</td>
<td>27.3 (114/418)</td>
<td>21.0 (54/257)</td>
</tr>
</tbody>
</table>

CONCLUSIONS: Leukocyte decline predicts SVR in patients undergoing Peg2b/RBV treatment of chronic HCV G1 infection. The magnitude of leukocyte decline as pharmacodynamic Peg2b effect seems in part reflect its antiviral activity.

THE ASSOCIATION BETWEEN SUSTAINED VIROLOGICAL RESPONSE AND BASELINE LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN THE TREATMENT OF CHRONIC HEPATITIS C PATIENTS WITH GENOTYPE 1

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BACKGROUND AND AIM: Little is known about the relationship between low-density lipoprotein (LDL) and virological response in chronic Hepatitis C (CHC) treatment. The aim of this study was to evaluate the effect of baseline LDL cholesterol levels on sustained virological response (SVR) in CHC patients with genotype 1.

METHODS: Study data were obtained from 5 tertiary centers of Turkey. Consecutively, selected data of patients who were treated 48 weeks with pegylated interferon alpha-2a or alpha-2b and ribavirin for CHC were obtained retrospectively. SVR was defined as undetectable HCV-RNA in six months after the end of treatment. Baseline HCVRNA, baseline Histology Activity Index (HAI) and fibrosis score, liver enzymes and baseline LDL-cholesterol and total cholesterol level as well as hepatic steatosis were analyzed at initiation of treatment. A total of 224 patients were enrolled in the study. 47 cases were excluded due to incomplete data. 9 cases with different genotype were excluded. In total, the data of 168 CHC patients were evaluated. Among 168 patients, 78 cases were included in the non-response group and 90 patients were included in the response group. SPSS program was used for statistical analysis.

RESULTS: Most of the patients were genotype 1. Data were sufficient for 168 patients (90 SVR positive and 78 SVR negative). The mean baseline LDL level was found 85.5 mg/dl in nonresponse group, while it was found 108.4 mg/dl in response group (p=0.0001). LDL-cholesterol level was more than 100 mg/dl in 20 nonresponse patients (25.6%) and 49 response patients (54.4%). The result was accepted significant (p=0.0001). Total cholesterol were less than 150 mg/dl in 42 nonresponse patients 42 (53.8%) and 24 response patients (26.6%), (p=0.003). The mean age (p=0.005), Baseline HAI (p=0.004) and fibrosis score (p=0.002), baseline GGT level (p=0.0001) were significantly higher in nonresponse patients.

CONCLUSIONS: Baseline LDL-cholesterol and total cholesterol levels were higher in response patients compared to nonresponse patients in genotype 1 chronic hepatitis C infection. Baseline elevated LDL levels were associated with higher SVR rates. Unsuccessful rates were higher among cases with high initial fibrosis levels and HAI, baseline elevated GGT. Advanced age negatively affects the response to treatment.

INTERLEUKIN-10 SERUM LEVELS ARE LOWER IN SUSTAINED VIROLOGICAL RESPONDERS (SVR) CARRYING IL-28B CC GENOTYPE (rs12979860) COMPARED TO SVR-CT/TT


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BACKGROUND AND AIM: IL28B gene encodes interferon I (IFNI), an innate immunity antiviral cytokine. IL28B polymorphisms can...
predict successful therapy for HCV. Conversely, interleukin 10 (IL-10), a cytokine of Th2 response antagonizes antiviral effects of Th1 response mediated mainly by INFg production. Therefore, IL-10 could interfere in the response of patients to pegIFN-a/RBV. Our aim was to associate the levels of IL-10 at the end of pegIFN-a/RBV treatment with IL28B polymorphisms.

**Methods:** Patients infected with HCV (n=487) and blood donors (n=234) were genotyped for IL28B at rs12979860. Treatment was done with pegIFN-a/RBV either 24 or 48 weeks. Patients were grouped in Sustained Virological Responder (SVR) and Non-Responder or Relapse (NR/RL). TAQMAN probes determined the IL28B SNPs at rs12979860, giving CC, CT or TT genotypes. Serum IL-10 was measured in 143 out of 487 patients using ELISA (R&D Systems, Inc.). Statistics employed chi-square test, 95% of CI, t-Student test or Mann–Whitney when appropriated, and p-value was considered significant when <0.05.

**Results:** The frequency of CT/TT was higher in HCV 75% vs. 68% blood donors group (p=0.02, OR 1.4 IC 1.0–1.96) and SVR group was associated with CC genotype (p=0.02 OR 2.2 IC 1.3–3.7). Confounding variables such as HCV genotype, METAVIR fibrosis scale, age and sex were not statistically different between groups. Forty percent of CC showed IL-10 at 8.5pg/mL, while 23% CT/TT presented levels of IL-10 at 14.1pg/mL, and p-value was considered significant when <0.05.

**Conclusions:** IL10 levels were lower in IL28B CC-SVR vs. CT/TT-SVR, suggesting that the IL-10 decrease effect caused by pegIFN-a/RBV seem to be more sustained in CC genotype, reflecting higher SVR rate in this group. Also, the results showed a higher CC genotype frequency in health blood donors compared to HCV group, which may be related to protection to infection as previously described.

### 915
**WORSE TOLERABILITY OF TRIPLE THERAPY IN SPECIAL POPULATIONS: CIRRHOTICS AND LIVER TRANSPLANT RECIPIENTS**

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**Introduction:** Triple antiviral therapy (TT) has become the standard-of-care for Hepatitis C virus (HCV) genotype 1 infected patients. Tolerability in special populations is of major concern and has not been fully described. We aimed to describe and compare the safety of TT (adverse events/management) in different groups of patients.

**Methods and Results:** Observational single-center study, comparing three groups (n=83): [A] Mild to moderate fibrosis (n=29); [B] Cirrhosis (n=38) and [C] Liver transplant (LT) recipients (n=16). Median time on treatment was 26 weeks. There were no significant differences regarding baseline characteristics between groups A, B and C: gender (69%, 63% and 81% male), median age (52, 57 and 57 years), IL28B genotype (24%, 16% and 25% CC), naïve (28%, 24% and 19%), prior null responders (28%, 34% and 44%). The protease inhibitor most frequently used was telaprevir in all groups (62%, 66% and 68%). Median MELD-score in group B was 8; 31% of LT recipients had graft cirrhosis (median MELD 14).

All patients developed adverse events (AE), especially hematologic. Only thrombocytopenia was significantly more frequent in special populations (A vs B and A vs C: p=0.001, B vs C p=ns). Anemia developed early (<12 weeks) in most patients (59%, 74% and 69%) and was severe (>5 g/dl drop from basal hemoglobin) in 41%, 63% and 31%, respectively. Median nadir hemoglobin levels were 10 g/dl, 9.5 g/dl and 8.4 in groups A, B and C (A vs B: p=0.015; A vs C: p=NS). EPO was used in 30%, 48% and 46%; and ribavirin dose-reduction in 55%, 62% and 54% of patients, respectively (all p=ns). Blood transfusions were significantly (p=0.047) more frequent in LT (54% vs 20% in group A, and 21% in group B). Treatment discontinuation rates were higher in group C (38%) than in groups A (24%) and B (13%). No patients died in groups A and B, vs 25% of deaths in LT (p=0.07).

**Conclusions:** Tolerability of TT is worse in special populations, leading to treatment discontinuation in many cases. Cirrhotics and LT recipients must be carefully monitored while on treatment.
62%, respectively) than in telaprevir treated patients (45% and 28%). Conversely, pruritus and erythema were more frequent with telaprevir compared with boceprevir (76 vs 37% and 50 vs 26%, respectively).

Conclusions: PI-based triple therapy leads to high rates of on-treatment virological response in previously non-responder patients. However, renal function after triple therapy is impaired as well as MELD score in cirrhotics. Cautious clinical monitoring should focus not only on haematological and dermatological side effects but also on renal function.

917 DEVELOPMENT OF PSYCHIATRIC SYMPTOMS DURING ANTIVIRAL THERAPY FOR CHRONIC HEPATITIS C: A RETROSPECTIVE STUDY ON 590 SUBJECTS

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Background and Aims: about 3% of worldwide population suffers from chronic HCV infection. Peg-IFNα and Ribavirin (Peg-IFN+RBV) are the treatment backbone and may favour development of psychiatric symptoms (PS) that represent a possible treatment contraindication or failure. We investigate the prevalence of mental disorders (MD) and the development of PS during Peg-IFN+RBV treatment in subjects with chronic hepatitis C.

Methods: Retrospective records evaluation of 590 patients (pts) treated with Peg-IFN+RBV from 2005 to 2011 in a hepatology center with a psychiatric counseling.

Results: Men were 53.6% and median age was 56 years (19–77). The most represented age group was 51–60 years (28%) followed by 41–50 (22%), 31–40 (11%), 71–80 (9%) and 18–30 (6%). 51.4% received Peg-IFNα2a and 48.6% Peg-IFNα2b. Treatment duration was 24 weeks (wks) in 45.8%, 48 wks in 26% and >48 wks in 28.2%. A MD history before treatment was present in 22.4% (558/590). Development of PS during therapy occurred in 67.9% and 43.4% of subjects with or without a MD history respectively (p = 0.000). Overall, 48.8% (288/590) developed the following PS: irritability (26.6%), sleep disorders (19%), depressed mood (17.7%), anxiety (10.9%), neurocognitive dysfunctions (6%) and others (4.2%). PS occurred most frequent in the age group 41–50 years (31.3%) followed by 51–60 (24.4%), 31–40 (18.3%), 61–70 (13%), 71–80 (6.9%), 18–30 (6.1%). PS appeared during the first 4 wks in 50.7%, from 5th to 12th wk in 25.5%, from 13th to 24th wk in 11.9%, from 25th to 48th wk in 10.1% and from 49th wk onwards in 1.8%. Pts who ended therapy, based on response or stopping rules, were 94.6% (558/590) while only 5.4% dropped-out: 3.7% (22/590) for non-PS adverse events and 1.7% (10/590) for PS. In this last group 1% (6/590) had a MD history and 0.7% (4/590) not. The type of Peg-IFNα not influenced PS development.

Conclusions: MD affect a large proportion of HCV-positive subjects. Pts develop in about half of pts especially during the first 12 wks of treatment and in subjects between 40–60 yrs. Psychiatric counseling represent an essential resource for the treatment of HCV disease.

918 VIROLOGICAL EFFICACY OF BOCEPREVIR (BOC) AND TELAPREVIR (TVR) PLUS PEGINTERFERON alfa-2a (P)/RIBAVIRIN (R) UNTIL WEEK 12 IN DRUG ADDICTED PATIENTS WITH GT-1 INFECTION: FIRST REAL LIFE REPORT

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Background: New triple treatments require a higher degree of adherence to treatment than dual combination, especially due to the 3-times daily intake scheme.

Methods: Between October 2011 and August 2012 1502 GT-1 patients (pts) starting hepatitis C treatment were included in the non-interventional study PAN conducted by Association of German Gastroenterologists in Private Practice (bng) and Roche. Of these, 137 were in a drug addiction program (OST = oral substitution treatment). We analyzed 49 pts with dual treatment compared to pts with triple treatment (27 with BOC/P/R and 61 with TVR/P/R). Precision of measuring HCVRNA are week 4, 8 or 12 was set to ±3d.

Results: Demographic data of pts with dual treatment (DT) vs. BOC containing or TVR containing therapy were mean age 36.6/41.4/22.2yrs, male gender 78/78/85%, BMI 25.9/26.8/26.0 kg/m², duration of infection 9.6/13.2/13.2yrs, pretreated pts 22/30/46%. 31/41/51% of pts got methadone, 31/44/25% polamidone, 22/7/21% buprenorphine (+− naloxone), 16/8/3% others. Alcohol consumption was documented in 14/26/12% of pts, parallel consumption esp. of benzodiazepine in 14/26/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts, parallel consumption esp. of benzodiazepine in 14/13/12% of pts.

Conclusion: Triple treatment showed higher week 4 (TVR) and week 8 (BOC) virological responses than dual therapy with P/R but not as distinct as in pivotal trial. Reason could be the excellent rapid and early virological response in drug addicted pts as shown elsewhere in literature. Due to higher rates of side effects a careful evaluation of drug addicted pts undergoing triple therapy seems to be reasonable.

Table: Virological efficacy in drug addicted patients

<table>
<thead>
<tr>
<th></th>
<th>Dual treatment</th>
<th>BOC/P/R</th>
<th>TVR/P/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 4</td>
<td>31.0% (n = 29)</td>
<td>12.5% (n = 16)</td>
<td>56.8% (n = 44)</td>
</tr>
<tr>
<td>Week 8</td>
<td></td>
<td>72.7% (n = 22)</td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>57.1% (n = 28)</td>
<td>72.7% (n = 22)</td>
<td>72.5% (n = 51)</td>
</tr>
<tr>
<td>Week 4, 8 and 12 adjusted to available data</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ANEMIA AND ITS MANAGEMENT IN PATIENTS TREATED WITH TELAPREVIR TWICE DAILY VERSUS EVERY 8 HOURS IN THE PHASE III OPTIMIZE STUDY

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Background and Aims: OPTIMIZE was a Phase III, randomized, open-label, international, non-inferiority study comparing twice daily (bid) versus every 8 hours (q8h) telaprevir (TVR) combined with peginterferon/ribavirin (PR) in treatment-naïve, genotype 1 HCV-infected patients (NCT01241760). We report analyses that describe anemia and its management in this study.

Methods: Anemia was defined as grouped adverse event (AE) terms, including hemoglobin (Hb) <10.5g/dL in females and <10.5g/dL in males. Ribavirin (RBV) dosing, including modifications for anemia, followed local prescribing instructions and was categorized by the amount and timing of dose reduction.

Results: Anemia was reported in 401/740 (54%) randomized patients, led to TVR discontinuation in <5% and was higher in patients with cirrhosis versus without (65% vs 52%, respectively; p=0.0165). In a multivariate analysis (N=731), anemia was associated (p<0.05) with lower baseline Hb (OR=1.63), RBV dose (OR=1.25 per mg/kg), age (OR=1.67 per decade) and cirrhosis (OR=1.76), but not with treatment assignment (bid vs q8h), gender, or TVR pharmacokinetics. 340 patients (46%) required interventions for anemia. RBV dose was reduced in 316 (43%; 153/316 [48%] vs 600mg/day), at a median of 9 weeks from TVR initiation and was followed local prescribing instructions and was categorized by the amount and timing of dose reduction.

Conclusions: Anemia was associated with lower baseline Hb, RBV dose, age and cirrhosis, but not TVR treatment arm (bid vs q8h). RBV dose reduction was frequently used to manage anemia and did not compromise SVR12, even in those with cirrhosis.
Multivariate analysis revealed IgG level (P=0.001) as the only predictor of prognosis. HLA data were available for 25/46 (54%) patients with PSC and IBD and 22/43 (51%) patients without IBD. Female with PSC and IBD had significantly higher positivity for HLA DR13 (P=0.04), DRw52 (P=0.0004) and DQ6 (P=0.05), compared to a higher positivity for HLA A2 (P=0.02) and DR4 (P=0.03) in females without IBD.

Conclusion: Females diagnosed as PSC without IBD are older at diagnosis, have an excellent prognosis and different HLA associations compared to females with both PSC and IBD.

Table 1. Comparison of end points between the two groups

<table>
<thead>
<tr>
<th>End point</th>
<th>PSC females with IBD, n (%)</th>
<th>PSC females with no IBD, n</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>7/46 (15%)</td>
<td>0/43</td>
<td>0.012</td>
</tr>
<tr>
<td>Liver decompensation</td>
<td>4/46 (9%)</td>
<td>0</td>
<td>0.11</td>
</tr>
<tr>
<td>Liver transplantation</td>
<td>1/46 (for CCA)</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>3/46 (6%)</td>
<td>0</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 2. Comparison of HLA associations between female patients

<table>
<thead>
<tr>
<th>HLA</th>
<th>PSC females with IBD, n=25</th>
<th>PSC females without IBD, n=22</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>6</td>
<td>13</td>
<td>0.02</td>
</tr>
<tr>
<td>B7</td>
<td>1</td>
<td>5</td>
<td>0.08</td>
</tr>
<tr>
<td>DR4</td>
<td>4</td>
<td>11</td>
<td>0.03</td>
</tr>
<tr>
<td>DR13</td>
<td>9</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>DRw52</td>
<td>19</td>
<td>5</td>
<td>0.0004</td>
</tr>
<tr>
<td>DQ6</td>
<td>7</td>
<td>1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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HEPATIC LYMPHOCYTE IMMUNOPHENOTYPING STUDIES IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS: IL-12 RECEPTOR-γ2 EXPRESSION AND INFILTRATING INTRAHEPATIC T-REGULATORY CELLS

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Background: Primary biliary cirrhosis (PBC) is a progressive autoimmune liver disease leading to cirrhosis and often, transplantation. Experimental studies implicate interacting innate, humoral and cellular mechanisms in disease pathogenesis, on a background of genetic and environmental risk. Recent genome wide association studies have demonstrated genetic risk associated with specific immune activation pathways including the IL-12 pathway. Signalling via IL-12Rβ2 is key for the STAT4-dependent development of Th1 cells and impaired expression of IL-12Rβ2 leads to abortive differentiation of regulatory T cells (Treg).

Aims and Methods: We isolated liver-infiltrating lymphocytes from non-enzymatic mechanical dissociation (Miltenyi Biotech) of human liver tissue followed by density gradient separation and used 8-colour flow cytometric analysis to phenotype T cells (CD4+ and CD8+), Tregs (CD4+ CD25+ CD127low), NK cells (CD3-CD56+), B cells (CD19+), monocytes (CD14 +/- CD16+), and dendritic cells (HLA-DR+, CD86+) and real-time PCR to quantify mRNA in diseased and normal tissue.

Results: 41% of isolated intrahepatic lymphocytes were T cells (range: 21%-60%), with the remaining majority expressing an NK phenotype. The ratio of CD4:CD8 intrahepatic T cells in PBC (n=4) was 60:40 compared to 50:50 in all other diseases combined (n=7). 50% (range: 43%-58%) of liver infiltrating Treg isolated from patients with PBC expressed IL-12Rβ2 compared to 32% in cells isolated from patients with alcoholic cirrhosis (n=2) and <2% in patients with cryogenic cirrhosis or in peripherally circulating Treg isolated from healthy volunteers (n=4). No differences were detected in the expression of IL-12Rβ2 by no-Treg between any diseases. Real-time PCR demonstrated a 3-fold increase in STAT4 expression in PBC liver compared to normal donor liver whilst a similar induction of protein expression was detected by western blotting.

Conclusions: Our studies demonstrate increased expression of IL-12Rβ2 on infiltrating T-regulatory cells and STAT4 activation in patients with PBC. This is the first study to suggest that the IL-12R pathway in PBC may be functional and linked to disease pathogenesis. Further functional characterisation is required to determine the downstream effects of this pathway on the immunopathogenesis of PBC.
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EMERGENCE OF A NEW OPPORTUNISTIC INFECTION IN EUROPE: HEPATIC ALVEOLAR ECHINOCOCCOSIS. A FIFTY-CASE REPORT

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Background: Isolated cases of alveolar echinococcosis (AE), a rare parasitic liver disease, were described in patients with immune suppression (IS) in the past few years in various endemic European countries. This prompted us to get a more precise figure of AE as an opportunistic infection.

Methods: AE cases diagnosed in patients with the following IS conditions (IS/AE cases): cancer, malignant hematological disorders (MHD), chronic inflammatory diseases (CID), transplantation, or AIDS, were extracted from the AE French Registry database (1982–2012); additional details were obtained from their physicians by phone interviews; patients’ characteristics were compared to those of non-IS AE patients.

Results: Fifty among 516 Registry cases had a concomitant condition associated with IS. There was no difference in gender-ratio, median age, residence area and risk factors, but less people lived on agriculture among IS/AE patients. There was a significant increase in the percentage of IS/AE cases over time, with a marked progression after January 2004. IS conditions were: 30 cancers, 9 MHD, 14 CID, 5 transplantsations, and 1 AIDS (2 associated IS conditions in 9 cases). AE diagnosis was fortuitous in 3/4 patients, on symptoms in 1/4, including unusual liver abscess-like presentations. Diagnosis of AE and IS condition was concomitant in 16 patients; median time period between diagnosis of the underlying disease and AE diagnosis was 48 months in other cases. Available retrospective imaging in 15 patients did not show AE lesions. After first AE symptom/image, diagnosis was delayed in 50% of patients. Specific EM2+ serology was negative in 11 patients; PCR assessed diagnosis in 7 patients. All IS/AE patients but 7 took IS drugs; 5 patients received biological agents. Median follow-up was 43 months, with 8 deaths non-related to AE. Albendazole was efficient in all cases but associated with side-effects in 19 patients.

Conclusion: AE is definitely an emerging opportunistic disease coincident with more agressive IS treatment of malignant and inflammatory diseases in Europe. Early differential diagnosis is difficult but should be attempted since non-diagnosed AE-related signs and symptoms are misleading regarding the patient’s initial condition staging and management, and albendazole may control the disease despite the IS situation.

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EVIDENCE OF EFFECTIVE INTRALESIONAL DIFFUSION OF ALBENDAZOLE SULFOXIDE IN PATIENTS WITH LIVER ALVEOLAR ECHINOCOCCOSIS

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Background and Aims: Albendazole (ABZ) therapy, alone or in combination with surgery, remains the standard of care for human alveolar echinococcosis (AE). ABZ sulfoxide (ABZ-SO) is the main active metabolite of ABZ and regular monitoring of ABZ-SO blood levels of patients is recommended. A few data are available on the pharmacokinetics of ABZ in cystic echinococcosis (CE) patients, especially on the concentration in the liver cysts and cyst fluid. However, no data on target site concentrations are available in AE patients. Our study aimed to provide data on intralesional ABZ-SO concentration in patients with liver alveolar echinococcosis.

Methods: AE patients with ABZ treatment and who benefited of surgical removal of AE lesion over a 5-year period (2008–2012) were included in this prospective study. ABZ-SO concentrations were determined using HPLC methods according to a standardized protocol in several specimens: non-infected liver tissue and AE lesion (nmol/l), blood during surgery (µmol/l), and gallbladder bile. Clinical, radiological, serological, and pathological data were prospectively collected for each patient, as well as pre-operative ABZ monitoring data.

Results: Twelve patients with AE liver resection were included. Operative specimens were sampled 10 to 19 hours after last ABZ intake (median time: 16h). Mean ABZ-SO concentrations were respectively 0.55 µmol/l [min: 0.21; max: 3.47] in per-operatively-collected plasma. ABZ-SO were significantly higher in AE liver lesion with a mean concentration at 1.52 nmol/g (<0.10; 8.97) than in non-infected liver tissue with a mean concentration at 0.42 nmol/g (<0.10; 1.6) (p = 0.025 Wilcoxon test). Bile were obtained in 4 patients, with a mean ABZ-SO concentrations reaching 0.48 µmol/l (versus mean at 0.41 µmol/l in blood for these 4 patients).

Conclusion: Our study provides for the first time evidence of effective diffusion of ABZ into AE lesion in the liver, with significant higher ABZ-SO concentrations observed in AE lesion versus non-infected liver tissue. This fact might be explained by accumulation and/or lower clearance of ABZ-SO from the lesion. Further studies are needed, focusing on potential relationship between the type of lesion and intralesional ABZ-SO diffusion.
FACTORS ASSOCIATED WITH POOR OUTCOME IN PRIMARY BILIARY CIRRHOSIS

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Background: In primary biliary cirrhosis (PBC), progression to liver failure is variable. Risk stratification at baseline is advantageous to optimize management. It is well-established that biochemical response to ursodeoxycholic acid (UDCA) strongly predicts long-term outcome. Using a cohort of almost 4000 patients recruited to the UK-PBC Project, we sought to identify additional variables that might refine prognostic models based on UDCA-response.

Methods: We undertook time-to-event analysis using the Kaplan–Meier method and the Cox model. The entry point was the date of diagnosis and the endpoint was the date of “failure” (death from liver failure; liver transplant [LT] for PBC, or serum bilirubin ≥100 μmol/L) or censor (date of most recent hospital attendance).

Results: Follow-up data were available for 3567 patients, including 623 LT recipients (14.6%). In this cohort, age at diagnosis predicted outcome, with younger patients having worse prognosis (Hazard Ratio [HR]=0.978, P<0.0001). Baseline clinical data were available for a subgroup of 1370 patients, including 72 LT recipients (5%). In this subgroup, multivariate analysis showed that outcome was independently predicted by the baseline bilirubin, transaminases, sodium and creatinine, and by UDCA-response (Table 1). Of note, elevated transaminases predicted worse outcome in the univariate analysis (HR=1.70, P=0.0002) but better outcome in models including bilirubin. This suggested an interaction between bilirubin and transaminases, confirmed by introducing an interaction term into the multivariate model.

Conclusions: This study defines additional prognostic factors, independent of UDCA-response, which may be used to refine existing models. It is interesting that younger patients have worse prognosis. Statistical interaction between bilirubin and transaminases is also interesting, in essence showing that in patients with elevated bilirubin, those with elevated transaminases do better than those with normal transaminases, probably reflecting greater inflammatory activity with better potential for a response to treatment.

Table: Multivariate time-to-event analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>195</th>
<th>195</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN bilirubin</td>
<td>3.181</td>
<td>2.423</td>
<td>4.177</td>
<td>&lt;2 × 10⁻⁶</td>
</tr>
<tr>
<td>LN (AST or ALT ratio)</td>
<td>0.634</td>
<td>0.459</td>
<td>0.877</td>
<td>0.006</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.927</td>
<td>0.871</td>
<td>0.986</td>
<td>0.016</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.997</td>
<td>0.994</td>
<td>0.999</td>
<td>0.020</td>
</tr>
<tr>
<td>Treatment failure (Paris I criteria)</td>
<td>8.361</td>
<td>4.574</td>
<td>15.278</td>
<td>5 × 10⁻¹²</td>
</tr>
</tbody>
</table>

PRIMARY BILIARY CIRRHOSIS AND METABOLIC SYNDROME

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PBC is characterized by a long natural history and a low incidence of cardiovascular events despite high cholesterol serum levels. However, the role of metabolic conditions (obesity, hypertension, insulin resistance) eventually associated to PBC has not been analyzed.

Aim: To assess the influence of metabolic syndrome (MS) on either the response to UDCA and the survival in PBC patients.

Methods: The historic database collection (1975–2011) was used. The mean follow up was 123 months, (range 6–425 months). All patients were treated with UDCA (15 mg/kg/day). Hypercholesterolemia was treated with statins or fibrates when total cholesterol was >240 mg/dl. Responders to UDCA were defined subjects who reached at least a 40% decrease in alkaline phosphatase after 1 year. MS was defined MS according to the American Hearth Association criteria. Survival was analyzed with Kaplan–Meier curves.

Results: A total of 171 PBC patients were considered eligible for the study; 55 of them (32.1%) at presentation fulfilled the criteria for MS. LFTs and Mayo score were comparable in patients with MS and in those without MS; only GGT resulted significantly higher in the group with MS (243 vs 159 IU/ml, p=0.038). Histological stages and fibrosis score were similar in the two groups at baseline. The occurrence of cardiovascular events during the follow-up was significantly higher in patients with MS (p<0.01). The response to UDCA was higher in the group with metabolic syndrome (p<0.05). Kaplan–Meier curves were similar until a 200 month interval, thereafter survival was worse in the group with MS, but the difference was not statistically significant.

Conclusion: MS when associated to PBC should be carefully treated, due to the risk of cardiovascular events and the reduced response to UDCA.

RISK FOR HEPATOCELLULAR CARCINOMA IN AUTOIMMUNE HEPATITIS – IS THERE AN INDICATION FOR SURVEILLANCE?

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Background: Autoimmune hepatitis (AIH) is an autoimmune liver disease, which is diagnosed by elevated aminotransferases, presence of autoantibodies (ANA, SMA, LKM), elevated IgG, liver biopsy findings and by ruling out infectious hepatitis. Retrospective studies have shown that the annual risk of hepatocellular carcinoma (HCC) is 1.1% in patients with AIH and cirrhosis. Surveillance for HCC is recommended when the annual risk is over 1.5%. Nevertheless surveillance of AIH patients is recommended in the latest guidelines.

Aim: To investigate the risk of HCC in a large and well-characterized cohort of AIH patients.

Methods: A cohort of 634 AIH-patients (73% women) has been established. Median age at diagnosis was 59 years (range 43–77). Swedish citizens have a unique personal number, which is searchable in the National cancer registry. All new cancer diagnoses are mandatory to report. The expected number of cases used to calculate standard incidence ratios (SIRs) was obtained by calculating age- and calendar-specific person-years in the cohort
with the corresponding matched incidence in the entire Swedish population.

**Results:** In the cohort 248 persons had cirrhosis at diagnosis (n = 179) or follow-up (n = 69). The total follow-up time in the cohort was 3158 years (mean 12.3, range 0–40.5 years). Patients followed were followed to liver transplantation or death. Most of the patients in this study were not subjected to screening.

We identified 8 patients with HCC, none had hepatitis and all had cirrhosis. This gives a life-time-risk of 3.2% (8/248) during the observational period and a yearly risk of 0.25% (8/3158). SIR was 54.6 (CI 19.9–100.0) for patients with cirrhosis and for the whole cohort 17.14 (95%CI 6.3–37.3).

**Conclusions:** There is a significant over risk for HCC in AIH patients with established cirrhosis. The annual risk is, however, very low and in line with other studies. The true prevalence may be underestimated since the autopsy frequency is declining and clinicians are not searching for HCC in patients beyond curable therapy.

Based on our findings we cannot recommend HCC surveillance in AIH. The very low annual risk of HCC indicates that surveillance is not cost effective in these patients.

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AUTOIMMUNE HEPATITIS IN ISRAEL: A MULTICENTER STUDY OF 15 YEARS FOLLOW-UP

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**Background and Aims:** The epidemiology and outcome of autoimmune hepatitis (AIH) in Israel are unknown. We aimed to assess the incidence, prevalence and clinical outcomes of AIH in the Israeli population.

**Methods:** Case-finding methods and population-based administrative data were used to evaluate the epidemiology and prognostic factors of outcome in AIH from 1995 through 2010.

**Results:** We identified 100 cases of AIH (95% women; median age at diagnosis 51 years; type I 92%; 21 cirrhotic patients). Most cases were of Jewish origin (80%) while Arabs represented only 20% of the cohort. During the period of study (1995–2010) the average annual prevalence and incidence of AIH in southern Israel were 110.1 cases per million and 6.7 cases per million respectively. Among 100 incident cases with a total follow-up of 600 persons-years from diagnosis, the estimated 1-year and 10-year probabilities of survival for AIH patients diagnosed were 96.5% and 89.7% respectively. Out of 100 patients, 97 cases were treated with a combination of steroids and azathioprine or steroids alone (prednisone and azathioprine 71%; budesonide and azathioprine 11%; prednisone alone 5% and budesonide alone 10%). Remission was recorded in 58% of patients. Incomplete response and failure to treatment were noted in 26% and 16% respectively of the patients. In multivariate analysis independent predictors of remission were fibrosis degree [mild vs. bridging fibrosis (F3) and cirrhosis (F4)] [OR 0.04; 95% CI 0.004–0.31; P = 0.003], and albumin level [OR 5.17; 95% CI 1.16–22.93; P = 0.031].

**Conclusions:** These findings suggest that the prevalence rate of AIH in Israel is quite similar to other European Caucasian populations, with a relatively long-term good prognosis, despite a lower rate of remission to immunosuppressive therapy.

**Table: Worldwide prevalence and incidence rates of AIH**

<table>
<thead>
<tr>
<th>Country</th>
<th>Period of study</th>
<th>Prevalence (per 100000)</th>
<th>Incidence (per 100000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
<td>1984–2000</td>
<td>42.9</td>
<td>No reported</td>
</tr>
<tr>
<td>Norway</td>
<td>1986–1995</td>
<td>16.9</td>
<td>1.9</td>
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<tr>
<td>Sweden</td>
<td>1990–2003</td>
<td>10.7</td>
<td>0.85</td>
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<tr>
<td>Spain</td>
<td>1990–2003</td>
<td>11.6</td>
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<td>New Zealand</td>
<td>2006–2008</td>
<td>24.5</td>
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<td>Israel</td>
<td>1995–2010</td>
<td>11</td>
<td>0.67</td>
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PHARMACOCGNETICS OF AZATHIOPRINE IN AUTOIMMUNE HEPATITIS: RELATIONSHIP OF THIOPURINE METHYLTTRANSFERASE AND INOSINE TRIPHOSPHATE PYROPHOSPHATE POLYMORPHISMS TO AZATHIOPRINE METABOLITE LEVELS AND TO ADVERSE EVENTS

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**Introduction and Aims:** Azathioprine (AZA) is widely used to treat autoimmune hepatitis (AIH). However, 20% of patients are intolerant of AZA and a further 18% are unresponsive. AZA metabolism is complex and in childhood leukaemia, genetic polymorphisms in thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) have been associated with AZA toxicity and differences in active metabolite production. This has been little studied in AIH. We aimed to assess the association of these polymorphisms with AZA toxicity and with accumulation of AZA derived thioguanine nucleotide (TGN) and methylmercaptopurine metabolites (MeMPNs) in AIH.

**Methods:** We studied 151 patients with AIH (123 female; median age at diagnosis 55 (range 2–81) years). Subjects were genotyped for the presence of TPMT *3, TPMT *2 and ITPA 94C>A and IVS2+21A>C variant alleles. TGNs and MeMPNs were measured in patients who remained on AZA.

**Results:** For TPMT, 138 patients were wildtype and 13 (9%) were heterozygous (1 TPMT*T2, 11 TPMT*T3A and 1 TPMT*T3C). For ITPA, 95 were wildtype, 50 (33%) heterozygous (10 94C>A and 40 19S2+-21A>C) and 6 homozygous/compound heterozygous. There were 57 adverse events (AE) in 54 (36%) patients – in 32 (21%) AZA was withdrawn. TPMT wildtype and heterozygous patients had a similar incidence of leucopenia (18 vs 17%, p = 0.9) and of non-myelotoxic AEs (21 vs 8%, p = 0.5). Likewise, ITPA wildtype, heterozygous and homozygous patients had a similar incidence of leucopenia (19, 10 and 16% respectively, p = 0.2). ITPA heterozygous and homozygous patients had a similar incidence of leucopenia (19, 10 and 16% respectively, p = 0.2) and of non-myelotoxic AEs (21 vs 8% p = 0.5). Compared to wildtype patients, TPMT heterozygotes accumulated higher concentrations of TGNs (413 vs 212 pmol/8x108RBCs, p = 0.009) and lower MeMPNs (111 vs. 1000 pmol/8x108RBCs, p < 0.001), despite being a lower doses of AZA (1.0 vs. 1.7 mg/kg/day, p < 0.001). Comparing ITPA wildtype, heterozygous and homozygous patients, there was no difference in TGNs (222, 212 and 176 pmol/8x108RBCs respectively, p = 0.76) and MeMPNs (957, 957 and 713 pmol/8x108RBCs respectively, p = 0.7).
DECREASE OF LIVER STIFFNESS IN PATIENTS WITH PRIMARY BILARY CIRRHOSIS AND BIOCHEMICAL RESPONSE TO URSODEOXYCHOLIC ACID

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Background and Aims: Transient elastography (Fibroscan) is a non-invasive procedure for assessment of liver fibrosis and portal hypertension evaluation in liver diseases and estimation of fibrosis and histological stage in primary biliary cirrhosis (PBC) as well. Therapeutic response to ursodeoxycholic acid (UDCA) in PBC is defined using different biochemical criteria such as decrease >40% or normalization of alkaline phosphatase after one year of treatment (Barcelona criteria). However, it is unknown whether these biochemical changes are associated with a lack of disease progression. Therefore, this study evaluates changes in liver stiffness after a long-lasting period of UDCA treatment based.

Patients and Methods: 112 PBC patients treated with UDCA who underwent liver elastography (Fibroscan, Echosens) and it was repeated after 6.4±0.1 years. Patients were divided into responders and non-responder according to the Barcelona’s criteria. Changes in cholestasis, development of complications of portal hypertension and whether patients died or were transplanted were recorded as well.

Results: 78 patients (69.5%) had good biochemical response to UDCA. These patients had lower indices of cholestasis and baseline liver stiffness than non-responders. Moreover, there was a significant improvement in liver stiffness in the responder group (from 6.69±0.30 to 6.07±0.27 kPa, p < 0.005), while in the non-responder group there was an increase in liver stiffness (from 8.98±1.1 to 10.4±2.35 kPa, p > ns). These changes paralleled those observed in alkaline phosphatase, which decreased in non-responders (from 439±46 to 425±39 UL/L, p > ns). Three and six responders and non-responders, respectively had had liver stiffness levels above 13 kPa, level which is associated with severe fibrosis or cirrhosis. Two non-responders were transplanted.

Conclusions: Patients with good biochemical response to ursodeoxycholic acid decrease liver stiffness in parallel with the improvement of cholestasis, suggesting that transient elastography is a good procedure for assessing the course and response to therapy in primary biliary cirrhosis.

THE PROGNOSTIC ROLE OF MAGNETIC RESONANCE CHOLANGIOGRAPHY IN PRIMARY SCLEROSING CHOLANGITIS (PSC)

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Background: Magnetic resonance cholangiography (MRC) has become the diagnostic imaging modality of choice when PSC is suspected. The prognosis of this disease, however, is difficult to be determined, considering the survival variability due to the recurrence of complications and the loss of an effective medical therapy.

Aim: To evaluate the prognostic role of MRC in patients with PSC.

Methods: 20 consecutive patients with ERCP-proved PSC (10 males, 10 females; median age 40 yrs, range 23–73 yrs) underwent MRC at different intervals during the follow-up. The prognostic value of MRC was assessed adapting the original validated Amsterdam classification adopted for ERCP (1). MRC imagings were scored from 1 (no abnormalities) to 5, based on the severity of biliary strictures and dilatations, both intra- and extrahepatic. The MRC scores were correlated with: liver function tests, the Mayo risk score, the occurrence of clinical events (bacterial cholangitis, pancreatitis, cancer, major strictures requiring an endoscopic/surgical treatment).

Results: The median follow-up was 103 months. According to the overall imaging score patients were divided in two groups: MRC stage ≤3 (9 patients, 45%), MRC stage >3 (11 patients, 55%). Patients included in the latter group had a significant lower age at the time of diagnosis than those with MRC score ≤3 (39 vs 25yrs). No significant correlation was observed between MRC score and: the occurrence of bacterial cholangitis (22.2% vs 36.4%, p=n.s.), the development of pancreatitis/cancer/major strictures (55.6% vs 27.3%, p=n.s.) and LFTs, except for AST levels at 10 years from diagnosis (0.7 vs 2.1 ULN, p < 0.05). Logistic regression analysis showed a strong correlation between the MRC stage and Mayo score prognostic index (p < 0.05).

Conclusions: MRC offers a good performance for prognostic purposes in PSC. Therefore, its validation is warranted in a larger cohort of PSC patients.

A FREQUENT PNPLA3 VARIANT IS A SEX SPECIFIC DISEASE MODIFIER IN PSC PATIENTS WITH BILE DUCT STENOSIS

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Background and Aims: Primary sclerosing cholangitis predominately affects males and is an important indication for liver transplantation. The rs738409 variant (I148M) of the PNPLA3 gene is associated with alcoholic and non-alcoholic liver disease and we evaluated its impact on the disease course of PSC.

Methods: The I148M polymorphism was genotyped in 121 German PSC patients of a long-term prospective cohort and 347 Norwegian PSC patients. PNPLA3 gene expression was performed in cholangiocyte cell lines and biliary tract tissues.

Results: In the prospective German cohort, actuarial survival free of liver transplantation was significantly reduced for I148M carriers (p = 0.011) compared to wildtype patients. This effect was restricted to patients with severe disease, as defined by development of dominant stenosis (DS) requiring endoscopic intervention. Patients with a DS showed markedly decreased survival (p = 0.004) when carrying the I148M variant (13.8 years; 95% confidence interval: 11.6–16.0) compared to wildtype patients (18.6 years; 95% confidence interval: 16.3–20.9) while there was no impact on survival in patients without a DS (p = 0.87). Furthermore, we observed a sex specific impact of the I148M polymorphism, since the effect on survival was further restricted to male patients (mean survival 11.9 years; 95% confidence interval: 10.0–14.0 in I148M carriers vs. 18.8 years; 95% confidence interval: 16.2–21.5 in wildtype; p < 0.001) while female patients were unaffected by the polymorphism (p = 0.65). These sex specific findings were validated in a second, independent Norwegian cohort showing markedly reduced survival for male I148M carriers (mean survival 7.8 years; 95% confidence interval: 4.9–10.6 vs. mean 13.4 years; 95% confidence interval: 6.9–19.9 in male wildtype; p = 0.013) while not affecting female patients. Using RT-PCR we were able to detect
PNPLA3 gene expression in the biliary system of humans, mice and cholangiocyte cell culture lines.

Conclusions: By following a sexual dimorphism, the common I148M variant of the PNPLA3 gene is a risk factor for reduced survival in male PSC patients with bile duct stenosis requiring intervention.

933 LIVER X RECEPTOR β CONTROLS HEPATIC EXPRESSION OF AQUaporIN-1 AND AQUaporIN-4 AND A GENDER-SPECIFIC, CAVEOLIN-DEPENDENT CELLULAR LOCALIZATION OF AQUaporIN-4

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Introduction: Aquaporins (AQPs) are a family of channel proteins that facilitate the osmotic movement of water and small solutes across biological membranes. In the hepatobiliary tract, AQPs participate in formation and flow of bile. We have previously shown that Liver X Receptor β (LXRβ), a nuclear receptor activated by oxysterols, controls the expression of AQP-1 in pancreas and kidney.

Aim and Methods: In the present study we aimed to investigate the role of LXRβ in controlling water channel homeostasis in the hepatobiliary tract, in-vivo. Both male and female WT and LXRβ−/− mice were studied at the age of 12 months.

Results: In WT mice, LXRβ protein was strongly expressed in the nuclei of intrahepatic cholangiocytes while a weak expression was detected in hepatocytes. Male LXRβ−/− mice demonstrated a significant increase of serum levels of both total bilirubin and alkaline phosphatase, indicating the presence of a mild cholestasis. In the whole liver, there was a significant reduction in the levels of both the mRNA of AQP-1 and AQP-4 in male LXRβ−/− mice. Interestingly, AQP-4 protein expression was detected on the plasma membrane of male WT hepatocytes while in LXRβ−/− mice, the immunoreactivity was identified in the cytosol, suggesting a possible defect in its transport to the membrane. No AQP-4 was detected in female mice either WT or LXRβ−/−.

In the kidney, AQP-2 trafficking is controlled by caveolin-1, a major component of caveolae, that is involved in transcytosis, endocytosis and signal transduction. In prostate cancer cells, caveolin-1 is upregulated by androgens and acts as Androgen Receptor (AR) co-regulator being involved in non-genomic effects of AR. In LXRβ−/− male mice, strong immunoreactivity of caveolin-1 was detected in the cytosol while in WT mice it was on the cell surface. Thus there is impaired translocation of caveolin-1 in the mutant mice. Interestingly, in LXRβ−/− male mice, serum testosterone levels were in normal range.

Conclusion: LXRβ regulates the level of AQP-1 and AQP-4 mRNA as well as the cellular localization of AQP-4, via caveolin-1, in male mice.

934 DEFFECTIVE CD39 EXPRESSION BY Tregs IN AUTOIMMUNE HEPATITIS PREVENTS SUPPRESSION OF IL17 PRODUCTION AND IS ASSOCIATED WITH IMPAIRED ATP-HYDROLYSIS

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Background and Aims: Autoimmune hepatitis (AIH) is an inflammatory liver disease associated with both numerical and functional CD4posCD25high regulatory T-cell (Treg) defects. Tregs express the ectonucleotidase CD39, which initiates an adenosine tri/diphosphate (ATP/ADP) hydrolysis cascade culminating in the production of the immunomodulatory nucleoside adenosine. CD39 defects have previously been linked to human autoimmune disease. We have found a reduced frequency of CD4posCD25highCD39pos Tregs in AIH compared to healthy subjects (HS) but the functional implications of this observation remain unknown.

Methods: We studied 10 AIH type-1 patients (8 female, median age: 13 years) and 6 healthy subjects (HS; 2 female, median age: 29 years). ATP hydrolysis by immunomagnetically purified CD4posCD25high and CD4posCD25low cells was assessed by quantifying the concentration of free phosphate – the bi-product of ATP hydrolysis – after ATP addition. CD39 expression was assessed by flow cytometry. CD4posCD25high, CD4posCD25highCD39pos and CD4posCD25highCD39pos reporter Tregs and CD4posCD25pos responder cells were isolated by fluorescence-activated cell sorting. The ability to suppress proliferation was assessed after 72 hours by thymidine incorporation. Suppression of IL17 production was ascertained by intracellular cytokine staining.

Results: CD4posCD25pos cells from AIH patients were less able to hydrolyse exogenous ATP compared to HS (phosphate concentration: 7.79±3.03 versus 70.46±19.69μM, p = 0.05). In HS, CD25pos cells generated greater concentrations of phosphate compared to CD25pos cells (0.83±0.72 versus 70.46±19.69μM, p = 0.01), and this was reflected by higher CD39 expression on a population (3.63±0.40 versus 24.53±4.61, p = 0.002) and per-cell basis (2239±147 versus 2901±213, p = 0.06). Percent suppression of proliferation by CD4posCD25high and CD39pos Tregs from AIH patients was lower than in HS (CD4posCD25high, 10.97±3.16 versus 41.86±11.48, p = 0.04; CD39pos: −11.5±8.07 versus 40.33±25.43, p = 0.02). CD39pos Tregs were poor suppressors of proliferation in both AIH and health (−2.54±2.55 versus 9.22±8.68, p=NS) although they were potent suppressors of IL17 production. Suppression of IL17 by CD4posCD25high and CD39pos Tregs was weaker in AIH than in health (CD4posCD25high: 29.3±8.3 versus 54.4±8.3, p = 0.04; CD39pos Tregs: 10.3±13.1 versus 53.0±13.3, p = 0.04).

Conclusion: Lower expression of CD39 by Tregs in AIH is associated with reduced ability to hydrolyse ATP and less effective suppression of IL17 production, suggesting that CD39pos Treg defects contribute to impaired immunoregulation in AIH.

935 OVEREXPRESSIO OF CREM ALPHA IN CD4+ T CELLS ACCELERATES ACUTE ConA-INDUCED HEPATITIS BUT DOES NOT INFLUENCE FIBROSIS

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Background and Aims: The cAMP response element modulator CREM is overexpressed in T cells of patients with autoimmune diseases such as systemic lupus erythematosus (SLE). In SLE T cells produce decreased amounts of IL-2 and increased amounts of IL-17. Similarly, mice overexpressing CREMα specifically in T cells (CD2-Cremα) also show enhanced IL-17, decreased IL-2 production and T cell proliferation, leading to accelerated contact dermatitis compared to wildtype (wt) mice. Based on the involvement of IL-17 producing T cells (Th17 cells) for autoimmune hepatitis, we investigated in this study the functional relevance of CREMα in immune-mediated hepatitis and liver fibrosis.

Methods: Wildtype (wt) and CD2-Crem-overexpressing (CD2-Cremα) mice were subjected to Concanavalin A (ConA)-induced hepatitis or chronic liver fibrosis by repetitive carbon tetrachloride (CCL4) injections. Liver damage, inflammation and fibrosis were evaluated by histological analysis. Our study also evaluated the functionality of CREMα in T cells in terms of their ability to activate the RORγt+Th17 cell axis.

Conclusion: The autoinflammatory response in CD2-Cremα mice has a significant impact on liver damage and fibrosis development. CD2-Cremα mice showed a higher degree of liver damage compared to the wildtype controls. The Th17 cells from CD2-Cremα mice expressed higher levels of CREMα, which correlated with an increased expression of pro-inflammatory cytokines such as IL-17 and TNF-α. This suggests that CREMα overexpression in Th17 cells may be a key contributor to the accelerated autoimmune hepatitis and fibrosis observed in CD2-Cremα mice.

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development were assessed by biochemical methods, histology, flow cytometry and qPCR.

**Results:** Upon ConA treatment CD2-Crem<sup>−/−</sup> mice showed elevated transaminase levels and increased mortality rate compared to wt mice. In contrast, chronic injury and fibrosis development upon CCl<sub>4</sub> were comparable between wt and CD2-Crem<sup>−/−</sup> mice. In both models, CD2-Crem<sup>−/−</sup> mice displayed less infiltration of immune cells to the liver, while immune cell numbers in the spleen remained similar to wt mice. Crem<sup>+</sup> expression in hepatic and splenic CD4 T cells decreased after ConA or CCl<sub>4</sub> treatment, along with increased IL-2 and IL-17 expression. Interestingly, Crem-overexpressing CD4 T cells from the liver showed higher levels of IL-2 and lower levels of IL-17 compared to wt T cells, while in splenic CD4 T cells expression of these cytokines did not differ between both strains. We could not detect differences in CD4 T cell differentiation or activation between CD2-Crem<sup>−/−</sup> and wt mice. Reduced hepatic T cell numbers in CD2-Crem<sup>−/−</sup> mice was not caused by overactivation and cell death through higher levels of IL-2, because pretreatment of wt mice with IL-2 did not accelerate ConA-hepatitis.

**Conclusions:** Overexpression of CREM in CD2<sup>+</sup> T-cells enhances acute immune-mediated hepatitis but does not influence fibrosis. CREM<sup>+</sup>-overexpression does not induce a predominant Th17 response in intrahepatic T-cells, thus suggesting compartmental differences of T cell activation pathways between liver and other organs in autoimmunity.

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**VITAMIN D MODULATES BILIARY FIBROSIS IN ABCB4 DEFICIENT MICE**

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**Background:** Vitamin D deficiency is common in chronic liver diseases. Especially impaired vitamin D-VDR signalling seems to represent an aggravating factor during liver injury in particular in biliary-type liver diseases (Chignard et al. 2012). To assess the effect of vitamin D on biliary fibrosis, we treated ATP-binding cassette transporter knockout (Abcb4<sup>−/−</sup>) mice an established model of biliary fibrosis with 25-hydroxyvitamin D (vitD).

**Methods:** Abcb4<sup>−/−</sup> mice and wild-type mice were fed a vitD supplemented diet (2400 IE vitD/kg food), a deficient- (100IE/kg) or a control- (600IE/kg) diet for 12 weeks. Serum vitD concentrations were measured by chemiluminescence immunoassays. Liver injury was determined by liver enzyme activities (ALT, AP), histopathological stages of fibrosis and hepatic collagen contents. Steady-state mRNA expression levels of ColIa2, Timp1, Tgfβ and Camp were analyzed by quantitative RT-PCR.

**Results:** Serum vitD levels depend on genotype and diet. Abcb4<sup>−/−</sup> mice on control diet display lower vitD concentrations as compared to wild-type mice (38.4±1.9 vs. 46.8±1.7 ng/ml; p<0.01), whereas after vitD supplementation Abcb4<sup>−/−</sup> animals demonstrate higher levels in comparison to wild-type mice (67.2±2.4 vs. 42.0±2.3 ng/ml; p<0.001). Fibrosis stages differ significantly between Abcb4<sup>−/−</sup> animals fed different diets, while hepatic collagen contents in wild-type mice are not affected by diet. Abcb4<sup>−/−</sup> mice receiving vitD-deficient diet develop significantly higher fibrosis stages (F-score 2.8±0.1) as compared to knockout animals fed control or vitD-sufficient diets (F-scores 1.5±0.2 and 2.1±0.1). Additionally, we detected higher (p<0.05) collagen contents in Abcb4<sup>−/−</sup> mice fed the vitD-deficient diet (351±22 μg hydroxyproline/g liver) as compared to mice on control diet (294±15 μg/g); the lowest collagen contents were observed after vitD supplementation (275±4 μg/g). Furthermore, vitD deficient Abcb4<sup>−/−</sup> mice reveal reduced (p<0.05) expression of Timp1 and Tgfβ in comparison to Abcb4<sup>−/−</sup> animals on regular diet.

**Conclusions:** Liver fibrosis severity in Abcb4<sup>−/−</sup> mice depends on vitD status. Our findings indicate that vitD modulate biliary injury and fibrogenesis in vivo. We speculate that adequate vitD intake might have antifibrotic effects in patients with chronic liver diseases.

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**URSOLIC ACID MINIMIZES CHOLESTATIC LIVER INJURY IN COMMON BILE DUCT-LIGATED RATS**

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**Background:** Extended cholestasis of any etiologies often results in therapeutic difficulties. Recently, we have reported that ursolic acid (UA), a pentacyclic triterpenoid of herbal plants origin, ameliorates chemical-induced hepatic fibrosis through induction of apoptosis in hepatic stellate cells (HSCs). In this study, we evaluated the effect of UA on cholestatic liver injury caused by bile duct ligation (BDL) in the rat.

**Methods:** Male, 8-week-old Wistar rats underwent triple ligations of common bile duct under light ether anesthesia, and maintained for 2 weeks after operation. Rats were then given repeated intraperitoneal injections of UA (50 mg/kg body weight, 3 times/week) for 1 week. Serum levels of liver enzymes, total bilirubin and bile acid were measured colorimetrically, and liver pathology was evaluated by H-E staining. Hepatic mRNA levels for multi-drug resistance-associated protein (MRP)-2, bile salt exporting pump (BSEP), Na+-taurocholate cotransporting polypeptide (NTCP), and farnesoid X receptor (FXR), were analyzed by real-time RT-PCR.

**Results:** Rats given BDL operation showed remarkable increases in serum aminotransferases and alkaline phosphatase levels, in combination with elevated total bilirubin and bile acid levels, the values being significantly blunted when UA was given 3 times for the third week after BDL. BDL induced pathological changes in the liver comprising enlargement of portal area with marked proliferation of small bile ducts and fibrotic changes expanding from perportal area, all of which were remarkably ameliorated in UA-treated animals. Hepatic mRNA levels for MRP-2, BSEP, NTCP, and FXR were decreased in BDL-operated rats, the levels being around 70% of sham-operated rats. Interestingly, the decreases in all of these parameters were completely recovered when rats were treated with UA. Moreover, a single injection of UA at 2 weeks after BDL almost doubled urinary excretion levels of bilirubin.

**Conclusions:** UA obviously minimizes cholestatic liver injury caused by BDL. The mechanisms underlying the protective effect of UA most likely involve induction of FXR and anion/bile salt transporters in the liver, which in part enhances urinary excretion of bilirubin, in combination with anti-fibrotic effect through induction of apoptosis in HSCs. In conclusion, UA appears to be a promising therapeutic reagent for cholestatic liver diseases.

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**NATURAL HISTORY OF SCLEROSING CHOLANGITIS IN CRITICALLY ILL PATIENTS (SC-CIP): PREDICTORS AND OUTCOME**

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Sclerosing cholangitis in critically ill patients (SC-CIP) with sepsis and acute respiratory distress syndrome (ARDS) is a cholestatic liver disease with a rapid progression to liver cirrhosis and hepatic
failure. It is initiated by an ischemic injury to the biliary tree with subsequent stenoses of biliary ducts, biliary casts and infections, often with multi-resistant bacteria. Until now, little is known about the outcome of patients with SC-CIP.

Patients and Methods: Medical records of 52 patients (f=7, m=45) with SC-CIP were evaluated (between 2002 and 2010). The following parameters were analyzed: Cause of sepsis, duration of mechanical ventilation, duration of ICU treatment, age at diagnosis of SC-CIP, microbiology results, time point of diagnosis by ERCP, survival data, cause of death, and other parameters.

Results: In all patients SC-CIP was diagnosed by ERCP. SC-CIP was diagnosed within 3.6±1.8 months after begin of ICU treatment. The age at SC-CIP diagnosis was 56±15 years. Causes of sepsis were polytrauma (35%), post-operative complications (25%) or internal comorbidities (40%). Mean follow-up was 20±25 months. Twenty-eight of 52 patients died already, 21 of 28 patients within the first 6 months after diagnosis. The 1- and 3-year survival rates of all patients were 51% and 41%, respectively. Seventeen of 52 patients underwent liver transplantation (LT). The 1- and 3-year survival rate of the LT-patients was 68% and 61%, respectively, while the 1- and 3-year survival rate of the non-LT patients was 42% and 32%, respectively.

Conclusions: In patients with sepsis, long-term ICU therapy and hyperbilirubinemia SC-CIP must be kept in mind. Gold standard for diagnosis of SC-CIP is the ERCP. The prognosis of SC-CIP is unfavorable. LT is the only curative treatment of SC-CIP and is associated with a good outcome. Therefore, the option of LT should be evaluated in all patients with SC-CIP.

939 WHO MAY HAVE TREATMENT BENEFITS WITH FIBRATES IN PRIMARY BILIARY CIRRHOSIS: A SINGLE CENTER RETROSPECTIVE OBSERVATIONAL COHORT ANALYSIS

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Objectives: Fibrates, peroxisome-proliferator activator receptor alpha agonists, have been shown to improve liver biochemistry in primary biliary cirrhosis (PBC). Nevertheless, their long-term safety and efficacy, especially in the patients who have inadequate response to ursodeoxycholic acid (UDCA), is still obscure. The aim of the study was to investigate the characteristics of PBC that might be associated with the treatment response of fibrates.

Methods: Patients with PBC who were prescribed with fibrates at our center from 1991 to 2011 were retrospectively analyzed. Biochemical response (BR) was evaluated by the Paris II criteria, originally a predictive marker of prognosis of early PBC under UDCA (Corprecht C et al, J Hepatol 2011). Normalization of AST, ALT, ALP and total bilirubin was also referred as biological normalization (BN). Histological assessment was performed with Nakanuma’s staging and grading system (Hiramatsu K et al, Histopathology 2006). PBC-autoimmune hepatitis (AIH) overlap syndrome was diagnosed based on the Paris criteria (Poupon R et al, Hepatology 2006).

Results: 33 (21.9%) out of 155 PBC patients were introduced with fibrates; 6 out of 18 PBC-AIH overlap syndromes were included. Inadequate biochemical response was a reason for prescription (75.8%), whereas achieving BN was the other. The median period of time between initial diagnosis and introduction is 9.5 months (range 0–139). The median duration of prescription was 34 months (range 2–116); all but 2 cases were prescribed for longer than 1 year, while discontinuation due to worsening of liver biochemistry was needed in 2 cases within 6 months. 63.8% of patients achieved end of treatment BR by intention-to-treat analysis. Marked hepatitis activity (grade 3s) and advanced progression (stage 4) that was accompanied by severe bile duct loss were significantly associated with lack of BR (p=0.0134 and 0.0033, respectively). Pre-treatment AST and total bilirubin were accordingly higher in non-BR than in BR (p=0.0166 and 0.0065, respectively). Multivariate analysis revealed the stage as a risk factor for nonresponse (OR 6.496; 95%CI, 1.10–31.43, p=0.0387).

Conclusions: Fibrates may be more effective in early stage PBC patients who are refractory to UDCA. Benefits in late stage warrant further evaluation.

940 A FREQUENT PNPLA3 VARIANT IS ASSOCIATED WITH DECREASED PRURITUS IN PBC PATIENTS: NOVEL CHARACTERISTIC OF A PROFIBROGENIC VARIANT

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Background and Aims: The common PNPLA3 (adiponutrin) variant p.148M represents the major genetic determinant of severe non-alcoholic1 and alcoholic liver disease2. According to the latest study3, the PNPLA3 risk variant enhances the conversion of lysophosphatidic acid (LPA) into phosphatidic acid (PA), resulting in increased cellular diacylglycerol synthesis. Of note, LPA has been identified as critical mediator of cholestatic pruritus4. Here, we investigate the association between the PNPLA3 variant and liver phenotype as well as quality of life in patients with primary biliary cirrhosis (PBC).

Methods: We recruited 186 PBC patients (age range 22–83 years, 165 females). All patients fulfilled ESL criteria for the diagnosis of PBC. Liver functions tests and PBC-specific antibodies were determined by standard assays in fasted blood samples. PBC-related symptoms were quantified using the PBC-27 test. The PNPLA3 polymorphism was genotyped using a real time PCR-based assay with TaqMan probes and fluorescence detection.

Results: Overall, 68 patients presented with liver cirrhosis at inclusion. We detected a trend (P=0.06) for an association between liver cirrhosis and PNPLA3 genotypes [IM] and [MM]. The frequency of the PNPLA3 genotypes did not correlate with PBC-specific antibodies or with liver function tests (all P>0.05). Of note, carriers of the gain-of-function allele [M] reported significantly less itching in the PBC-27 test as compared to [II] individuals (3.8±0.5 vs 5.1±0.5, P=0.05).

Conclusions: Our findings demonstrate that the PNPLA3 variant may promote severe hepatic phenotypes in PBC. Conversely, carriers of the risk variant report less itching. We speculate that this novel finding is related to increased LPAAT activity in liver and/or skin, which eventually results in decreased levels of LPA mediating pruritus in chronic cholestasis.

Reference(s)
### POSTERS

**941**

**ALKALINE PHOSPHATASE VALUES ARE A SURROGATE MARKER IN PREDICTION OF TRANSPLANT FREE SURVIVAL IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS – AN INTERNATIONAL, COLLABORATIVE ANALYSIS**


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**Background:** Serum alkaline phosphatase (ALP) values are characteristically elevated in primary biliary cirrhosis (PBC). Various criteria related to ALP (e.g. ALP1.67×ULN) in patients treated with ursodeoxycholic acid (UDCA) have been associated with prognosis, and proposed for risk-stratification and end-points in drug development. We sought to evaluate ALP as a surrogate marker of survival in PBC.

**Methods:** In this investigator-initiated-project individual patient data on clinical characteristics, liver biochemistry and long-term outcomes were collected from 13 centres worldwide and updated to 2012. Cox-regression, stratified by centre and adjusted for age, gender, calendar-time and UDCA use along with a performance-measure (c-statistics) and goodness-of-fit-criteria (Akaike’s-Information-Criteria (AIC)) were used to analyze ALP in relation to transplant-free-survival.

**Results:** Data from 2111 PBC-patients (female: 91%; mean age: 54yrs; UDCA: 89%) were included. Mean follow-up was 7.7±5.7yrs; 427 patients received a liver-transplant or died. 10 year-transplant-free-survival: 76%. A log-linear association of ALP with transplant-free-survival was observed at different time-points (table), independent of bilirubin and UDCA.

ALP≥1.67×ULN was associated with a worse prognosis at entry, 1 and 2 years: (respectively Hazard Ratio (HR) = 1.7; 95%CI (1.3–2.3); p = 5.3x10⁻⁵; 2.3; 95% CI (1.8–2.8); p = 1.0x10⁻⁵; 2.5; 95% CI (1.9–3.3); p < 1.0x10⁻⁵). The c-statistics and the AIC levels of a range of ALP cut offs (1.0, 1.1, ..., until 3.0×ULN) were compared. These were optimal around the 1.67×ULN cut off (c-statistics =0.791).

At 1 year the optimal cut off was 1.8×ULN (c-statistics=0.793), but not significantly different from the 1.67×ULN. ALP remained an independent marker after adding bilirubin to the model (p-interaction=0.45; table).

**Conclusion:** In this large international PBC patient cohort a log-linear association was found between ALP values and transplantation-free-survival; higher values were associated with worse outcome. This was independent of use of UDCA, bilirubin values, follow-up time and across all centres. This study provides strong evidence for ALP as a surrogate marker for prediction of transplantation-free-survival and is logical to use as a clinical trial endpoint.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>At entry</th>
<th>At 1 year of follow up</th>
<th>At 2 years of follow-up</th>
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<tr>
<td>ALP log-linear</td>
<td>HR</td>
<td>1.4</td>
<td>1.2–1.6</td>
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<tr>
<td>ALP≥1.67×ULN</td>
<td>HR</td>
<td>1.5</td>
<td>1.1–2.2</td>
</tr>
<tr>
<td>ALP≥1.67×ULN + bili normal</td>
<td>HR</td>
<td>6.2</td>
<td>3.8–10.2</td>
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<tr>
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<td>HR</td>
<td>7.4</td>
<td>4.3–9.4</td>
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**942**

**RADIOLOGIC COURSE OF PRIMARY SCLEROSING CHOLANGITIS (PSC): ASSESSMENT BY MAGNETIC RESONANCE CHOLANGIOGRAPHY (MRC) AND PREDICTIVE FEATURES OF PROGRESSION**

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**Background:** PSC is a chronic bile duct disease leading to fibrotic biliary strictures and cirrhosis. Endoscopic retrograde cholangiopancreatography has been the gold standard in diagnosing PSC but MRC is now the primary diagnostic modality and is frequently repeated. The radiologic natural history of PSC remains poorly known. The aim of this study was to assess the radiologic course of PSC on MRC and to identify features able to predict progression.

**Methods:** This retrospective single center study included PSC patients with at least two MRC performed in at least one year interval. Two experienced radiologists scored MRC for a number of specifically defined items (strictures, dilatations and contrast enhancement of the common bile duct, left and right hepatic duct and intrahepatic ducts, liver heterogeneity after gadolinium injection, stones, lesions of cystic and Wirsung ducts, gallbladder dilatation, presence of mass, liver dysmophy, portal hypertension). Primary endpoint was overall radiologic progression: aggravation, stability or improvement assessed by radiologist’s judgment on all MRC done during the follow-up. Radiologic items were analyzed by alternating logistic regression.

**Results:** 289 MRC from 64 patients were analyzed (radiologic follow-up duration = 3.8 yrs [1–81], number of MRC per patient = 4 [2–10]) (median [extremes]). Patients characteristics at baseline were: age = 43 [19–66], male = 59%, IBD = 70%, ursodesoxycholic acid treatment in 73%, serum bilirubin= 8 [1–65] mg/l, alkaline phosphatase = 1.4 [0.3–4.6] ULN. The first MRC showed severe extrahepatic duct stenosis in 62.5%, and liver dysmophy in 53%. No patient developed radiologic improvement, 58% had aggravation and 42% had stability. Items associated with radiologic progression in multivariate analysis were liver heterogeneity after gadolinium (p = 0.016) and liver dysmophy (p = 0.0007). Severe bile duct stenosis was not a prognostic factor. A simple score calculated with these two items was significantly associated with progression: p < 0.0001 with AUC of 83% ±4%.

**Conclusion:** A majority of PSC patients develops radiologic aggravation upon MRC over 4 years. A radiologic score based on two parenchymal features (liver heterogeneity and liver dysmophy) can predict radiologic progression. An external validation is needed and the prognostic value should be tested.
Background and Aims: Primary sclerosing cholangitis (PSC) is a chronic liver disease characterised by progressive inflammation, fibrosis and destruction of the bile ducts. The aetiology of PSC remains enigmatic although lymphocytic infiltration in areas of portal damage suggests an autoimmune mediated disease. CD28 is an important T cell co-stimulatory molecule and CD28− T cells have been reported as being involved in several autoimmune diseases. CD28− T cells are able to produce high amounts of IFNγ, resist to apoptosis and show enhanced cytolytic activity. Given this we sought to further characterise CD28− T cells in PSC.

Methods: Immunohistochemistry was used to localise CD4 and CD8 subsets in human liver tissue. Liver-infiltrating mononuclear cells (LIMCs) and peripheral blood mononuclear cells (PBMCs) were isolated from normal individuals and patients with PSC. Flow cytometry was used to determine the frequency and phenotypic characteristics of CD4 and CD8 T cells in association with CD28 expression.

Results: Immunohistochemical analysis revealed high CD4 and CD8 T cell infiltration in the portal areas of human PSC liver, which was much higher when compared to normal donor livers (n = 5). CD4+ and CD8+ T cells in peripheral blood of PSC patients were present at equal frequencies (ratio of CD4:CD8 1:1) whereas in PSC LIMCs an increased frequency of CD8 T cells was observed (ratio 1:1.5). CD4+CD28− T cells were present at a frequency of 5% in normal peripheral blood, 10% in PSC patients' peripheral blood and 52% in human PSC liver. 50% of the CD8+ T cells in normal peripheral blood were CD28−. In PSC patients, in both peripheral blood and liver the frequency of CD8+CD28− T cells was 75% and 71% respectively.

Conclusions: Our results show a 5-fold increase in the frequency of CD4+CD28− T cells in human PSC liver when compared to PSC patients' peripheral blood, and an increased frequency of CD8+CD28− T cells in both peripheral blood and liver of PSC patients. Our data suggest that CD28− T cells may be involved in the pathogenesis of PSC.

944 PHENOTYPIC AND FUNCTIONAL PLASTICITY OF CD4posCD25hiCD127neg AND CD4posCD25hiCD127pos T CELLS IN AUTOIMMUNE HEPATITIS

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Background and Aims: In autoimmune hepatitis (AIH) CD4posCD25hiFoxp3pos regulatory T-cells (T-regs) and CD4posCD25hiCD127pos T helper cells (Th) both contribute to the development of immune-tolerance, are numerically defective and impaired in their ability to control effector cell function. At variance with autoimmune sclerosing cholangitis, AIH is characterized by hypergamamoglobulinaemia, autoantibody seropositivity and interface hepatitis that, in AIH, are associated with bile duct abnormalities. CD4 T-cell immune responses are involved in the pathogenesis of AIH. Whether the extent and the type of CD4 effector immune responses differ between AIH and AISC is unknown.

Aim: To determine the frequency and the phenotype of CD4 effector cells in AIH and AISC.

Methods: We studied 9 patients with ANA/SMA+ AIH (6 females, median age: 13.9 years), 9 with AISC (5 females, median age: 14.3 years), and 10 healthy subjects (6 females, median age: 29.5 years). The frequency and phenotype of T-cells was evaluated by cytofluorimetry. Transcription factor and cytokine profiles were determined by intracellular staining.

Results: The frequency of CD3posCD4pos cells was lower in AISC compared to AIH-1 (p = 0.001) and HS (p = 0.002). The frequency of CD4posCD25posFoxp3pos regulatory T-cells (T-regs) and CD4posCD25hiCD127pos T-helper (Th) cells in AIH-1 and AISC were significantly lower than in HS (AIH-1: p = 0.001; AISC: p = 0.05); in contrast the frequency of CD4posCD25posFoxp3pos regulatory T-cells (T-regs) and CD4posCD25hiCD127pos T-helper (Th) cells in AIH-1 was lower than in AISC (p = 0.05), being in both higher than in HS (AISC: p = 0.005; AIH-1: p = 0.02). The frequency of CD4posCD8pos cells (Th2) did not differ among groups. While the frequency of IFNγpos cells was similar in AIH-1 and AISC, that of IL-17pos cells was lower in the former than the latter (p = 0.05). In AILD, the frequency of CD4posCD8pos cells correlated positively with AST levels (r = 0.54; p = 0.02). In AISC, the frequency of IL-17pos cells correlated positively with the levels of alkaline phosphatase (r = 0.91; p = 0.0007), and gamma-glutamyl-transpeptidase (r = 0.67; p = 0.05).

Conclusion: While Th1 effectors are increased in both AIH and AISC, Th17 cells predominate in the latter. The correlation between Th1 immune responses and AST in both groups of patients suggests...
that IFN-g orchestrates hepatocyte damage, whereas the correlation between Th17 immune responses and biochemical indices of cholestasis in AISC indicates that IL-17 is more likely to be involved in the bile duct damage characteristic of this condition.

946 SHOTGUN PROTEOMICS IDENTIFIES SPECIFIC PROTEIN PROFILES IN APOPTOTIC BODIES FROM BILIARY EPITHELIAL CELLS

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Background and Aims: Primary biliary cirrhosis (PBC) is characterized by an autoimmune reaction against mitochondrial antigens and the destruction on small biliary epithelial cells (BEC). A major unanswered question regarding the pathogenesis of PBC is the specific targeting of the small biliary duct epithelial cell; even thou all nucleated cells have mitochondria, only small BECs are the targets of the autoimmune attack in PBC. We have reported that after apoptosis, human intrahepatic BECs translocate the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) immunologically intact into apoptotic bodies, forming an apotope, that leads to an innate immune activation. The aim of the study was to identify unique proteins that could induce the signature inflammatory cytokine response from PBC.

Methods: We isolate apoptotic bodies from a primary culture of human intrahepatic biliary epithelial cells (HiBEC) (n=4), and from two control primary epithelial cell types: human renal proximal tubular epithelial cells (n=6) and human bronchial epithelial cells (n=6). We then used shotgun proteomics to define the proteome of the apoptotic bodies. The data comparing the hepatic blebs to each of the control groups were individually plugged into a graphical interface software package that reads standard output from protein assemblies and analyzed with a quasi-likelihood Generalized Linear Modeling. All reported proteins were identified with a minimum of 2 distinct peptides. The results were then sorted by a metric (quasi-P) that takes into account both the difference between the two groups and how consistent the values are within that group. Only p<0.05 were considered significant.

Results: Overall analysis identified a total of 40,843 distinct peptides and 6,160 protein groups; 13 proteins were identified to be specifically located within blebs from HiBEC. The pathway analysis of the identified proteins led in NF-kB activation pathway, ERK pathway, Notch signaling pathway, and IL8 and CXCR2-mediated signaling events.

In conclusion, this study determined the proteome of apoptotic bodies of HiBEC and identified 13 specific proteins with a potential pathogenic role in PBC that suggest that they are more than an innocent victim in the pathogenesis of PBC.

947 POSSESSION OF HLA DR3 PREDICTS GOOD RESPONSE TO IMMUNOSUPPRESSIVE TREATMENT IN CHILDREN WITH AUTOIMMUNE LIVER DISEASE

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Background: Childhood autoimmune liver disease (AILD) comprises autoimmune hepatitis type 1 (AIH-1), - type 2 (AIH-2) and autoimmune sclerosing cholangitis (ASC). It has been shown previously that HLA class II genes DRB1*03 (DR3), -07 (DR7) and -13 (DR13) are the predisposition genes for AIH-1, AIH-2 and ASC respectively.

Aim: To define whether in AILD there is an association between HLA predisposition genes and response to immunosuppressice treatment (steroids plus azathioprine).

Patients and Methods: A total of 216 children with AILD were followed up for a median of 9.2 years (range 1 month to 35 years until August 2012). Patients were defined as: 1. responders (rapid and sustained remission); 2. non-responders (no remission leading to liver transplantation/death, or frequent relapsers after remission).

HLA DRB antigens were defined by PCR/sequence specific primers (SSP) using kits obtained from Biotest (Dreieich, Germany).

Results: Overall, 75/87 (86%) DR3 positive patients were responders, compared to 25/49 (51%, p<0.0001) DR7 positive and 26/38 (68%, p<0.05) DR13 positive patients. Amongst 91 patients with AIH-1, the proportion of responders (36/42, 86%) was higher within DR3 positive than DR7 positive patients (12/19, 63%, p=0.046), but similar to that within DR3 positive patients (3/4, 75%, p=NS). Among 49 patients with AIH-2, the proportion of responders tended to be higher within DR3 positive (14/19, 74%) than DR7 positive patients (11/22, 50%, p=0.012), but was similar to that within DR13 positive patients (3/4, 75%, p=NS). Among 76 ASC patients, the proportion of responders was higher (25/26, 96%) within DR3 positive than DR7 positive (4/10, 40%, p<0.0002) and DR13 positive patients (11/19, 58%, p<0.005).

Conclusion: Though possession of at least one HLA DR3 allele is a risk factor for the development of autoimmune liver disease, it also predicts good response to immunosuppression in all three AILD subtypes. In contrast, possession of DR7 in all three subtypes and DR13 in ASC predicts a poor response to immunosuppressice treatment.

948 INCREASING INCIDENCE OF AUTOIMMUNE HEPATITIS


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Introduction: Autoimmune hepatitis is a rare disease, with an estimated annual incidence of nearly 1.9/100,000 inhabitants and a prevalence of 16.9/100,000. Over the past thirty years, an increase in the incidence of autoimmune diseases (Cohn’s disease, type 1 diabetes, multiple sclerosis) in industrialized countries has been reported. The aim of this study was to confirm our clinical feeling of a parallel increase in the incidence of autoimmune hepatitis.

Patients and Methods: A 12 years (2001–2012)-retrospective study has been conducted in our Hepatology department: all patients diagnosed in the department with autoimmune hepatitis, according to the revised International Autoimmune Hepatitis Group, were included.

Results: Between 2001 and 2012, 139 new cases of autoimmune hepatitis have been diagnosed, with a constant increase: 2001–2002: 14, 2003–2004: 17, 2005–2006: 23, 2007–2008: 21, 2009–2010: 33 et 2011-T3.2012: 31 (Figure 1). Over these 139 new cases, 101 (72.7%) were women (with a stable sex ratio over time), with a mean age of diagnosis of 50 years (51 for women and 47.5 for men) and a trend of an older age at diagnosis, particularly during 2011–2012 with a median age at diagnosis of 59 years. The percentage of cirrhosis was 25% and the rate of overlap syndrome was 20.1%. Combined therapy with azathioprine and regressive dose of corticosteroids avoided liver transplantation and controlled hepatitis activity in 95.2% of patients. Most of cirrhosis had biopsy-proven cirrhosis reversal. Another autoimmune disease was observed in 39.8% patients. A comparison of this cohort with the 86 cases of autoimmune hepatitis which were diagnosed before 2001 will allow to determine whether or not clinical
and biochemical features (presentation, prognosis, response to treatment) also evolved over the last 20 years.

**Figure 1.** New cases of autoimmune hepatitis during 2001–2012.

**Conclusion:** Incidence of autoimmune hepatitis is increasing, as other autoimmune diseases in developed countries. The main recognized hypothesis is the ‘hygienist theory’ (improvement of other autoimmune diseases in developed countries. The main hypoglycaemia is the ‘hygienist theory’ (improvement of hygiene leading to a decrease in infections) with few putative non-exclusive mechanisms involving antigenic competition, extension of immune regulation induced by exogenous bacterial antigen or Toll Like Receptors.

### 949 IDENTIFICATION OF TWO CLINICALLY DISTINCT SUBTYPES IN AUTOIMMUNE HEPATITIS BY COMPARATIVE FUNCTIONAL GENOMICS

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Autoimmune hepatitis (AIH) is a heterogeneous inflammatory liver disease of unknown etiology. Recent advances in diagnosis and treatment have effectively reduced the risk of progressive disease in AIH patients, but the exact molecular mechanism(s) generating a pleiotropic spectrum of different phenotypes of the disease remain largely unknown. Here, we used a comparative genomic approach to define the molecular characteristics and to test the predictive value of gene expression profiles in this important liver disease. Gene expression profiles of 124 patients with different chronic liver diseases (AIH, HBV, HCV) were generated from FFPE samples using Illumina DASL assay and computationally integrated with expression data from a T-cell induced mouse model of autoimmune liver disease.

Whole transcriptome analysis of AIH patients and patients with chronic viral hepatitis revealed three distinct clusters of samples with AIH specimens separated in two of these main clusters. A 1743 gene AIH signature was generated with differential activation of functional networks involving immune response, apoptosis and oxidative stress within the two AIH subtypes. Clinically, the signature was associated with response to standard therapy and a distinct pattern of auto-antibodies. Application of comparative functional approach using a murine AIH model further demonstrated high concordance with related molecular pathways specific for human AIH patients. Furthermore, unsupervised cluster analyses revealed that AIH mouse samples were closely linked to the subpopulation of human AIH patients with poor response to standard therapy, indicating that the model could be useful to study molecular alterations of these patients.

In summary, we provide the first in-depth genomic analyses of human AIH patients and defined two AIH subclasses. The AIH gene signature was associated with distinct clinical features and might be relevant for diagnostic and therapeutic interventions. Furthermore, cross-species comparison demonstrated the utility of a mouse model that closely mimics the genomic characteristics of human AIH. These results might be helpful to identify novel biomarkers for diagnosis, allow precise risk stratification and improve existing therapies by targeting relevant genes and pathways in this important liver disease.

### 950 PROGNOSIS OF PRIMARY BILIARY CIRRHOSIS WITH FEATURES OF AUTOIMMUNE HEPATITIS OVERLAP SYNDROME. ROLE OF URSEDOXYCHOLIC ACID IN LONG-TERM SURVIVAL

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**Background and Aims:** The coexistence of primary biliary cirrhosis (PBC) and autoimmune hepatitis is called overlap syndrome (OS). Its diagnosis is challenging and the natural history has not been completely assessed. Therefore, we have investigated the long-term course as well as the response to ursodeoxycholic acid (UDCA) therapy in a cohort of patients with PBC with biochemical features of OS at diagnosis of the disease.

**Patients and Methods:** 785 patients (91.3% females) followed for a mean period of 9.1 years. Biochemical features of OS were defined by baseline alanine amino transferase at least 5 times the upper limit of normal (ULN) and/or serum IgG levels at least 2 ULN. Baseline features of liver disease and biochemistries were assessed as well as the treatment with UDCA and the final outcome (dead or transplantation).

**Results:** 80 patients (10.2%) had features or OS. Patients with OS were younger than patients without OS (46.6±1.3 vs 53.2±0.5 years, p < 0.001). Moreover pruritus, jaundice and more severe disease as indicated by higher bilirubin (4.3±0.7 vs 1.9±0.2 mg/dl, p < 0.001), alkaline phosphatase (1179±94 vs 611±21 U/L, p < 0.001) and gammaglobulin (20.0±1.0 vs 17.5±0.3 g/L, p = 0.01) and IgG, IgA and IgM levels and lower albumin concentration and prothrombin index were observed in OS. Most patients with and without OS (88 and 85%, respectively) were treated with UDCA (13–16mg/kg/d). Nineteen patients with OS (24%) and 108 patients without OS (16%) died of were transplanted (p: n.s.). Survival free of liver transplantation was not significantly different in patients with and without OS, but it was significantly better in patients under UDCA (p < 0.001) regardless of OS features. By contrast, survival was significantly lower in patients with and without OS who were not treated with UDCA (p < 0.001).

**Conclusions:** Biochemical features of overlap syndrome are observed in approximately 10% of patients with PBC. Although the baseline severity and the clinical features of liver disease are worst in patients with OS, the long term survival is related to UDCA therapy but not to the features of overlap syndrome.
POSTERS

951 THE IMPACT OF LIVER TRANSPLANTATION ON THE PHENOTYPE OF PRIMARY BILIARY CIRRHOSIS (PBC) IN THE UK-PBC COHORT
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PBC is the underlying liver disease in up to 10% of patients listed for liver transplantation in the UK, and although effective in improving survival in end-stage liver disease as a result of PBC, the beneficial impact of transplantation on systemic symptoms of PBC such as fatigue is less clear. This study aimed to use the comprehensive UK-PBC cohort, including 383 post-transplant and 2185 non-transplanted patients, to explore the demographic and symptom phenotype differences between transplanted and non-transplanted patients.

A cross-sectional study examining demographic (gender, age at diagnosis, age at study) and treatment (ursodeoxycholic acid[UDCA] treatment and response[Paris criteria], disease years until transplant, immuno-suppression used) data of transplanted and non-transplanted PBC patients from the UK-PBC cohort was undertaken. Transplanted patients were then matched for gender, age at diagnosis, and age at study to a non-transplanted PBC patient for comparison of symptoms assessed in all UK-PBC participants by the following validated measures: PBC-40, Epworth Sleepiness Scale (ESS), Orthostatic Grading Scale (OGS), and Hospital Anxiety and Depression Scale (HADS).

Over 25% of transplanted patients were grafted within 2 years of diagnosis. Transplanted patients were significantly younger at presentation than non-transplanted (mean 7 years). >35% of patients presenting under 50-years had already undergone liver transplantation at the study point, and >50% of those diagnosed under 50-years were classed as treatment failures (post-transplant or unresponsive to UDCA). Systemic symptom severity (fatigue, cognitive symptoms) was identical in matched female post-transplant and non-transplant groups and was unrelated to disease recurrence or immune-suppression type. Non-transplanted males had milder systemic symptoms than females, but post-transplant symptoms worsened in males to match levels experienced by both post- and non-transplanted females.

Conclusion: Age at PBC presentation (<50 years) is a major risk factor for treatment failure and progression to transplantation. Although confirmatory longitudinal studies are required to assess individual symptom variation post-transplantation, we found no evidence of improved systemic symptoms after liver transplantation in PBC (in fact worsening in males) and patients should be advised accordingly. Consideration needs to be given to enhancing rehabilitation approaches to improve these systemic symptoms known to impact on function and life quality after liver transplant in PBC.

952 THE IMPACT OF PRIMARY BILIARY CIRRHOSIS (PBC) ON PERCEIVED QUALITY OF LIFE (QoL): THE UK-PBC NATIONAL STUDY
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PBC is the commonest autoimmune liver disease, and has a complex clinical phenotype. Debate about the extent and specificity of frequently described systemic symptoms such as fatigue has complicated efforts to effectively manage the significant extra-hepatic symptom burden seen in PBC. This study aimed to use a national patient cohort recruited from all clinical centres in the UK, to comprehensively quantify and explore the relationships between a variety of systemic PBC symptoms and assess their impact on perceived QoL.

A cross-sectional cohort of patients recruited to the UK-PBC Cohort, created to carry out the UK-PBC genetics studies, completed the following established and validated symptom assessment tools: PBC-40, Epworth Sleepiness Scale (ESS), Orthostatic Grading Scale (OGS), and Hospital Anxiety and Depression Score-Depression (HADS-D). Participants were also assessed for perceived quality of life impairment and health status. Controls were recruited via a ‘best-friend’ method, with Newcastle UK-PBC patients selecting an age- and sex-matched friend to complete a version of the PBC-40 developed and validated for use in controls, along with all other symptom measures.

2353 PBC patients and 196 controls completed all assessment measures, making this the largest study of clinical expression of PBC. Perception of poor QoL was significant in PBC patients vs controls (35% vs 6%, p < 0.0001), as was perceived impaired health status (46% patients vs 6% controls). Fatigue was the symptom with the greatest impact on QoL, with multivariate analysis highlighting that the additional presence of social dysfunction symptoms increased this impact. Depression was a significant factor, but appeared to be a manifestation of complex multi-symptom burden, rather than a primary event.

Conclusion: The symptom burden in PBC, which is unrelated to liver disease severity or ursodeoxycholic acid treatment response, is complex and results in significant deficits in quality of life compared to a control population. The complex interaction of symptoms, such as the worsening of fatigue impact when accompanied by social dysfunction, highlights that specific approaches to symptom management are warranted which address both symptom biology and factors modifying social impact.
FUT2 POLYMORPHISM IS ASSOCIATED WITH DOMINANT STENOSIS AND CANDIDA INFECTION IN PRIMARY SCLEROSING CHOLANGITIS

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Background and Aims: A recent genome-wide association study (GWAS) identified FUT2 secretor status and genotype defined by the rs601338 single-nucleotide polymorphism (SNP) as a potential risk factor in primary sclerosing cholangitis (PSC) that significantly influences biliary microbial composition. We aimed to analyze influence of this SNP in a well-characterized PSC cohort and report about its impact on clinical and microbial parameters.

Methods: We analyzed 215 PSC patients that were treated at our tertiary care center. ERCS and bile culture of patients were analyzed. During ERC 639 biliary cultures were attained and available for microbial analysis. Clinical data was obtained by chart review.

Results: 69 patients (32.1%) were homozgyous wildtype (GG), 97 (45.1%) patients were heterozogous (AG) and 49 patients (22.8%) were homozygous mutated (AA). Patients with AA genotype developed more often dominant stenosis (40/49; 81.6%) than patients with AG (61/97; 62.8%) or GG genotype (35/69; 50.7%) (p = 0.002). 639 biliary cultures were collected during ERCS of which 596 in 140 patients were also tested for fungal specimen. In biliary cultures of GG patients candida species were less abundant (4/52; 7.7%) compared to heterozygous (18/57; 31.6%) or homozygous (22/57; 39.6%) patients (p = 0.009). Microbiome analysis by culture biliary cultures revealed 54 different bacterial strains with genotype specific changes in distribution depending on FUT2 genotype.

Conclusions: Beside genotype specific changes in microbial bile composition, FUT2 genotype is associated with dominant stenosis, episodes of cholangitis and presence of candida species in PSC. FUT2 genotype is an important genetic risk factor for host-microbial diversity and risk factor for disease progression in PSC.

PRIMARY SCLEROSING CHOLANGITIS WITH FEATURES OF AUTOIMMUNE HEPATITIS: CHARACTERISTICS AT FIRST PRESENTATION AND LONG TERM OUTCOME IN A LARGE, MULTICENTER COHORT FROM GERMANY

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Background and Aims: Primary sclerosing cholangitis (PSC) can present with features of autoimmune hepatitis (AIH). There is no consensus on the indication for immunosuppression and few patients are described with long term follow-up. We therefore aimed to characterize PSC patients who received immunosuppression for their inflammatory type of PSC.

Methods: We retrospectively reviewed clinical records of patients with PSC and immunosuppression given for their liver disease (PSC-IS) from seven tertiary care centers in Germany. This cohort was compared to patients with classical PSC (PSC). A total of 277 patients [PSC-IS 101/PSC 176] were included in the analysis.

Results: PSC-IS patients were significantly younger at presentation (27 vs. 34 years, p < 0.001), but there was no difference in gender distribution (2/3 male). Fewer patients in the PSC-IS group suffered from inflammatory bowel disease (51 vs. 66%, p = 0.004). At the start of treatment with UDCA, patients with PSC-IS had significantly higher aminotransferase activities (ALT: 150 vs. 103 U/L, p = 0.017), IgG levels (18.2 vs. 13.6 g/L, p < 0.001), and more autoantibodies (ANA 64 vs. 43%, p = 0.03, and SMA 32 vs. 16%, p = 0.05). PSC-IS patients, although younger at diagnosis, presented with a higher rate of liver cirrhosis (p < 0.001). After a similar median observation period (8 vs. 7 years), a significantly higher number of patients had developed cirrhosis (64 vs. 33%, p < 0.001), suggesting a more aggressive course of disease in PSC-IS patients. However, mortality (PSC-IS: 8.2 vs. PSC: 6.1%) and rate of liver transplantation (PSC-IS: 19.8 vs. PSC: 19.5%) were similar between both groups. Of note, the risk of developing cholangiocarcinoma was not increased in the PSC-IS group (PSC-IS: 5.1 vs. PSC: 6.1%).

Conclusions: We here present the largest study reported to date on PSC patients with signs of AIH. Typical clinical characteristics of patients who received immunosuppression for their inflammatory type of PSC could be identified, which may help to guide treatment decisions of these rare and difficult to treat patients. Despite the aggressive course of disease, the similar rates of death, liver transplantation and cholangiocarcinoma may argue for some efficacy of immunosuppression in these patients.
Conclusion: We here demonstrate that the protection from autoimmune cholangitis which was observed in male mice was mediated by testosterone. Indeed, testosterone supplementation induced resistance to disease in otherwise susceptible female mice. The increased inflammation observed in female mice was associated with increased IL-17 expression by endogenous CD4 positive T cells which seems to be recruited to the liver via an increased expression of chemokines that recruit T cells to the liver. These results may stimulate future investigations into gender differences observed in autoimmune liver diseases.

956 RISK FACTOR PREDICTIVE OF TRANSPLANT FREE SURVIVAL IN A LARGE COHORT OF PSC PATIENTS IN THE UK
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Background: Primary Sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown aetiology. Risk factors for severe disease remain unknown. We therefore analysed the significance of cholangiographic changes on time to liver transplantation in a large UK PSC cohort.

Methods: All patients were recruited to the UK-PSC Consortium between August 2008 and February 2012. The diagnosis of PSC was confirmed using characteristic ERCP, MRCP or histological changes. All patients completed a descriptive phenotypic questionnaire. A clinical questionnaire was completed by the recruiting physician. Demographic and relevant clinical data were extracted from questionnaires. Patients were classified as having intra-hepatic disease or both intra-hepatic and extra-hepatic (beyond second order ducts) disease.

Results: Data on cholangiographic findings and the duration to transplantation/censor was available for 587 patients who were analyzed. Median age at diagnosis was 45 years (range = 10–85). 62% were male (male to female ratio 1.6:1). 69% (403/587) reported recent cholangiography: 42.5% patients had both intra-hepatic and extra-hepatic disease. 30% of patients with both intra-hepatic and extra-hepatic disease were transplant recipients (74/250), in comparison to 19% of patients with only intra-hepatic disease (63/337). The median duration to transplantation was 17 years and 29 years respectively [log-rank p-value = 0.01; HR = 1.50 (1.08–2.10)] (Figure 1).

Conclusion: Cholangiography (ERCP or MRCP) remains the cornerstone in diagnosis of PSC and allows differentiation between intra-hepatic and extra-hepatic disease. We have shown that patients with both intra-hepatic and extra-hepatic disease have a significantly reduced transplant-free survival in comparison to patients with only intra-hepatic disease. It is possible that patients with extensive bile duct strictureing/disease have more marked cholestasis resulting in persistent inflammation and fibrosis. Well-designed prospective trials are needed to confirm these findings.

957 CALPROTECTIN LEVELS IN BILE ARE ASSOCIATED WITH PRIMARY SCLEROSING CHOLANGITIS AND SEVERITY OF THIS DISEASE
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Background and Aims: Primary sclerosing cholangitis (PSC) is a chronic cholestatic disorder of unknown etiology. PSC is characterized by progressive inflammation and fibrosis of the intra- and extrahepatic bile ducts leading to biliary cirrhosis and eventual liver failure. Markers for disease activity and predictors for clinical endpoints are still missing. Calprotectin, a heterodimeric protein established as a fecal marker of inflammation in patients with inflammatory bowel disease, may also play a role in the inflammation of the bile ducts in patients with PSC. As the action of PSC takes place at the biliary epithelium, we hypothesized that calprotectin in bile is elevated and may serve as a disease marker. Therefore, our aim was to evaluate the diagnostic potential of calprotectin in serum and bile of patients with PSC.

Methods: Calprotectin levels were measured in patients with PSC (n = 56), cholangiocarcinoma complicating PSC (n = 13), cholangiocarcinoma (CC) (n = 30) and choledocholithiasis (n = 38) in serum and bile by enzyme-linked immunosorbent assay. PSC patients were categorized by Mayo risk score (MRS) to characterize the disease severity of each patient.

Results: Median calprotectin level in bile was significantly higher in PSC patients (237 mg/l, interquartile range (IQR) 88–500) in comparison to the other groups (p < 0.05). Patients with cholangiocarcinoma complicating PSC (20 mg/l, IQR 9–120), CC (71 mg/l, IQR 17–142) and choledocholithiasis (33 mg/l, IQR 7–120) displayed lower concentrations. Stratification of PSC patients by MRS showed elevated biliary calprotectin concentrations in the high MRS group (380 mg/ml, IQR 158–1001) in comparison to the low MRS group (121 mg/l, IQR 56–367) (p = 0.45). In contrast, serum calprotectin levels did not differ between the groups and was not associated with disease severity.

Conclusions: Calprotectin in bile is associated with PSC and serves as an inflammatory marker. In addition, patients with advanced disease have higher calprotectin concentrations. Therefore, calprotectin may be an interesting candidate for the assessment of disease activity and therapy monitoring.
Background and Aims: The risk not only of biliary but also of extrhepatic malignancies is increased in patients with primary sclerosing cholangitis (PSC). Furthermore many patients with progressive PSC will require liver transplantation as the only effective treatment for this disease. Since transplant recipients seem to have an increased rate of de novo malignancies, the aim of our study was to better characterize in a large patient cohort with a very long follow-up which extrhepatic malignancies develop in recipients transplanted for PSC.

Methods: We retrospectively collected clinical, surgical and laboratory data, data on inflammatory bowel disease (IBD), on immunosupression, on outcome and on extrhepatic malignancies of 297 patients who underwent LT for PSC in 6 German transplant centers between 01/1990 and 12/2006.

Results: After exclusion of 22 patients with a follow-up of less than 3 months 275 patients remained (68.4% men, mean age 38.1 years, 74.4% with IBD). These patients were transplanted 8.4 years (mean) after initial PSC-diagnosis and were followed for 109.3 months (mean, range 3–266 months). The one-year-, five year- and overall recipient survival was 97.1%, 90.2% and 81.5% respectively.

Methods: Using the multireader Light’s kappa or the Washington intraclass observer reproducibilities.

Conclusions: The comprehensive implementation of existing post-LT- and IBD-surveillance programs however helps to improve the overall quality and robustness of the histopathological results in PBC.

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THE FXR AGONIST GW4064 INCREASES LIVER INJURY LEADING TO AN INHIBITION OF THE TRANSCRIPTIONAL ACTIVATION OF UGT1A GENES IN BILE DUCT LIGATED tgUGT1A WT MICE

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Background and Aims: In obstructive cholestasis (bile duct ligation; BDL) accumulation of toxic bile acids and production of reactive oxygen species (ROS) lead to inflammation and liver injury. Bile acids (via farnesoid X receptor, FXR etc.) are candidate in injury. Bile acids (via farnesoid X receptor, FXR etc.) are candidates in injury. Bile acids (via farnesoid X receptor, FXR etc.) are candidates in injury. Bile acids (via farnesoid X receptor, FXR etc.) are candidates in injury.
for detoxification and elimination by glucuronidation, which is also regulated via the xenobiotic-activated arylhydrocarbon receptor (AhR) and the oxidative stress sensor nuclear factor erythroid 2-related factor 2 (Nrf2). Aim of this study was to elucidate transcriptional UGTIA regulation in vivo during obstructive cholestasis and the effects of exposure to the potentially beneficial FXR agonist GW4064.

Methods: TgUGTIA WT mice were subjected to BDL for 5 days, 4 days intraperitoneal (i.p.) injection of GW4064, or 4 days i.p. GW4064 injection after BDL. Liver histology, serum bilirubin levels and aminotransferase assays (AST, ALT) as well as UGTIA gene expression (TaqMan-PCR in liver, jejunum and colon) were determined. Additionally, hepatic AhR-, Nrf2-, FXR-, IL6- and TNFα-mRNA-expression was quantified.

Results: In BDL/BDL+GW4064 mice, upregulation of hepatic IL6 and TNFα expression (2.7-, 2.4-fold) was observed in addition to significantly increased bilirubin and AST/ALT levels correlating with increased histological injury. While no effect on hepatic Nrf2 expression was seen in BDL/BDL+GW4064 mice, AhR expression in the liver was profoundly reduced. In GW4064 treated mice, UGTIA gene expression was significantly activated in the liver (2–5-fold) and colon (2.4–45-fold). After BDL, hepatic UGTIA genes showed a 2–4-fold increase, contrasting intestinal activation observed only for UGT1A3 (3–7-fold) and UGT1A4 (2–27-fold). In BDL+GW4064 mice, only UGT1A4 (6-fold) and UGT1A6 (1.4-fold) were significantly induced in colon; no further significant induction was observed in liver or jejunum.

Conclusion: Unexpectedly, administration of the putative protective FXR agonist GW4064 during cholestasis resulted in an increased inflammatory response (IL6, TNFα), increased liver injury (ALT, AST, histology) and inhibition of hepatic AhR expression, rather than FXR-mediated UGTIA upregulation. As previously shown (Kalhoff et al. JBC, 2010), transcriptional UGTIA activation requires coactivation of both Nrf2 and AhR, which is impaired by eliminating an AhR response demonstrated in this study. This inhibits a protective UGTIA response. Therefore, the development of more specific candidate substances for the treatment of cholestatic liver diseases is needed.

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EFFECT OF URSODEOXYCHOLIC ACID WITHDRAWAL IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS
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Background and Aims: Recent AASLD Guidelines recommend against the use of ursodeoxycholic acid (UDCA) in adult patients with PSC. In this study we investigated the effect of UDCA withdrawal on liver biochemistry, bile acids (BA) profiles and health-related quality of life (HRQoL) in a group of well characterized subjects with primary sclerosing cholangitis (PSC).

Methods: Twenty-six patients with PSC (16 males/10 females, mean age 33±10.5 years) were included. They have all been treated for a period of at least 12 months with a low dose of UDCA (range 10–15 mg/kg b.w.). Paired blood samples for liver biochemistry and BA were collected one day before UDCA withdrawal and 3 months after. Liquid chromatography coupled with mass spectrometry (LC-MS/MS) was used for the identification and quantitation of 18 plasma BA metabolites. VAS score was applied for the assessment of pruritus and PBC-40 questionnaire for evaluation of HRQoL.

Results: Significant elevation of ALP (from 249U/l to 439U/l, p<0.0001); GGTP (from 301U/l to 656U/l, p<0.0001); ALT (from 81U/l to 133U/l, p<0.005) and total bilirubin (from 1.6mg/dl to 2.4mg/dl, p<0.05) was seen after UDCA withdrawal (all biochemistry data shown as mean). BA analysis has shown, as expected, a significant decrease of UDCA and its taurine/glycine conjugates in BA pool. It also revealed that UDCA discontinuation causes a significant decrease of lithocholate (p=0.001), sulfo-lithocholate (p=0.002), glycolithocholate (p=0.01) but not taurolithocholate (p=0.12). No effect on cholate, chenodeoxycholate, deoxycholate and their taurine/glycine conjugates, hyocholate and hyodeoxycholate concentrations was seen after UDCA withdrawal. Five (19%) patients experienced worsening of their pruritus in VAS score. Six (23%) and eight (31%) reported deterioration in fatigue and social/emotional domain of PBC-40 questionnaire respectively after UDCA withdrawal.

Conclusions: Discontinuation of low-dose UDCA in patients with PSC causes significant deterioration of liver biochemistry. It also affects concentrations of bile acids metabolites causing decrease of lithocholate and its sulf- and glyco-conjugates. Furthermore UDCA discontinuation exerts a negative effect on HRQoL in a proportion of patients with PSC.

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HEPATOCELLULAR CARCINOMA DOES NOT SIGNIFICANTLY CONTRIBUTE TO THE RISK OF HEPATOBILE MALIGNANCY IN CIRRHOTIC PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS
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Background and Aims: Primary sclerosing cholangitis (PSC) is associated with an increased risk of hepatobiliary and gastrointestinal malignancy. There are few studies evaluating the risk of hepatocellular carcinoma (HCC) in PSC cirrhosis and a frequency of HCC in PSC patients of 2% has been suggested. The aim of this study was to determine the risk of HCC in PSC cirrhosis and to compare it to the risk of cholangiocarcinoma (CCA), gall bladder cancer (GBC) and colorectal cancer (CRC).

Methods: We included 509 patients with well defined PSC receiving a regular surveillance program with ultrasound and/or MRI of the liver into this retrospective study. Liver cirrhosis was assumed if ascites, oesophageal varices, a transient elastography value >14kPa or a positive liver histology were present. Survival and risk analyses were performed using the Kaplan–Meier method and the Cox proportional hazard model. As no HCC was observed, the upper limit of the 95% confidence interval (CI) for its instantaneous risk was estimated as the −ln(0.05)/patient-years.

Results: A total of 3954 patient-years were analyzed. 67% of patients were male. In 119 patients (23%) liver cirrhosis was diagnosed at first presentation or during follow-up. Not a single HCC was detected in 245 patient-years with cirrhosis. Therefore the estimated upper limit of the 95% confidence interval for the instantaneous risk of HCC in cirrhotic-stage PSC was 1.2%. CCA (n = 35) was the most frequent malignancy followed by CRC (n = 9) and GBC (n = 3). 20% of CCA developed within the first year of diagnosis. Excluding these cases the cumulated risk of CCA after 10 and 20 years was 4.7% and 14.4%. The development of CCA was independent from sex and smoking and there was a trend towards a lower risk for CCA of patients treated with azathioprine (HR 0.16, 95% CI 0.02–1.17, p = 0.07, versus no azathioprine). Median transplantation-free survival in the whole cohort was 15 years (95% CI 13.7–16.3 years).

Conclusions: Whereas we here confirm that the risk of hepatobiliary malignancy is high in PSC, the risk of HCC in PSC cirrhosis was low. A surveillance strategy for HCC in PSC cirrhosis may not be warranted.
963 SORTILIN-DEFICIENT HEPATOCYTES ARE LESS SUSCEPTIBLE TO BILE ACIDS-INDUCED INFLAMMATION AND APOPTOSIS
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Background and Aims: Sortilin is a trafficking molecule directing newly synthesized molecules from the trans-Golgi network to secretory pathways or cell surface. One of the molecules trafficked by sortilin is acidic sphingomyelinase (ASMase), whose product ceramide participates in apoptosis signaling. We hypothesized due to less production of ceramide during liver injury, hepatocytes from sortilin−/− mice would be less sensitive to apoptosis. We have investigated liver injury, apoptosis and necrosis, inflammation and fibrosis in a bile duct ligation model in wild type (WT) and sortilin−/− mice.

Methods: We have assessed liver damage and fibrosis 7 days after BDL using WT and sortilin−/− mice. The effect of bile acids on hepatocyte apoptosis and expression of inflammatory cytokines was determined in vitro in primary hepatocytes from WT and sortilin−/− mice.

Results: Sortilin−/− mice displayed reduced liver damage compared to WT mice, as determined by lower serum AST, ALT, alkaline phosphatase and bilirubin. Sortilin−/− mice also had fewer areas of hepatocyte necrosis that result from bile infarcts, reduced hepatocyte apoptosis compared to WT mice, as well as reduced mRNA levels of collagen I. We determined the in vitro effect of bile acids on hepatocyte apoptosis and expression of pro-inflammatory cytokines and observed strong reduction of apoptosis and inflammatory cytokines in sortilin-deficient hepatocytes.

Conclusion: Sortilin−/− mice have attenuated fibrosis and liver damage after BDL, due in part to reduced hepatocyte apoptosis and reduced inflammatory cytokines.

14. EU PUBLIC HEALTH

964 IMPLEMENTING EASL & AASLD GUIDELINES FOR HEPATOCELLULAR CARCINOMA SCREENING IS COST-EFFECTIVE IN LONDON’S ETHNICALLY DIVERSE POPULATION
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Introduction: Recent guidance from the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Disease (AASLD) recommends screening for hepatocellular cancer (HCC) in all cirrhotic patients and in certain non-cirrhotic HBV-infected patients. EASL do not specify ethnicity, whereas AASLD recommends surveillance in selected Black and Asian patients although these broad racial terms are not defined. The Royal London Hospital serves one of the most ethnically-diverse communities in the UK. We sought to assess the impact of introducing society guidelines into practice by auditing HCC surveillance in our Unit.

Method: The Electronic Patient Records (EPR) were reviewed for all patients who attended the Royal London Hospital Hepatology Clinics in one month. Demographic and clinicopathological data were used to identify patients in whom surveillance was recommended, using the most inclusive interpretation of ethnicity.

The EPR was used to identify physicians’ intention to survey, the nature of surveillance and reasons for non-surveillance.

Results: 425 patients (70% non-Caucasian) were seen in August 2011; 160 (38%) were eligible for surveillance. 64/160 (40%) were non-cirrhotic HBV-infected patients (43 Black and 21 Asian). An intention to survey for hepatoma was evident in the EPR in all but 2 eligible patients (99%). 18 patients (11%) did not attend a follow-up appointment in the 12 months following the index encounter. 121/140 patients (86%) received ultrasound-based surveillance and the remainder were tested for alphafetoprotein but did not attend ultrasound appointments.

In 2011, 19 cases of HCC were diagnosed through our surveillance programme. Based on these data, the estimated cost of surveillance in our unit is £355,000; or £18,700 per HCC identified.

Discussion: The recent UK Chief Medical Officer’s Report highlights the rising burden of liver disease and in particular HCC. This study highlights the cost-effectiveness of HCC screening, even in an ethnically-diverse, young and mobile population such as ours. Attendance at clinic and radiology appointments is clearly essential in delivering cancer surveillance.

14. EU PUBLIC HEALTH
COMMUNITY TRANSMISSION OF HEPATITIS C VIRUS AMONG EGYPTIANS: ANALYSIS OF A 10 YEARS CASE–CONTROL STUDY


Background and Aims: Egypt has the highest prevalence of hepatitis C worldwide, with 10% of 15–59 years chronically infected. Previous studies have identified several iatrogenic (eg invasive medical procedures) and community (eg injecting drug use) risk factors associated with past and current hepatitis C virus (HCV) transmission in Egypt. There are though numerous cases, especially among young people, for which no risk factor has been identified yet. The aim of this study was to identify the current risk factors for HCV transmission among Egyptians.

Methods: A 1:1 matched case control study was conducted in four fever hospitals in Egypt (2 in Greater Cairo, 1 in Alexandria and 1 in Assiut). Case patients were incident acute symptomatic hepatitis C. Control subjects were acute hepatitis A identified at the same hospitals and matched to cases on age (±1 year) and gender. A questionnaire sheet was designed to cover iatrogenic, community and household exposures to HCV in the six months prior to onset of symptoms. Conditional logistic regression was used to identify risk factors associated with acute hepatitis C.

Results: From April 2002 to May 2012, 321 HCV patients and 653 controls were recruited, out of which 190 cases and 190 controls were included in this study. Independent risk factors associated with acute HCV infection were illiteracy (Odds ratio (OR)=3.6, 95% Confidence interval (CI)=1.6–7.8), recent marriage (<1 year) (OR=4.0, 95% CI=1.0–15.7), intravenous infusion (OR=3.3, 95% CI=1.0–10.1), sutures (OR=4.0, 95% CI=1.4–11.6), shaving at barbers (OR=4.7, 95% CI=1.5–14.6), injecting drug use (IDU) (OR=8.4, 95% CI=2.4–29.2) and multiple sexual relations (OR=7.7, 95% CI=1.2–49.6).

Conclusion: This study highlighted that additionally to HCV transmission in healthcare settings through invasive medical procedures, there is on-going HCV transmission in the community, whether as a result of recent marriage, shaving at the barber, injecting drug use or having multiple sexual relations, and this was more common among illiterates. Health promotion campaigns should be carried out to increase awareness about community transmission of HCV.

Background and Aims: In Germany, antenatal hepatitis B antigen (HBsAg) screening is mandatory from 32 weeks of gestation onwards. Antiviral treatment has recently been suggested as a possible intervention to further reduce the risk of mother to child transmission of hepatitis B virus (HBV) in highly viremic women. To estimate the number of pregnant women in Germany who might be eligible for such an intervention we describe herein hepatitis B surface antigen (HBsAg) positivity rates and HBV DNA levels detected during routine antenatal care.

Methods: Between April 2011 and October 2012, HBsAg and – if positive – additional serum markers of HBV infection (HBeAg, anti-HBc, anti-HBe) were determined in 41,942 serum samples from pregnant women using enzyme immunoassays according to the manufacturer’s instructions (DiaSorin S.P.A.). Additionally, in HBsAg positive cases HBV DNA levels were assessed by COBAS TaqMan HBV Test (Roche Diagnostics). Age and migration background were also documented.

Results: HBsAg was detected in 258 women. Thus, the rate of HBsAg-positive women in our antenatal population was 0.615%. Viral load was determined in 183 HBsAg-positive women of which 17 had HBV DNA levels >105 IU/ml. Thus, HBV DNA levels >105 IU/ml were detected in 9.28% of HBsAg-positive pregnant women. 70.6% of the cases with a high viral load >105 IU/ml were determined HBeAg-positive whereas in the group of viral load <105 IU/ml the HBeAg positivity rate was considerably lower (<5%). Median gestational age at testing was 34 weeks, median maternal age in HBsAg-positive as well as in HBsAg-negative pregnant women was 31 years. Migration background was assigned in 74% of HBsAg-positive pregnant women.

Conclusions: We estimate from our present data that about 400 of 700,000 pregnant women per year would be eligible for prophylactic antiviral treatment in Germany due to high viral load. Women with migration background are overrepresented in the population of HBsAg-positive pregnant women. Therefore, targeted HBsAg testing of risk groups should be considered earlier in pregnancy than the general HBsAg screening as currently recommended by the German Maternity Directives of the Joint Federal Committee.
INCIDENCE AND ROUTES OF TRANSMISSION OF HEPATITIS B VIRUS IN FRANCE, 2003–2011

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Background: In the French adult population, an estimated proportion of 0.65% of HBs Antigen (HBsAg) carriers, of whom 55% ignore their status, constitutes Hepatitis B virus (HBV) reservoir. In addition, anti-HBV vaccination coverage is low (<60% in children aged two years in 2009) although vaccination has been recommended for toddlers and pre-teens since 1995. Vaccination is also targeted to populations at-risk of HBV infection. To assess the impact of vaccination strategy, we estimated the incidence and described the modes of transmission of acute HBV infection.

Methods: Acute HBV infection was defined as the first detection of 1) anti-HBc IgM or 2) HBsAg and total anti-HBc with elevated ALT. Chronic HBV infections were excluded.

The modes of transmission were analysed based on mandatory notifications of HBV acute infection during 2003–2011. As the annual number of reported cases was underestimated, we conducted a national survey among a random sample of 1,412 laboratories to estimate the 2010’ incidence of acute infection. Multiple imputation was applied to estimate missing data.

Results: Overall, the estimated incidence of acute infection in 2010 was 2.5 per 100,000 (=1,622 cases), higher in men than in women (3.0 versus 2.1/100,000). It ranged from 1.1/100,000 in the 0–19 to 3.6/100,000 in the 20–39 years age groups. Estimates of total acute infections (symptomatic and asymptomatic) and chronic infections will be presented.

Among the 1,218 notified cases in 2003–2011, 71% were men, older than women (median age: 40 vs. 34 years). At least one HBV at-risk exposure (in the six months preceding the diagnosis) was reported in 63% of cases (multiple sexual partners: 35%, travel to high endemic countries: 31%; men who have sex with men: 21%, HBsAg carrier sex partner: 13%; family exposure: 12%; intravenous drug use: 4%). Among them, at least 53% should have been vaccinated. A fulminant hepatitis was reported in 45 patients (4%), leading to liver transplantation (n=18) and death (=14). Among these 45 cases, at least 56% corresponded to a vaccine indication.

Conclusions: In France, incidence of acute HBV infection is low. However, it should be definitively even lower if vaccine recommendations were better applied.

COST-EFFECTIVE STRATEGY FOR THE DIAGNOSIS OF HEPATITIS C INFECTION WITH VIREMIA: UTILIZATION OF THE HIGH ANTIBODY LEVEL IN LOW PREVALENCE POPULATION

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Background: CDC guidelines recommend confirmatory testing with recombinant immunoblot assay (RIBA) and polymerase chain reaction (PCR) to all individuals with a positive antibody to HCV (anti-HCV).

We propose a new anti-HCV classification strategy in three levels according to the signal-to-cutoff ratio of the anti-HCV value (>20 for high, 4.5–19.9 for low and 4–4.49 for very low), testing with RIBA and RNA when the value is low or high but no further testing when it is very low. Our aim is to determine the most cost-effective strategy for the diagnosis of HCV infection with viremia in low-risk populations (<1%).

Methods: With the information of 47,847 Mexican blood donors previously published (Contreras AM, et al. Salud Publica Mex 2011; 53 suppl I: S13-S18) we performed an economic analysis from the perspective of the company hired by the Mexican Institute of Social Security (IMSS). Mexican pesos (Mex$) were converted to North-American dollars (US$) of the same year using the one-year average exchange rate of Mex$12.629 per dollar. We used the chemiluminescent immunonassay Vitros® to measure the anti-HCV level, and PCR for RNA HCV testing. The strategies were defined as follows:

1. IMSS-1: Three level anti-HCV->RNA ->RIBA or RIBA->RNA (No RIBA or RNA if anti-HCV is very low)
2. IMSS-2: Three level anti-HCV->RNA->RIBA or RIBA->RNA (RIBA or RNA if anti-HCV is very low)
3. IMSS-3: Two level anti-HCV (low ≤19.99, high >20)->RIBA->RNA or RIBA->RNA
4. CDC-1: One level Anti-HCV->RNA->RIBA
5. CDC-2: One level Anti-HCV->RIBA->RNA
6. CDC-3: Two level Anti-HCV (low ≤7.99, high >8)->RNA->RIBA or RIBA->RNA

Results: The strategy IMSS-1 was dominant (Table 1) with an average cost per patient studied of US$312 and an average cost-effectiveness ratio per viremic detected of US$363 compared with the strategy with the highest cost, CDC-1, with US$300 and US$974 respectively.

Conclusion: The strategy IMSS-1 is the most cost effective. The use of this alternative can save significant resources and could improve the diagnosis in low risk population.

Table 1. Cost-effectiveness of strategies for the diagnosis of hepatitis C with viremia in patients with positive anti-HCV

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Cost (US$) per case studied</th>
<th>Effectiveness</th>
<th>Cost-effectiveness ratio (Average (US$)* Incremental</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMSS-1</td>
<td>112</td>
<td>0.3085</td>
<td>363</td>
</tr>
<tr>
<td>IMSS-3</td>
<td>163</td>
<td>0.3085</td>
<td>553</td>
</tr>
<tr>
<td>IMSS-2</td>
<td>171</td>
<td>0.2946</td>
<td>556</td>
</tr>
<tr>
<td>CDC-3</td>
<td>215</td>
<td>0.3064</td>
<td>700</td>
</tr>
<tr>
<td>CDC-2</td>
<td>244</td>
<td>0.3082</td>
<td>790</td>
</tr>
<tr>
<td>CDC-1</td>
<td>300</td>
<td>0.3082</td>
<td>974</td>
</tr>
</tbody>
</table>

The alternatives are ordered from lowest to highest cost per case.
*Cost per patient diagnosed with hepatitis C with viremia.

AWARENESS OF HEPATITIS B AND C SCREENING AND PATIENT MANAGEMENT GUIDELINES AMONG HEALTH PROFESSIONALS IN SIX EUROPEAN COUNTRIES

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Background and Aims: As part of the European project “HepScreen”, the aim of this study is to identify the hepatitis B and C screening and patient management guidelines in place in 6 European countries (Germany, Italy, The Netherlands, United Kingdom, Spain, Hungary) and to assess the awareness of these guidelines among health professionals.

Methods: A systematic search of the scientific and grey literature was done to retrieve hepatitis B and C screening and management
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guidelines. Additionally an online survey was conducted both to identify additional guidelines and to verify whether different groups of health professionals (e.g. those working in public health, antenatal care, primary care, care for asylum seekers/refugees, sexual health, and gastroenterology/hepatology) are aware of these guidelines.

Results: The systematic literature search revealed 10 national guidelines: Scotland (1); the UK (3); Italy (2); Germany (2); and the Netherlands (2). Through the survey, 68 additional hepatitis B/C related guidance documents were retrieved: Germany (16); Spain (9); Hungary (14); Italy (7); the Netherlands (5); the UK (17). Specific professional guidelines were identified by 29% (for HBV) and 21% (for HCV) of GPs, 52% (HBV) and 26% (HCV) of antenatal care providers, zero for both HBV and HCV among those working with asylum seekers/refugees, and 61% (HBV) and 56% (HCV) of specialists. Of national public health officials, 46% mentioned the existence of GP guidelines for HBV and 39% for HCV, and specific guidelines for migrants were mentioned by 23% (HBV) and 14% (HCV).

Conclusions: the significant proportion of health professionals, especially GPs (>70%) and antenatal care providers (48% for HBV), who didn't mention HBV/HCV guidelines may indicate that specific professional guidelines are either lacking or that awareness is low. It is however striking that more public health officials mention specific GP guidelines than GPs themselves. To ensure that screening, management and treatment is consistent with the best available evidence, best practice guidelines for specific professional groups need to be developed. To achieve wide application of best practice guidelines at national and European level, these guidelines need to be tailored to the needs of different professional groups and actively promoted.

971 CHRONIC HEPATITIS B AND C PATIENT MANAGEMENT AND TREATMENT RESTRICTIONS – CURRENT PRACTICE IN SIX EUROPEAN COUNTRIES

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Background and Aims: Literature describing and comparing patient management for chronic hepatitis B/C is very limited. Single European country studies suggest that a significant proportion of positive patients do not receive appropriate specialist care. As part of a European study (HEPscreen) into best practice in screening and care for viral hepatitis among migrant populations, we studied current practice in specialist secondary care in six European countries (UK, Germany, Netherlands, Italy, Hungary and Spain). Methods: A survey was developed using the content of clinical practice guidelines identified by a systematic literature search. Experts representing the views of professionals within their speciality were identified and invited by email to complete the survey online. The survey was available in 6 languages and could be completed between July and September 2012. The EASL guidelines were used as a reference for recommended treatment. Results: The response rate was 27% (63/235). The majority of respondents were gastroenterologists/hepatologists involved in the care of patients who see chronic hepatitis B/C patients weekly. Patients tended to be referred to specialists from GPs or IDU services. A lack of consensus emerged concerning the referral from midwives/antenatal care providers; 27% indicated it was very common compared to 30% who indicated they never receive patients from these services. Divergence in treatment restrictions between and within countries was observed. For hepatitis B, no one reported that Entecavir and/or Tenofovir cannot be prescribed. Restrictions were most common for the new protease inhibitors for hepatitis C (Boceprevir and Telaprevir). Other restrictions were based on clinical indications such as viral load, genotype and contra-indications (e.g. cirrhosis). All countries reported significant or complete restrictions for treatment of undocumented migrants. Conclusions: Our data suggest that, other than from GPs, current referral practice is highly divergent within and between countries. For hepatitis B treatment, it is encouraging that the options recommended in the EASL guideline are generally available. However, for hepatitis C, availability of the newest drugs varies considerably. Caution should be taken in interpretation as health care system context (as opposed to quality of care or provision) may explain some of these differences.

972 SCREENING FOR VIRAL HEPATITIS AMONG MIGRANTS IN THE EU – THE QUEST FOR ‘GOOD SCREENING PRACTICE’

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Background: Two large-scale campaigns in the USA have demonstrated successful screening of migrants for viral hepatitis. Although the increased risk among migrants is well known, comparable campaigns in the European Union (EU) have not been performed. To summarise available data on screening and to highlight critical factors for success we performed a. a literature review; b. an in depth analysis of five recent and successful campaigns.

Methods: a. EMBASE, MEDLINE and Google Scholar databases were searched for migrant screening campaigns conducted after 2000. The identified studies were further selected in a three-step-process based on title, abstract and full text. b. Hand searching was used to identify five campaigns in countries of the HEPscreen Project (Germany, Hungary, Italy, Netherlands, Spain, United Kingdom).

Results: a. After the selection process 11 articles of 323 originally identified were included. b. The campaigns were conducted in the Netherlands and UK in populations ranging from 170 to 4833. Targeted ethnicities were Bengali, Chinese, Indian, Pakistani and Turkish. Testing locations varied widely and included outreach locations. Awareness was raised using different media. Most campaigns used serology; only one used oral fluid testing and had to amend the procedure during the study to eliminate false positives. Other results are outlined in Table 1 (displayed as means and ranges).

Table 1. Parameter All participants HBV-infected HCV-infected

| | Male participants | 53% (34–85) | 56% (41–75) | 71% (50–100) |
| | First generation migrants (FGM) | 86% (74–94) | 97% (94–100) | 99% (97–100) |
| Rate followed-up (if available) not applicable | 85% (58–100) | 88% (76–100) |
| Rate under treatment (if available) not applicable | 22% (0–43) | 71% |

Conclusions: There is a deficit in literature on migrant screening for viral hepatitis in the EU. Male sex and FGM are overrepresented among the infected. The second generation of migrants still carries a significant disease burden and is not adequately reached by current programmes. Information on follow-up and treatment is
lost in a percentage of infected migrants. Key factors for a successful screening campaign are support from the local ethnic community, a favourable location and point in time, interpreters available on site and a sensitive and specific testing method.

973
A COMPETENCE FRAMEWORK TO SUPPORT NURSES AND HEALTHCARE PROFESSIONALS WORKING IN PRIMARY AND SECONDARY CARE WITH PATIENTS WITH OR AT RISK OF DEVELOPING LIVER DISEASE
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Background: The rising burden of liver disease in England (Figure 1) and the ever increasing rise in deaths from liver disease especially in the age group of 35–55 has pushed liver disease to the top of the public health agenda in England. The Department of Health (DH) commissioned a liver strategy, this aimed to improve detection, prevention and treatment of liver disease. The key challenges from a public health viewpoint are alcohol, viruses and non alcoholic fatty liver disease (NAFLD). A lack of knowledge and understanding amongst healthcare professionals (HCP) was seen as one of the barriers to implementing the strategy in the public health field.

Aims: To develop a competence framework for all nurses looking after patients at risk of, or developing or having liver disease. The framework must encompass both primary and secondary care, and have relevance to other HCP’s who come into contact with those at risk or have liver disease.

Methods: A Specialist Nurse Group identified nine domains of care closely related to early detection and prevention of liver disease, supporting patients with or at risk of liver disease and those who have liver disease. A competence framework has been developed to encompass the opportunity to make every contact with individuals count. See Table 1 for example of competence.

Conclusion: In recognising the significant burden of liver disease it is essential to ensure that a workforce is competent and confident to deliver high quality services. The competence framework whilst aimed mainly at nurses is broad enough to include other HCP’s working in the public health care setting to lead the fight to prevent patients from dying prematurely with liver disease.

Table 1 (abstract 973). Example of a competence framework that could be used in both primary and secondary care

<table>
<thead>
<tr>
<th>Level</th>
<th>Competence</th>
<th>KSF</th>
<th>Performance criteria</th>
<th>Knowledge and understanding of:</th>
<th>Attitudes and Behaviours</th>
<th>Contextual factors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–4</td>
<td>Signposts patients (and families/carers) to information about their condition</td>
<td>HWB1, HWB2, HWB3, HWB5, HWB6, Core 1 Core 3</td>
<td>Signposts patients, families and carers to further information and organisations</td>
<td>• range of approved information sources available in local area</td>
<td>• Aware of role limitations and when to obtain help.</td>
<td>Generic</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• organisations and agencies providing support</td>
<td>• Person centred and compassionate</td>
<td>• NMC documents and guidance</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• when to alert registered nurses about information requested and provided to enable follow through.</td>
<td>• Listening.</td>
<td>• Royal College of Nursing documents and guidance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Knows how to.</td>
<td>• Understanding.</td>
<td>• RCS Gastrointestinal Nursing Forum</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>• access further supplies</td>
<td>• Welcoming.</td>
<td>• British Association for the Study of the Liver Nurses</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• recognise own boundaries, competence and responsibility</td>
<td>• Open to receiving feedback.</td>
<td>• Forum (BASLNF)</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td>• Confidential.</td>
<td>• British Liver Nurses Forum (BLNF)</td>
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<td></td>
<td>• Pride in work.</td>
<td>• Department of Health (DH)</td>
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<td></td>
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<td></td>
<td>• Respectful.</td>
<td>• DH (2008) Making a Difference</td>
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<td></td>
<td>• NHS Liver Care</td>
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<td></td>
<td></td>
<td></td>
<td>• NHS Knowledge and Skills Framework</td>
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<td></td>
<td>• Gold Standards Framework for people nearing the end of life</td>
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<tr>
<td>5</td>
<td>Signposts patients (and families/carers) in their understanding of their condition through patient education and health promotion</td>
<td>HWB1, HWB2, HWB3, HWB5, HWB6, Core 1 Core 3</td>
<td>a. Assesses knowledge and understanding of patient’s and carer’s own condition, causes, risk factors and consequences</td>
<td>• risk factors, such as, drugs, obesity, alcohol</td>
<td>• Aware of role limitations.</td>
<td>• NHS core competences for end of life care 2009</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>b. Provides tailored education to patient and family/carers on condition, treatments and side-effects.</td>
<td>• Recognises own level of competence, able to identify learning needs.</td>
<td>• NICE</td>
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<td></td>
<td>c. Provides information about lifestyle factors, consequences and services available to support lifestyle changes.</td>
<td>• Accountable.</td>
<td>• Mental Capacity Act 2005</td>
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<td></td>
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<td>d. Refer to other appropriate health care professionals if required.</td>
<td>• Empathetic.</td>
<td>• DH (2012) Safeguarding vulnerable adults.</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>• Encouraging.</td>
<td>• DH Consent 2001</td>
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<td>• Data Protection Act 1998</td>
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<td></td>
<td>• NHS Choices</td>
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<td>• NHS Institute for Innovation and Improvement</td>
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<td></td>
<td>• Quality Outcome Measures, such as CQS/INS, PROMS etc.</td>
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<td></td>
<td>• Key quality assured patient and carer information and support from key charities and organisations, such as:</td>
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<td></td>
<td></td>
<td></td>
<td>– British Liver Trust</td>
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<td>– Children’s Liver Disease Foundation</td>
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<td></td>
<td>– Alcohol Concern</td>
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<td></td>
<td>– The Hepatitis C Trust</td>
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<td></td>
<td></td>
<td></td>
<td>– Hepatitis B Foundation UK</td>
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<td></td>
<td></td>
<td></td>
<td>– Haemochromatosis Society</td>
</tr>
</tbody>
</table>

*Some contextual factors omitted.

Figure 1. England – Movements in mortality 1971–2007. Deaths per million of population. Liver disease mortality trend dramatic increase relative to other major disease groups (WHO/Europe, European HFA Database, January 2012).

974
SEROPREVALENCE OF HEPATITIS E IN THE SOUTH PACIFIC ISLANDS
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Background and Aims: Hepatitis E (HEV) is hyperendemic in many parts of the developing world and typically causes waterborne outbreaks in regions with poor sanitation. Most patients recover,
but mortality is high in pregnant women. HEV is also endemic in industrialised countries where it is a porcine zoonosis caused by HEV genotypes 3 and 4. There are no data regarding prevalence of HEV in developing countries in the South Pacific where maternal mortality is high. However, HEV genotype 3 has been found in pigs in this region.

**Aim:** To determine the seroprevalence of anti-HEV IgG in humans in Papua New Guinea (PNG), Fiji and Kiribati.

**Methods:** Samples were collected between 2003 and 2005 from healthy volunteers as part of a study on the epidemiology of viral hepatitis. Samples were stored at −20°C and evaluated using the Wantai (PE2) HEV IgG ELISA kit. Equivocal results on repeat testing were defined as negative.

**Results:** The overall seroprevalence for HEV was 10.5% (110/1050). Prevalence in PNG was highest (15.2%), followed by Kiribati (8.8%), and Fiji (2.2%). There was no significant difference in seroprevalence between adults and children in any country tested, (age <16 years seroprevalence: 11.4%, 13.4% and 3.3% for PNG, Kiribati and Fiji respectively). In PNG, 85 paired parent/child samples were collected: of the 11 HEV IgG+ children, none had parents who were also HEV IgG+, suggesting transmission within families is not predominant. HEV was more prevalent in highland than lowland communities of PNG, (20.4% vs. 9.7%, p = 0.01).

<table>
<thead>
<tr>
<th>Total no. of samples</th>
<th>Median age (yrs)</th>
<th>Gender M/F</th>
<th>HEV + Prevalence</th>
<th>Prevalence Adult/Child (&lt;16yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNG</td>
<td>546</td>
<td>223/323</td>
<td>83/546</td>
<td>15.2%</td>
</tr>
<tr>
<td>Kiribati</td>
<td>239</td>
<td>97/142</td>
<td>21/239</td>
<td>8.8%</td>
</tr>
<tr>
<td>Fiji</td>
<td>265</td>
<td>124/141</td>
<td>6/265</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

**Conclusions:** HEV seropositivity varies significantly in the South Pacific. It is highly prevalent in PNG and rare in Fiji. This compares to a seroprevalence of 4% in New Zealand using the same diagnostic assay [1]. The high seroprevalence in children suggests recent infection and implies active viral circulation in these communities. This is an uncommon finding in other seroprevalence studies in developing countries. The reason for this difference remains to be determined. HEV is more prevalent in highland than lowland PNG, possibly explained by zoonotic transmission in highland regions where pig keeping is more common.

**Reference(s)**


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**POSTERS**
HBV infected subjects are eligible for antiviral therapy but most of them are unaware of their own HBV status with very poor access to care and treatment.

977 HOSPITAL COST OF THROMBOCYTOPENIA RELATED TO HEPATITIS C INFECTION: ANALYSIS OF THE FRENCH HOSPITAL DISCHARGE DATABASE

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Background and Aims: There are about 4 million carriers of hepatitis C virus (HCV) in Europe, around 370,000 in France. Thrombocytopenia (TCP) is a common complication of HCV, particularly among those with advanced liver disease (liver cirrhosis and cancer), which make numerous HCV patients ineligible to treatment. In clinical trials of interferon-based antiviral therapy, more than one third of patients experience a decrease in platelet counts, which may lead to dose reductions, interruption, or discontinuations of interferon, potentially jeopardizing the chances of achieving sustained virologic response. Few data are available on the economic burden of TCP in HCV. Our objective is to estimate the costs related to TCP in hospitalized HCV patients population in France.

Methods: All 2011 hospital discharge forms including both D69.a (all causes of TCP) and B18.2 (HCV) ICD-10 diagnosis codes were identified in the French national database (so-called PMSI). Total and mean hospital costs were calculated according to the 2012 National Hospital Tariffs.

Results: 2,264 stays (corresponding to 1,469 patients) were extracted with both TCP and HCV diagnoses: 1,462 stays (64.6%) were considered as not directly related to TCP and/or HCV (especially D69.0 to D69.2, D69.8 and D69.9), and excluded from analysis; 193 stays (8.5%) were considered as directly related to TCP with an HCV associated diagnosis (group 1) and 609 (26.9%) as directly related to HCV (with/without complications, including liver cirrhosis and hepatocarcinoma), together with TCP as an associated diagnosis (group 2). The total annual cost of stays related to TCP (groups 1 and 2) in France, 2011, was 2,751,991 €. The per year per patient (PPPY) cost of HCV infected patients hospitalized for TCP was estimated at 3,241 € (group 1); the PPPY cost in patients in group 2 was 5,325 €.

Conclusions: When compared to other studies on HCV related costs, the PPPY of patients with TCP (3,241 € to 5,325 €) is higher than in patients hospitalized for non-complicated HCV in France (ie in 2009, HCV related costs = 1,080 €), which corroborates other findings showing that HCV patients with thrombocytopenia have higher liver disease-related costs than patients without thrombocytopenia.

978 LOW RISK FOR HEPATOCELLULAR CANCER (HCC) IN HEPATITIS B VIRUS (HBV) INFECTED ASIAN MIGRANTS: IMPLICATIONS FOR CANCER SURVEILLANCE

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Background: The global incidence of HCC is rising and it is the third most common cause of cancer-related death worldwide. Surveillance of at risk patients has been recommended by expert guidelines to detect early cancers that are amenable to curative treatments. AASLD has recommended HCC surveillance for non-cirrhotic HBV-positive Asian males over 40 and females over 50yrs of age. However, the evidence to support this recommendation is limited and is derived from studies conducted in Asia. Results from such studies may not be applicable in the West, due to differences in environmental risk factors and availability of HBV treatment. Implementation of such a recommendation would place a burden on healthcare resources, and may not be justified if the risk for HCC is substantially lower than previous estimates in this population.

Aims: To assess the incidence rate of HCC in non-cirrhotic Asian patients infected with HBV from a Western centre.

Methods: A retrospective study was carried out of all Asian patients undergoing follow up for HBV infection from 1990–2012. Patients were classified as cirrhotic or non-cirrhotic according to clinical, biochemical, radiological and histological results. Follow-up was until September 2012, and was censored at time of death, development of cirrhosis or loss to follow-up.

Results: Among 316 identified Asian patients with HBV, 73 non-cirrhotic patients fulfilled the proposed AASLD surveillance criteria, either at time of initial referral or during the period of follow-up. The median at-risk follow up period (as defined by AASLD guidelines for non-cirrhotic Asians) was 57 months (range: 0–354 months). HCC was diagnosed in one non-cirrhotic patient after 77 months of follow up (male, 60yrs), two patients became cirrhotic after 49 and 89 months (male, age 46 and 55yrs) and no deaths occurred. The overall incidence of HCC in the non-cirrhotic cohort meeting the AASLD surveillance criteria was 1 per 429.5 patient-years of follow-up (0.23% per patient-year).

Conclusion: The incidence of HCC in Asian patients with non-cirrhotic HBV is low in our cohort. This low incidence challenges the rationale for surveillance in this group of patients. More studies are needed to assess the benefit of such approach.

979 COST-EFFECTIVENESS OF INCREASING HCV CASE-FINDING FOR PEOPLE WHO INJECT DRUGS VIA DRIED BLOOD SPOT TESTING IN ADDICTION SERVICES AND PRISONS

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Background: Over 80% of HCV is among people who inject or have injected drugs (PWID). Cohort testing is unlikely to be cost-effective in countries with HCV infection rates of less than 0.05% in the adult population. We determine the cost-effectiveness of increasing HCV case-finding among PWID in addiction services and prisons.

Methods: A dynamic HCV transmission model among PWID was developed, including disease progression, diagnosis, treatment, injecting status, incarceration, and addiction services contact. A systematic review identified HCV dried blood spot (DBS) testing as an intervention that could increase case-finding. The model was parameterized to the UK. Costs (in UK £, ~1.25 Euro) and utilities (quality adjusted life years, QALYs) were attached to each state and the incremental cost effectiveness ratio (ICER) determined. Multivariate uncertainty and one-way sensitivity analyses were performed.

Results: For a £20,000 per QALY gained willingness-to-pay threshold, DBS testing in addiction services is cost-effective (ICER of £14,600 per QALY gained). Under the base-case assumption of £14,600 per QALY gained. Results are robust to changes in HCV prevalence; increasing PWID treatment rates to those for ex-PWID considerably reduces the ICER (£4,500 and £30,000 per QALY gained for addiction services and prison, respectively). If continuity of care is >40%, the prison DBS ICER falls below £20,000 per QALY gained.
Conclusion: Increasing case-finding can be cost-effective in addiction services especially if treatment rates are increased. However, case-findings is only cost effective in prisons if continuity of treatment/care is ensured.

980 INCREASING ACCESS TO DIAGNOSIS AND TREATMENTS FOR HEPATITIS C (HCV) IN RESOURCE LIMITED SETTINGS (RLS), HOW SHOULD WE MOVE FORWARD?
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Background and Aims: Worldwide 150 to 180 million of people are infected with the hepatitis C virus. Most of them live in resource limited settings. Two to five millions people living with HIV-AIDS are co-infected with HCV.

New technologies may revolutionise the ability to diagnose patients and monitor treatment efficacy. Oral treatments are being developed which will drastically change the way HCV-infected people will be treated. The question is how can we make them accessible and affordable for people living in RLS?

Methods: We conducted grey literature review analyses, key informant interviews, and expert meetings. The objective was to identify the main access barriers to diagnosis, monitoring and treatments for HCV-infected people, and identify the potential solutions and game changers for scaling-up access to HCV care in RLS.

Results: The main determinants of access to HCV diagnosis and treatment in RLS identified to date are: Lack of reliable epidemiological data, no civil society mobilization, no rapid diagnosis test (RDT) for HCV suitable for RLS, no access to HCV viral load/genotyping and non invasive markers of liver fibrosis, Peg Interferon (Peg IFN) not part of WHO Essential Medicines List, price of Peg IFN, lack of a international evaluation scheme for biosimilar version of Peg IFN alpha, lack of access to oral HCV drug regimen, and no financing mechanism.

The solutions or game changers identified are: Countries and WHO epidemiological surveillance systems, political will allowing right to care for all, no discrimination of vulnerable groups, decriminalization, WHO pre-qualification of HCV screening tests and RDT, point of care HCV viral load and genotyping tests, multi-analyte molecular tests, compatibility with dried blood spots, access to Fibroscan and to simple biological markers of fibrosis, Peg IFN alpha inserted in the WHO EML, price negotiations with originators, price monitoring and transparency, WHO to establish a feasible pathway for evaluation of biosimilar Peg IFN, oral drug regimen adapted to RLS at affordable price, quality assured generic versions; and finally innovative mechanisms for HCV (UNITAID, Global Fund, domestic funding).

Conclusion: Access to care for HCV-infected people is first a question of political will.

981 PREVALENCE AND RISK FACTORS FOR HEPATITIS B AND C AMONG PATIENTS ATTENDING A GERMAN EMERGENCY DEPARTMENT – IS A RISK ASSESSMENT QUESTIONNAIRE A USEFUL PRE-SCREENING TOOL?
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Background: Germany is a low prevalence country for Hepatitis B (HBV) and C (HCV) infections. Current guidelines only recommend testing individuals with associated risk factors. However, a high number of undiagnosed cases, especially of HCV, are suspected.

Conclusions:
1. HBV prevalence rates were confirmed in our study.
2. A general screening of all immigrants from high prevalence countries for HBsAg should be advocated.
3. Anti-HCV seroprevalence in our study (1.6%) was much higher than estimates (0.4%).
4. A questionnaire on known risk factors seems not helpful in detecting HCV infections in our study.

982 BINGE DRINKING, ALCOHOL ABUSE AND DEPENDENCE: SCREENING STUDY IN AN EMERGENCY DEPARTMENT
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Background and Aims: Alcohol is associated with many health problems and with 15.000–20.000 death/year in Italy. In Hospital Emergency Departments are examined alcohol related problems as consequence of short-term exposure to alcohol as well as consequences of long term alcohol use. The aims of this study are to investigate: the patterns of alcohol consumption among emergency room patients (risk or hazardous drinking, harmful drinking, alcohol abuse and dependence); the share and the characteristics of alcohol consumption subgroup; the feasibility of screening for alcohol problems in the emergency room.

Materials and Methods: The study was carried out at S. Anthony Hospital Department during a period from 8th January 2012 to 16th May 2012, 4 weeks were randomly selected. In three weeks screening was led from 8 am to 8 pm, in one week from 7 pm to 12 pm. Patients ≥18 years old were approached after they had been triaged, before or after being visited. AUDIT and CAGE tests were self-administered only to patients who accepted to perform the test in this way, the others were interviewed by the same interviewer. The statistical analysis was carried out with Pearson test.

Results: 1520 patients were evaluated in emergency department during the period of the study. 1000 (65.8%) were examined. 874 (57.4%) were interviewed. 19.5% of interviewed patients had
alcohol related problems on the basis of either CAGE or AUDIT. Higher rates were found for men (p < 0.001), young people aged 18–20 years (p < 0.028), divorced or single (p < 0.003), unemployed subjects (p < 0.001), homeless (p = 0.005), immigrants (p < 0.001). There was a lack of significant correlation between positive tests and day of presentation and need of admission.

Conclusions: Our data indicate that a large amount of emergency room patients have alcohol-related problems. Emergency Department may be the initial point of healthcare contact for patients with alcohol problems. Social outcast persons present higher risk of alcohol disorders. Screening can be useful to provide the first step of intervention in this group and is needed for early prevention and health care intervention.

983 INCIDENCE AND MORTALITY TRENDS FOR BILIARY TRACT CANCERS IN AUSTRIA
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Background: The epidemiology of biliary tract cancers (BTC) varies between geographic regions and has changed over time globally. Epidemiological data of BTC in Austria are widely unknown. We investigated the incidence and mortality trends of patients diagnosed with BTC over a 20-year period in Austria.

Methods: National databases were used to analyze epidemiological trends of Austrian patients diagnosed with BTC between 1990 and 2009. Data on incidence were obtained from the Austrian National Cancer Registry which compiles data on all newly diagnosed cancers in Austria. Data on mortality were obtained from the national death registry (Statistics Austria).

Results: Between 1990 and 2009, 15201 patients were diagnosed with BTC (m/f=42/58%; median age, 74 years), of which 20/17/35/0.2/1.5/19% had intrahepatic cholangiocarcinoma (iCCC)/extrahepatic CCC (eCCC)/ampullary carcinoma/gall bladder carcinoma (GBC)/overlapping lesions/biliary tract, unspecified/liver, unspecified. In iCCC, the age-adjusted incidence (m and f, 0.41 to 2.16 per 100000 (WHO standard population in 2001) and 0.22 to 1.52) and mortality (m and f, 0.12 to 1.83 and 0.11 to 1.38) rates increased from 1990 to 2009 in both, men and women. In eCCC, the age-adjusted incidence (m and f, 0.93 to 0.87 and 1.06 to 0.80) and mortality (m and f, 0.91 to 0.86 and 0.93 to 0.68) rate decreased over time in both sexes. In ampullary carcinoma, the age-adjusted incidence slightly decreased from 1990 to 2009 in men (0.45 to 0.28) and remained stable in women (0.26 to 0.32 per 100000). The age-adjusted mortality rate remained stable in both sexes (m and f, 0.26 to 0.30 and 0.11 to 0.18). In GBC, the age-adjusted incidence (m and f, 1.75 to 0.58 and 3.54 to 1.22) and mortality (m and f, 1.63 to 0.50 and 3.18 to 0.83) rate dramatically decreased from 1990 to 2009 in both, men and women.

Conclusions: GBC and iCCC were the most common entities amongst BTC over a 20-year period. While incidence and mortality rates of the cancers decreased in men and women over time, incidence and mortality rates of GBC decreased in both sexes. Other carcinomas of the biliary tract including eCCC and ampullary carcinoma were rarely diagnosed.

984 EMERGING HEPATITIS E GENOTYPE 4 INFECTION IN FRANCE
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Background and Aims: Until recently, reported autochthonous infections by Hepatitis E virus (HEV) in Europe were due to genotype 3 strains with swine as the main reservoir. Genotype 4 (G4) was limited to Asia. In 2008, a G4 infection was reported in a German patient and G4 strain was detected in Belgian swine in 2011. In France, autochthonous G4 infections were reported for 3 patients in 2011 and 2012. The objective was to characterize the genetic diversity of HEV G4 identified in French patients in 2011 by the French Reference Centre for HEV.

Methods: HEV infections with detectable RNA were reviewed for year 2011. Clinical characteristics of patients were recorded: age, sex, immunosuppression, and ALT level. HEV RNA detection was performed by a real time RT-PCR targeting the ORF3 region (Ceeram®, La Chapelle sur Edre, France). Phylogenetic analyses were conducted from ORF1, nt 1055–991 and ORF2, nt 5996–6343 regions by using Mega 4 software.

Results: Among 280 viremic infections, HEV genotyping was available in 260 cases. Nine HEV G4 infections were identified (3.5%). Only 1/9 G4 infection was associated with a travel to China, which sequence clustered with Chinese sequences in both ORF1 and ORF2 analyses, as did the previously reported German case. By ORF1 analysis, the remaining 8 sequences from autochthonous cases formed a single cluster, distinct from the previously published G4 sequences. These autochthonous sequences were close to but distinct from the Belgian swine. By ORF2 analysis, autochthonous sequences also formed a distinct cluster which included the tree G4-associated cases described in France. Cases were distributed throughout France. All G4 cases presented with higher ALT than G3 cases (1652±1895 vs. 1706±1433 UI/L; p < 0.0001). In addition, severe infections with prothrombin factor <50% were observed in 4/9 cases.

Conclusions: Though genotype 4 was not yet described in French swine, it may circulate in France as shown by the genetic divergence from Asian strains. Our data are consistent with previous reports suggesting a more severe clinical course of HEV genotype 4 in humans.

985 INCREASING RATES OF ACUTE HEPATITIS C ALONG WITH HIGH RATES OF SYPHILIS IN HIV-POSITIVE MEN WHO HAVE SEX WITH MEN IN MADRID
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Background: Outbreaks of acute hepatitis C in HIV-positive men who have sex with men (MSM) are being reported in large cities in western countries. Increasing rates of sexually transmitted infections are occurring in parallel.

Methods: All HIV-infected individuals attended at a large outclinic in Madrid within teh last 5 years were examined. Incident syphilis was diagnosed based on RPR reactivity, being negative previously or showing >4-fold increase. Acute hepatitis C was diagnosed based on HCV antibody seroconversion and/or positive serum HCV-RNA after being negative within the last year.

Results: A total of 859 episodes of syphilis and 19 of acute hepatitis C were diagnosed during the study period. Syphilis was recognized in 65/2094 (3.1%) individuals attended in year 2008 and rose up to 261/2521 (10.4%) in year 2012 (p < 0.001). Acute hepatitis C was diagnosed in only 1 subject in year 2008, rising up to 7 in year 2012 (p = 0.12). All 19 HIV patients with acute hepatitis C were MSM. Syphilis was diagnosed concomitantly in 7 of them. All 18 individuals that were treated with peginterferon plus ribavirin were cured, whereas none of the rest experienced spontaneous HCV clearance. Two clusters of infections caused by HCV genotypes 4 and 1a, respectively, were identified by phylogenetic analysis.

Conclusions: The incidence of acute hepatitis C is low but steadily increasing in HIV-positive MSM in Madrid (<1% yearly), despite
very high rates of syphilis (currently 20% yearly in HIV-positive MSM). Preventive measures for sexually transmitted infections and periodic HCV screening are warranted in this population, given that treatment of acute hepatitis C is very effective.

986 PREVALENCE OF VIRAL HEPATITIS, HUMAN IMMUNODEFICIENCY VIRUS AND SYPHILIS AMONG INMATES OF BULGARIAN PRISONS

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Objective: To determine the prevalence of viral hepatitis, HIV and syphilis and examine risk factors for those infections among inmates of Bulgarian prisons.

Methods: This study was carried out in 5 of the 11 Bulgarian prisons (for men and women) and a juvenile correctional facility. Anonymous cross-sectional data were collected for prisoners who agreed to participate in the study and who were interviewed using a standard questionnaire including demographic, imprisonment and syphilis history and viral hepatitis, HIV and syphilis related risk behaviors items. Thereafter, the blood drawn from the participants was tested for anti-HAV; anti-HBc, HBsAg and HBeAg; anti-HCV; anti-HDV and anti-HIV by ELISA tests and immunochromatographic rapid Treponema pallidum test kit.

Results: A total number of 658 inmates participated in our study. Four hundred and sixty eight (71.1%) were men and one hundred and ninety (28.8%) were women. The overall rate of antibody positivity for anti-HAV was 486 (73.8%), anti-HBc – 396 (60.2%), anti-HCV – 188 (28.6%) and anti-HDV – 62 (9.4%). Four (0.6%) of the prison inmates were HIV positive. One hundred and twenty four (18.4%) of the prisoners had reactive syphilis test. The presence of huge number of prisoners with viral hepatitis B and C are due to use of i.v. drugs, unprotected sexual contacts, tattoo and other manipulations with skin and mucous lesions.

Conclusion: The seroprevalence of viral hepatitis among prisoners in comparison with the general population in Bulgaria is very high suggesting their probable transmission in prisons through intravenous drug use, unsafe sexual behaviour and tattooing. The data indicate a lower prevalence of HIV in correctional facilities. Our results indicate the importance of policies to prevent transmission of viral hepatitis during and following incarceration.

987 DIFFERENTIATION OF NASH PREVALENCE IN POPULATION OF URALS, SIBERIA AND EUROPEAN PART OF RUSSIA. RESULTS OF OPEN MULTICENTRIC PROSPECTIVE STUDY DIREG_L_01903

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Introduction: NAFLD is the most common liver disease in the world [1]. Nonalcoholic steatohepatitis (NASH), the aggressive form of nonalcoholic fatty liver disease, is associated with an increased risk of liver-related mortality.

Aim: To compare the prevalence of NASH in the population of Siberia, Urals and European part of the Russian Federation.

Methods: We examined 30417 persons (13209 males and 17208 females, average age – 47.8 years) in 16 cities of Russia. Diagnostic algorithm of NASH was substantially based on Dionysus study diagnostic criteria [2]. NASH was established on the basis of careful physical examination, serum biochemistry (ALT, AST, GGT, lipid spectrum, glucose), analysis of alcohol consumption, viral hepatitis markers and abdominal ultrasonic scanning.

Results: The prevalence of NASH in the population of Siberia (1) was 5.9%, in the Urals (2) – 5.8%, in the European part of Russia (3) – 3.8% (p1–3 <0.001, p2–3 <0.001). Risk factors for NAFLD were obesity, diabetes mellitus type 2, hyperglycemia, hypercholesterolemia, metabolic syndrome in all regions. But the prevalence of risk factors was higher in the population of Siberia and Urals than in the European part of Russia (Table).
individuals. In general, higher HCV prevalence was found in programs in countries with intermediate to high HCV prevalence, in psychiatric clinics, and in programs that used a prescreening selection based on HCV risk factors. Only 6 programs used a comparison group for evaluation purposes, and 1 program used theory about effective promotion for screening. Comparison of the programs and their effectiveness was hampered by lack of reported data on program characteristics, clinical follow-up, and type of diagnostic test.

Conclusions: The published studies identified a relatively small proportion of the estimated HCV-infected population. A prescreening selection based on risk factors can increase the efficiency of screening in low-prevalence populations, and we need programs with comparison groups to evaluate effectiveness. Also, program characteristics such as type of diagnostic test, screening uptake, and clinical outcomes should be reported systematically.

989 FACTORS ASSOCIATED WITH ALT LEVELS IN A LARGE REAL-LIFE POPULATION: FOCUS ON THE RELATIONSHIP BETWEEN ALT AND AGE
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Background and Aims: Several studies, mostly carried out in blood donors, identified anthropometric and metabolic factors impacting alanine-aminotransferase (ALT) levels. Nonetheless, the relationship between ALT and age remains poorly understood. We aimed to verify factors associated with ALT levels in a large real-life population, with particular attention to age.

Patients and Methods: Data were extracted from a validated software employed by 120 general practitioners in the area of Naples, in charge of about 170,000 subjects. After exclusion criteria, data from 45,301 patients were analyzed.

Results: Male sex ($\beta = 0.18$), age ($\beta = -0.14$), BMI ($\beta = 0.14$), triglycerides ($\beta = 0.06$), creatinine ($\beta = -0.05$), glucose ($\beta = 0.05$), total cholesterol ($\beta = 0.04$) and HDL-cholesterol ($\beta = -0.03$), were all independently associated with ALT levels after multivariate analysis ($p < 0.0001$ for all except for HDL, $p < 0.01$). Age showed a nonlinear relationship with ALT activity and the best fitting curve for this association was shaped as an inverted-U ($r = 0.18$, $p < 0.0001$) (Figure 1).

Dividing our population into two groups (younger/older) by the age correspondent to the zenith of ALT (50 years), age retained its independent association with ALT only in the older group ($\beta = -0.19$, $p < 0.0001$), while, in the younger one, this association was no more significant when metabolic parameters were present in the model ($p = 0.7$). Indeed, excluding subjects with metabolic disorders, a marked reduction in the slope of the ascending arm of the curve was observed, while the slope of the descending arm was not affected and was consistent with a reduction of mean ALT levels of approximately 2 U/L for every decade of age ($p < 0.0001$).

Conclusions: In a large real-life population, metabolic factors were confirmed as significant determinants of ALT levels probably through their association with liver fat content. Proceeding to 50ties, ALT activity raises mainly due to increasing metabolic conditions, while, afterwards, it decreases progressively for still unidentified reasons, suggesting the need to lower the ALT upper normal limit in the older population.

990 HEPATITIS A VIRUS (HAV) IMMUNITY AMONG NON INJECTING DRUG USERS: VACCINATION SHOULD BE RECOMMENDED IN DEVELOPING COUNTRIES?
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Background and Aims: Crack use is highly prevalent in young, marginalized and urban populations, and associated with extensively elevated morbidity and premature mortality risks. In developing countries, better health and hygiene conditions have been observed in last years diminishing the prevalence of HAV infection in general population. However, HAV immunization is not available for most of individuals due to the high cost of this vaccine. The aim of this study is to evaluate HAV immunity among crack users from two geographical regions of Brazil in order to evaluate the potential need of vaccination among this risk group.

Methods: Our study assessed two community-recruited samples of street-involved young, regular crack users recruited from inner-city neighborhoods in Rio de Janeiro (n=81) (Southeast region) and Salvador (n=79) (Northeast region). Following eligibility confirmation and informed consent, study participants were assessed on drug use, health and socio-demographic characteristics by way of anonymous interviewer-administered questionnaires. Blood samples were tested for antibodies against HAV (anti-HAV) using commercial enzyme immunoassays. All reactive samples were re-tested for results confirmation.

Results: Most of individuals were male (77.4%) and age range was 18–25 years; 48.1% reported to live in unstable housing (e.g., homeless) and 41.9% were arrested in the past year. Participants reported numerous crack use episodes per day; the majority (58.1%) reported sharing of crack use paraphernalia, as well as co-use of alcohol (48.1%), tobacco (80.6%), cocaine (36.9%) and marijuana (66.2%) in the last 30 days. Anti-HAV prevalence was 78.8% increasing according to age.

Conclusions: We found overall anti-HAV prevalence elevated in this Brazilian crack users compared with the general Brazil population. This rate could indicate an excess risk of HAV infection and the potential need to offer hepatitis A vaccination among susceptible individuals.
01c. LIVER TRANSPLANTATION/SURGERY: ACUTE LIVER FAILURE – CLINICAL & EXPERIMENTAL

991 ACUTE LIVER FAILURE IN HIV INFECTED PATIENTS: A SINGLE CENTER EXPERIENCE


Background: Management and results of liver transplantation (LT) in HIV + patients with acute liver failure (ALF) and fulminant hepatitis (FH) are poorly known.

Aim: To describe the evolution of ALF in HIV + patients and the results of LT in this indication.

Patients and Methods: Between June 2002 and January 2012, 15 HIV + patients (9 male, mean age 40 years [25–53]) were admitted to our centre for ALF (PT/FV <50%). Eleven patients were treated by HAART (3 of them by d4T/ddI) and 5 had medical history of opportunistic disease.

Results: On admission, the average level of total bilirubin, ALT, creatinine, INR and CD4 count were respectively: 233 mmol/L [50–620], 2472 IU/L [208–7929], 22 mmol/L [34–773], 5 [1.4–15], 345 cp/ml [50–1200]. HIV viral load was <20 cp/ml in 8 patients. Four patients were HBV+, 1 patient HCV+ and 2 patients had an alcohol abuse (>40gr/day). Pathological examination (METAVIR) showed no fibrosis (F0) in 11 patients and chronic liver disease (F2) in 4. HAS’ cause was: drug-induced hepatitis (nevirapine= 2, acetaminophen= 2, abacavir/ritonavir= 1, isoniazid/rifampicin= 1), IRIS (n=3), DRESS syndrome (n=1), herpetic hepatitis (n=1), HBV reactivation (n=1), unknown for 3. 7 patients experienced spontaneous recovery and 1 patient died of ventricular haemorrhage. 7 patients, 2 with positive HIV PCR (2.1 and 2.9 log, respectively) were listed for emergency LT due to FH with a mean waiting time of 1 day [0–2]: 2 patients died before LT due to cerebral oedema and 5 patients were transplanted. One patient died 40 days after LT because of severe sepsis, one patient had hemi-colectomy for ischemic colitis and one patient experienced seizures. Post transplantation evolution of 4 pts was characterized by: median survival of 24 months [3–64], no opportunistic infection, last mean CD4 count of 530 giga/L [270–731] and HIV viral load <20 cp/ml on HAART.

Conclusions: In case of acute liver failure, the prognosis of HIV+ patients is severe with 50% evolving to fulminant hepatitis. Preliminary results of liver transplantation for fulminant hepatitis in HIV+ patients are encouraging with 80% survival at 24 months.

992 HYPOXIC HEPATITIS: PREDICTIVE FACTORS OF MORTALITY

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Background: Hypoxic hepatitis (HH) is a liver injury characterized by a centrilobular liver cell necrosis which affects critically ill patients in a context of hemodynamic instability. Based on 3 criteria, HH diagnosis requires:
1. an increase of serum aminotransferase levels;
2. in a clinical setting of cardiac, circulatory or respiratory failure;
3. the exclusion of other putative causes of liver cell necrosis.

HH could lead to an acute liver failure (ALF). Previous studies suggest that the severity of multiple organ failure is the main prognostic factor regardless the hepatic function. The aims of this retrospective study were to determine epidemiological parameters of HH patients in our center compare to patients admitted with an ALF without HH, and to evaluate the predictive factors of mortality in HH patients, especially the impact of the liver injury in this population.

Results: From 2006 to 2011, 148 patients were admitted for an ALF (HH, n = 79; No-HH, n = 69) in our intensive care unit. Differences between HH-patients and No-HH-patients were a higher IGS score (81 versus 39, p < 0.01), a higher rate of mortality (81% versus 19%, p < 0.01) and a higher age (66 years-old versus 42, p < 0.01) in the HH group. Among the HH patients (Male: 49%, mean age = 66±17 years old), the meantime follow up was 9.5±13 days. The overall mortality was 81%. At baseline, mean prothrombine time (PT) was 24±12% and the MELD score was 37±5. Among the other organ deficiencies regardless the liver, 75% required a dialysis, 100% a vasoactive therapy and 96% an invasive mechanic ventilation. At admission, the mean SOFA score was 17±3 and IGS score was 81±22.

In multivariate analysis, the prognosis factors of mortality were IGS score (p < 0.01), lactate level (p = 0.05), MELD score (p < 0.01), PT (p < 0.05). Others organ failures were not associated with mortality in this study. MELD score over 34 was discriminant to predict mortality (AUC: 0.77).

Conclusion: Hypoxic hepatitis is associated with poor prognosis among other cause of ALF, and the outcome is strongly influenced by the severity of liver impairment, estimated by the MELD score.

993 IN VITRO INHIBITORS OF MITOCHONDRIAL PERMEABILITY TRANSITION DO NOT REDUCE INTRANCRANIAL PRESSURE OR BRAIN EDEMA IN RATS WITH PORTACAVAL ANASTOMOSIS AND HYPERAMMONEMIA

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Background and Aims: Cerebral edema and intracranial hypertension are potentially fatal complications of acute liver failure. Ammonia is considered to play a crucial role by induction of impaired mitochondrial function and astrocyte swelling. In vitro studies of cultured astrocytes have demonstrated that inhibitors
POSTERS

of mitochondrial permeability transition (MPT) can attenuate the cellular oedema associated with exposure of ammonia. In this study we evaluated the effect of two MPT inhibitors in an in vivo model of acute hyperammonemia. We studied the effect administration of either histidine or ciclosporine on intracranial pressure and cerebral water content in a rat model with portacaval anastomosis and acute hyperammonemia. We hypothesized that MPT inhibitors would attenuate the intracranial pressure and reduce cerebral water content.

Methods: 55 male Wistar rats with a surgically constructed portacaval anastomosis were randomized into 2x4 groups receiving intrathecal ciclosporin/vehicle and intravenous ammonia/saline infusion (experiment A) and intraperitoneal histidine/saline and intravenous ammonia/saline (experiment B). The ammonia/saline infusion was given intravenously for four hours, while intracranial pressure was recorded. At the end of the experiment the rats were decapitated and the brain water content was measured.

Results: We found that ammonia infusion led to significant higher ICP (experiment A: 8.4±1.1 mmHg vs. 3.2±0.38 mmHg (p<0.001, two-way ANOVA) and B: 6.5±0.91 mmHg vs. 2.0±0.26 mmHg (p<0.001)) and brain water content (experiment A: 80.6±0.3% vs. 79.1±0.3% (p<0.001) and B: 80.0±0.3% vs. 79.0±0.1% (p<0.05)). Treatment with ciclosporin and histidine did not lead to a reduction in intracranial pressure or an attenuation of the increased cerebral water content.

Conclusion: MPT inhibitors with in vitro neuroprotective features do not reduce intracranial hypertension or brain water content in rats with portacaval anastomosis and hyperammonemia.

994 DEVELOPMENT AND PILOT OF A CHECKLIST FOR MANAGEMENT OF ACUTE LIVER FAILURE IN THE INTENSIVE CARE UNIT

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Background and Aims: Checklists decrease medical error and improve adherence to best practices, particularly during complex tasks. We aimed to develop a checklist for the management of acute liver failure (ALF) in the intensive care unit (ICU).

Methods: Checklist development used published guidelines and expert opinion. Initial drafts were reviewed by experts and refined through consensus. We pilot tested use of the checklist at 8 sites in the US and Canada. Checklist users were asked to provide an assessment of the use and quality of the checklist. Results were reported using 5-point Likert scales.

Results: The checklist contains a list of tests to be performed on admission to establish the etiology of ALF. It also contains a list of best practices organized by organ system to be reviewed and performed on admission and daily thereafter. 38 surveys were completed at 8 sites by a variety of practitioners in the ICU (hepatologists, gastroenterologists, surgeons, internists, nurses and pharmacists). Pilot users found the checklist logical and useful and generally agreed or agreed strongly with each survey item (see table). They rated the overall quality of the checklist as good to excellent (mean 4.53±0.60). All pilot users would use the checklist again for future patients. Written comments were used to improve the checklist.

Conclusions: The acute liver failure study group checklist for the management of ALF in the ICU was easy to use, useful and accepted by a wide variety of practitioners at multiple sites. The impact of the checklist on the management of ALF is the subject of future study.

Table: acute liver failure (ALF) checklist survey results

<table>
<thead>
<tr>
<th>Survey Item</th>
<th>Scale 1–5, 1 = Disagree Strongly, 5 = Agree Strongly</th>
</tr>
</thead>
<tbody>
<tr>
<td>The checklist was easy to read</td>
<td>4.68±0.47</td>
</tr>
<tr>
<td>The checklist was easy to use</td>
<td>4.65±0.48</td>
</tr>
<tr>
<td>The items on the checklists are categorized logically</td>
<td>4.63±0.54</td>
</tr>
<tr>
<td>The time to complete the checklist did not interfere with delivery of appropriate and safe patient care</td>
<td>4.66±0.63</td>
</tr>
<tr>
<td>The time to complete the checklist was not excessively burdensome</td>
<td>4.54±0.65</td>
</tr>
<tr>
<td>The checklist allowed me the freedom to use my clinical judgment</td>
<td>4.34±0.78</td>
</tr>
<tr>
<td>The checklist is a useful tool in the management of ALF</td>
<td>4.68±0.47</td>
</tr>
<tr>
<td>If I were a patient with ALF, I would want the checklist to be used</td>
<td>4.71±0.46</td>
</tr>
</tbody>
</table>

995 CYTOKINE AND CHEMOKINE PATTERNS IN HEPATIC VEIN SERUM DISTINGUISH PATIENTS WITH ACUTE LIVER FAILURE FROM OTHER LIVER DISEASES

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Background and Aims: Liver function test (LFTs) are not useful in distinguishing between various liver diseases and are suboptimal for monitoring their progression. Recently, efforts have been made in identifying new biomarkers that may serve as diagnostic and prognostic parameters. However, most relevant biomarkers have been measured in peripheral blood and it remains unclear whether the identified parameters are indeed of hepatic origin and whether they are causally linked to pathophysiological changes taking place in the liver. In our study we addressed this issue by measuring parameters in sera obtained simultaneously from hepatic and peripheral veins of patients with varying liver diseases.

Methods: Forty consecutive patients who underwent a transjugular liver biopsy at our center in 2011–2012 were prospectively included in this study (14 with acute liver failure, 9 with liver cirrhosis, 7 liver transplant recipients and 10 patients with cardiac failure and no primary liver disease who served as controls). Serum was drawn simultaneously from a hepatic and a peripheral vein. Fifty-four cytokines, chemokines and angiogenetic factors were measured in sera using multiplex technology (Bio-Plex System).

Results: Cytokine, chemokine and angiogenetic factor levels were analyzed with principal component analysis that revealed distinct biomarker patterns in the four patient groups. Up to 13 biomarkers displayed significant variation in the acute liver failure group compared to the other three groups, six of them (HGF, IL-18, IL-2Ra, SCGF, TNFp and M-CSF) were common in all groups. Measurements in sera obtained from hepatic rather than peripheral veins returned a higher number of biomarkers, which varied significantly (p<0.01) in patients with acute liver failure in comparison to other groups. Moreover, HGF was markedly elevated (p = 0.001) in both hepatic and peripheral serum of patients with acute liver failure lacking improvement in their liver function and subsequently being transplanted shortly after transjugular liver biopsy.

Conclusions: Measurement of biomarkers in hepatic vein serum distinguishes ALF from other liver diseases and provides us with evidence about the hepatic origin of several cytokines and chemokines. This approach may help elucidating the
pathophysiology and progression of liver disease and eventually turn into a useful diagnostic and prognostic tool.

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INCREASED HEPATIC FUNCTIONALITY OF THE HUMAN HEPATOMA CELL LINE HepaRG CULTURED IN THE AMC BIOARTIFICIAL LIVER
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Background and Aims: The clinical application of a bioartificial liver (BAL) depends on the availability of a human cell source with high hepatic functionality. The human hepatoma cell line HepaRG has a unique high hepatic functionality in monolayer culture. Characteristics of the HepaRG cells cultured in the AMC-BAL over time were compared with those in monolayer cultures.

Methods: HepaRG cells were cultured in laboratory-scale AMC-BALs for 21 days. 14-Day-old HepaRG-AMC-BALs were studied by immunohistochemistry. Hepatic functionality was studied at 7, 14, and 21 days. Functional parameters included ammonia elimination, urea production, conversion of 15N-ammonia into 15N-urea, 6β-hydroxylation of testosterone (cytochrome P450 3A4 activity), lactate metabolism, and apolipoprotein A1 production. Next, 14-day-old HepaRG-AMC-BALs were compared with 28-day-old monolayer HepaRG cultures for the same protein-normalized functional parameters, cell leakage (lactate dehydrogenase and aspartate aminotransferase), transcript levels of various hepatic genes, and amino acid metabolism.

Results: Immunohistochemistry of 14-day-old BALs demonstrated functional heterogeneity similar to that of monolayer cultures. Hepatic functionality of the HepaRG-AMC-BALs increased during 2–3 weeks of culture. The majority of the measured protein-normalized hepatic functions were higher in day 14 BAL cultures compared to monolayer cultures, including ammonia elimination (3.2-fold), urea production (1.5-fold), conversion of 15N-ammonia into 15N-urea (1.4-fold), and cytochrome P450 3A4 activity (7.9-fold). Lactate production in monolayer cultures switched into lactate elimination in the BALs, which is a hallmark of primary hepatocytes. Cell damage was 4-fold lower in 14-day-old BALs compared to monolayer cultures. In BAL cultures, transcript levels of cytochrome P450 1A2, 2B6, 3A4 and 3A7 genes and of the regulatory genes hepatic nuclear factor 4α and pregnane X receptor increased over time and were markedly higher than in monolayer cultures. In addition amino acid metabolism of HepaRG-AMC-BALs more resembled that of primary hepatocytes than monolayer HepaRG cultures.

Conclusions: BAL culture of HepaRG cells increases its hepatic functionality both over time as well as compared to monolayer. This is associated with a reduction in cell damage, upregulation of both regulatory and structural hepatic genes, and changes in amino-acid metabolism. These results confirm the high potential of HepaRG cells for BAL application.

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FULMINANT HEPATITIS LISTED FOR EMERGENCY LIVER TRANSPLANTATION IN FRANCE - EPIDEMIOLOGY AND OUTCOME
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The etiology of fulminant hepatitis (FH) varies in different countries. The aim of this retrospective study is to describe patients characteristics after wait-listing for emergency liver transplantation (ELT) in France and to determine their survival.

Patients: 808 patients were listed for LT on a super-emergency basis for FH from 1997 to 2010. At time of listing, 548 (68%) patients presented grade 3 or 4 hepatic encephalopathy. The mean values of total bilirubin, prothrombin time, and creatinine were 279 μmol/l, 16.6% and 176 μmol/l respectively. Data were obtained from the medical reports at Agence de BioMedecine and all liver transplant centers. Retrospective analyses was performed.

Results: The main causes of hepatitis were: viral 18.5%, paracetamol-induced hepatotoxicity 25.5%, non-paracetamol drug induced 9%, toxic 6%, indeterminate hepatitis 23%, and other causes 18%. The main cause of FH after 2002 was paracetamol compared to before 2002 (p < 10^-7). Overall, the peak of occurrence of FH was between 30 and 45 years (36%; n=258), and varied according to the etiology of FH. 587 (72%) patients underwent ELT, 109 (14%) presented grade 3 or 4 hepatic encephalopathy. The mean values of total bilirubin, prothrombin time, and creatinine were 279 μmol/l, 16.6% and 176 μmol/l respectively. Data were obtained from the medical reports at Agence de BioMedecine and all liver transplant centers. Retrospective analyses was performed.

Conclusion: Currently, in France, acetaminophen is the most common cause of FH. ELT in France is a system who allows LT in 94% of patients within 48 hours after listing. The survival is of 83% and 75% at 1 month and 1 year respectively. Criteria of LT should be reviewed because of the large number of patients listed but improved (14%).
THE INTENSIVE ARTIFICIAL LIVER SUPPORT SYSTEM IMPROVES SURVIVAL RATE OF FHF AND MAKES PERIOPERATIVE MANAGEMENT MORE APPROPRIATE

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Aim: The purpose of artificial liver support (ALS) is to sustain patients with fulminant hepatic failure (FFH) for long enough for the patient's liver to regenerate. In cases where the liver cannot regenerate, ALS should support liver function until transplantation is successfully performed. If ALS had the capability to sustain patients with FFH in a favorable condition, survival rates would be improved and the criteria for liver transplantation would be simpler and more accurate.

Method: Our study group of 159 patients comprised 90 cases of FH, 16 cases of LOHF, and 53 cases of severe acute hepatitis (SAH). Immediately after the onset of hepatic coma, patients were placed on ALS involving plasma exchange and hemodiafiltration using huge volumes of buffer. Treatment for underlying hepatitis consisted of immunosuppressive therapy and antiviral treatment.

Results: Of the 90 FH cases, 3 were the hyper-acute type and progressed to an ahepatic state. They were immediately placed on ALS, which sustained them in a good condition. One of the three patients subsequently underwent LDLTx and survived. Although the ALS system sustained the remaining two in a favorable condition for more than two weeks, they died because an organ donor was not found. Of the remaining FH cases, 42 were FH acute type and 36 of the 42 patients survived under ALS. The remaining 45 patients were FH subacute type and 32 of these survived. They were placed on the ALS system and underwent treatment for underlying liver disease. Four of the remaining 13 patients underwent LDLTx and 2 survived. The survival rate of LOHF patients under the same treatment as FH subacute type was 50%. Of the 53 SAH patients, 51 survived (96%). After several sessions of ALS, 109 of 116 (94%) patients regained consciousness and the 2-week survival rate was 107 of 116 (92.2%).

Conclusions: The Japanese treatment system for FH improved the prognosis of acute liver failure. The treatment system described in this study would sustain patients in good condition until the liver recovers or an adequate donor is found, and make perioperative management including organ sharing more appropriate.

THE USE OF CARDIOPULMONARY EXERCISE TESTING IN PREDICTING PRE- AND POST-OPERATIVE MORTALITY IN LIVER TRANSPLANT CANDIDATES

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Aims and Background: Accurate assessment of a patient's preoperative cardiorespiratory reserve could help identify those with high mortality risk on the liver transplant (LT) waiting list and postoperatively. Identification of such patients could help optimize the use of the limited organ donor supply. The aim of the study was to assess if variables measured in cardiopulmonary exercise testing (CPET) could predict death pre and post LT.

Methods: Patients with end-stage liver disease considered for LT were enrolled. Subjects underwent a maximally progressive CPET on an electronically braked ergometer. Expired gases were collected and analysed for minute ventilation (VE), ventilatory oxygen uptake (VO2), ventilatory carbon dioxide production (VCO2), peak work (watts), and anaerobic threshold (AT). Cardiac function was assessed with 12-lead electrocardiography. The test was terminated according to standard criteria including chest pain, fatigue, dizziness or failure to maintain the required rate on the ergometer. Standard biochemical and demographic data were also recorded. Continuous variables were expressed as medians with interquartile and analysed using the Mann Whitney U test. The chi squared test or Fishers exact test was used to analyse categorical variables.

Results: 107 Patients had CPET performed, disease etiologies included: ALD n = 34 (32%); HCV n = 16 (15%); NASH n = 3 (3%); PBC n = 5 (5%); PSC n = 9 (8.4%), HCC n = 18 (17%); Others n = 22 (21%), 75 (70%) of patients were male, median age 56 years (48–61 years). 36 (34%) patients had LT performed. Follow up post-LT was 694 days (IQR 283–1003). Of the pre-transplant variables assessed only AT predicted post LT mortality (p=0.04), (survivors AT 13 (12–15 mL/min/kg vs. death 10 (10.5–11)). 71 patients did not have a LT, of who 21 died (30%). The CPET variables that predicted death pre-LT were peak VO2 p<0.03 (survivors 21 mL/kg/min (18–25) Vs death18 (15–23)); and peak work, p=0.006 (survivors 115w (100–128) vs. death 78 (55–118)). A higher bilirubin (p=0.002), and higher UKELD also predicted death pre-LT.

Conclusions: CPET provides pre- and post LT mortality information. It should be used more commonly to prevent injudicious listing and LT in unsuitable candidates.

EARLY DIAGNOSIS OF LIVER FAILURE IN SEPTIC PATIENTS USING MAXIMAL LIVER FUNCTION CAPACITY TEST (LiMAX TEST). COMPARISON WITH CONVENTIONAL METHODS

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Introduction: Patients with bacterial sepsis often suffer from multiorgan failure. No reliable parameter exists for the exact estimation of the liver function. The indocyaninegreen-test (ICG-test) showed a correlation between liver function and mortality rate. The new Maximal Liver Function Capacity Test (LiMAX-test) is a realtime method to investigate the liver function. With the LiMAX-test we may detect a liver dysfunction in septic patients earlier than with other methods.

Methods: 30 septic patients were prospectively included in the study. The septic group was compared with a healthy group and a group of patients with a systemic inflammatory response syndrome (SIRS) after elective abdominal surgery. The LiMAX-test was performed on day 0, 2, 5 and 10 after sepsis onset and was compared with ICG-test and liver-specific laboratory parameters. The severity off illness was documented with the APACHE-II-score and during treatment with the SAPS-II- and SOFA-score. Primary endpoint was the mortality rate after 90 days. Secondary endpoint was the comparison with the ICG-Test. Beyond this, other parameters were correlated, such as LOS-hospital, LOS-ICU, dialysis and various infectious and liver parameters.

Results: The LiMAX-test showed low results initialy with increasing values on days 5 and 10. The 90 day mortality rate in patients with a low LiMAX-test on day 2 (<100 μg/kg/h) was significantly higher than in patients with a LiMAX-test >100 μg/kg/h. The LiMAX-test was comparable with the ICG-Test. Patients with indication for renal replacement therapy during hospital treatment showed significantly lower LiMAX-values than patients without dialysis. Other parameters such as Lactate, INR, Bilirubin, ASAT and ALT could not discriminate between mild and severe liver dysfunction.

Conclusion: With the LiMAX-test we can detect a liver dysfunction in septic patients early on day 0–2. The LiMAX-Test is equal with the ICG-Test and a result of <100 μg/kg/h on day 2 suggests a low probability of survival in septic patients.
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EXPRESSION ANALYSIS OF NF-κB IN PATIENTS WITH ACUTE LIVER FAILURE DURING PREGNANCY
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Background and Aims: There is paucity of information on the pathophysiological mechanisms of the hepatic damage during acute liver failure caused by hepatitis viruses. Nuclear Factor Kappa B transcription factor, a key regulator of genes involved in response to inflammation, infection and stress leads to production of inflammatory cytokines and apoptotic stimuli. The study was aimed to determine any role of NF-κB in the death of acute liver failure women during pregnancy.

Methods: A total of 60 patients were included in the study which constituted of 25 acute liver failure patients and controls consisting of 20 healthy blood donors, 10 healthy pregnant females and 5 autopsy cases. All the samples were subjected to serological analysis, PCR and nuclear protein extraction was carried out. Western blotting analysis was carried out to detect the expression of NF-κB using primary antibodies specific for p65 and p50 subunits.

Results: Mortality rate in the pregnant female acute liver failure patient was very high 75% (9/12) but no significant difference was observed between the pregnant and nonpregnant patients. The expression of p50 protein in blood was very high:78.94% (15/19) in the HEV infected acute liver failure cases while in majority of the cases 52.89% (11/19), p65 showed nil expression in the HEV infected cases. In the pregnant acute liver failure patients, expression of essential p65 was nil in 58.3% (7/12) cases and low in majority 60% (8/10) of the healthy pregnant patients showing nil expression in the HEV infected cases. The expression of p50 protein was very high in 6 cases (6/10, 60%) of the post mortem liver tissue whereas 3 (3/5, 60%) controls showed normal expression whereas in the surviving cases moderate expression of p50 was observed in the blood of all the six cases compared to p65 which showed low to nil expression in all the cases.

Conclusion: p65 is an essential component for normal functioning of the NF-κB complex and its absence causes increased apoptosis and liver degeneration leading to liver damage and death in acute liver failure patients confirming the anti-apoptotic role of NF-κB.

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INCREASED ACTIN PRODUCTION IN NEUTROPHILS IN ACUTE AND CHRONIC LIVER FAILURE REPRESENTS ADAPTATION TO FACILITATE CHEMOTAXIS, PHAGOCYTOSIS AND CYTOKINESIS
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Background: There is a marked propensity for patients with acute liver failure (ALF) and cirrhosis to develop sepsis culminating in multiorgan failure and death. Neutrophils are innate immune cells that play an active role during injury/infection. Neutrophil phagocytic dysfunction has been shown to be an important prognostic biomarker in ALF and cirrhosis but changes in the cytoskeleton, granules and mitochondria have not been studied. The aim of this study was to examine three specific ultrastructural components of the neutrophil: actin, mitochondria and lactoferrin granules in patients with acetaminophen-induced ALF (n=5) and cirrhosis (n=5) and in comparison with healthy controls (HC) (n=3). Actin is a 42kDa globular protein essential for microfilament production within the cytoskeleton. It is essential in cellular signalling, mobilisation and cytokinesis.

Methods: White blood cells from whole blood were isolated and prepared according to the Tokuyasu method. Thawed sections were initially stained with primary mouse (anti-actin and anti-mitochondria) and rabbit (anti-lactoferrin) antibodies independently and then with the corresponding secondary gold-conjugated antibodies. Stained sections were viewed on a FEI Tecnai 12 transmission microscope (TEM) operated at 120 kV. Images were acquired with an AMT 16000M digital camera. The gold particles present on the cells were counted using ImageJ software and relative labelling index (RLI) was calculated to determine the specificity of the stain (<1-random and non-specific labelling). The values were then analysed using chi-squared analysis. Plasma cytokines were also measured.

Results: Actin was increased in the cytoplasm of neutrophils in ALF and cirrhosis compared to HC [RLI: cytoplasm (>1) and nucleus (<1)] (Figure 1). There was no difference observed in the neutrophil mitochondria and lactoferrin particles between HC and patients. Plasma IL-6 and IL-8 concentrations were increased in the ALF and cirrhotic cohorts compared to HC.

Conclusion: Increased actin labelling within neutrophils isolated from patients with liver failure implies that these cells can appropriately adapt to facilitate cytoskeletal changes that will enable chemotaxis, phagocytosis and cytokinesis. How the increase in actin relates to increased susceptibility to infection warrants further investigation.

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LOW CIRCULATING HEPcidIN AND HIGH SERUM IRON LEVELS ARE ASSOCIATED WITH POOR PROGNOSIS IN ACUTE ON CHRONIC LIVER FAILURE (ACLF)
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Background: Acute-on-chronic liver failure (ACLF) is a serious ailment with very high mortality and limited treatment options. Heparicin is a major regulator of iron homeostasis. Deranged hepcidin synthesis accompanying liver failure could have a major impact on gastrointestinal iron absorption and recirculation from monocytes. Further, whether alterations of various proteins involved in iron regulation play a role in the pathogenesis of Multi Organ Failure (MOF) in ACLF is not clear.

Aim: To investigate the role of circulating hepcidin level in modulation of proteins involved in iron metabolism and predicting prognosis in ACLF patients with and without MOF.

Patients and Methods: We studied the associations between plasma levels of circulating hepcidin and iron regulating proteins (Ferritin, Transferrin, and Ceruloplasmin) and total iron in patients with ACLF-MOF (n=20), ACLF (n=20), chronic liver disease – CLD (n=20) and healthy controls (n=20).

Results: Circulating hepcidin level were found to be significantly down regulated in ACLF-MOF (1.09ng/ml) compared to ACLF
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(3.9 ng/ml), CLD (6.8 ng/ml), healthy control (6.6 ng/ml), [p = 0.02, 0.001, 0.001]. The Hepcidin/Iron ratio was found to be lowest (3.9%) in ACLF-MOF, compared to ACLF (19%) and CLD (22%) [p < 0.01]. Hepcidin/ferritin ratio was similarly lowest in ACLF-MOF (6.1%) than in ACLF (12%) or CLD (51%) [p < 0.01]. Hepcidin/transferin ratio was again lowest (37%) in ACLF-MOF than ACLF (84%), CLD (90%) [p < 0.01] and the ratio of Hepcidin/ceruloplasmin was 35%, 62% and 93% [<0.01] respectively. Circulating hepcidin levels inversely correlated to serum iron indices [r = -0.464, p = 0.003] in ACLF. ACLF patients with decreased circulating hepcidin and increased serum iron levels showed a significantly lower survival (p = 0.001, hazards ratio 13.19, CI 4.9–35.21), and higher development of MOF.

Conclusions: The ratio of hepcidin to other iron regulating proteins and total iron levels is reduced in patients with ACLF; more so in those with multiorgan failure. Altered levels of circulating iron and iron regulating proteins may help in predicting outcome and developing new treatment strategies in ACLF patients.

1004 PREDICTIVE CRITERIA FOR THE OUTCOME OF PATIENTS WITH FULMINANT HEPATITIS TREATED WITH MARS. UPDATE

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Background: The aim of this study was to confirm the improvement of prognostic parameters after treatment with the Molecular Adsorbent Recirculating System (MARS) in patients with fulminant hepatitis (FH) respect to analysis effected on 45 patients (pts) and published in 2009.

Materials: New twenty-one pts with diagnosis of FH were enrolled in this study for a total of 71 pts. Continuous variables were provided as medians and interquartile ranges. Continuous variables were compared with the Kruskal-Wallis test. A p-value <0.05 was considered statistically significant. A receiver operating characteristic (ROC) curve analysis was performed with the intent to validate the investigated score in predicting survival, need for LT or death: sensitivity and specificity were evaluated.

Results: The entire cohort was stratified in 2 groups: patients alive without LT (n = 28) and patients transplanted or dead before LT (n = 43). Thirteen pts (18.3%) died before LT and in 30 cases (42.3%) a LT was performed. ROC analysis was performed with the intent to evaluate the predictive role of the scoring system for LT or patient death. The score showed a high AUC (91.4%). The arbitrary cut-off values of 2 and 4 showed high sensitivity (81.4 and 48.8%) and specificity (92.9 and 96.4%), respectively. Patients corresponding to the low risk group (scoring points: 0–2; n = 36) did not experience deaths, and only 8 (23.5%) of them underwent a LT. Patients with intermediate risk (3–4 points; n = 22) were mainly transplanted (20 pts; 90.9%). In this group, only 2 patients survived without transplant. In the high risk group (5–6 points; n = 13), no patient survived. Only 2 (15.4%) pts were transplanted, while the remaining cases died before LT.

Conclusions: We were able to confirm that the following criteria: GCS ≥ 11 with ICP < 15 mmHg, lactate level < 3 mmol/L, TNF-α < 20 pg/ml, IL-6 < 30 pg/ml, and a change in hemodynamic instability from hyperkinetic to normal kinetic conditions, after 35 hours by the first MARS treatment, enabled us to divide patients into three groups and determine their outcomes.

1005 DOES LIVER IMPAIRMENT CORRELATE WITH EARLY MORTALITY IN HEART TRANSPLANT PATIENTS?

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Almost 25% of patients will die prematurely following heart transplantation (HT). Liver impairment, as decompensated cirrhosis, influence postoperative survival following cardiac non-transplant surgery. No data are available about liver impairment in non-cirrhotic patients previously to HT.

Aim: To analyze the prevalence of liver impairment at listing as a factor of early failure and death following heart transplantation.

Patients and Methods: Epidemiological and biological data in successive patients listing for HT between 2004 and 2011 were analyzed retrospectively. Exclusion criteria included combined transplantation (n = 4) and histological cirrhosis (n = 6). Uni and multivariate analysis with logistic regression then provided an evaluation of risks factors for early death (i.e. 3-months after transplantation – M3 death).

Results: 385 patients were analyzed (77.6% male), 49 ± 0.7 years-old, 35% redux, 49% UNOS I, 24% with ventricular assisted device (VAD). Four were on dialysis. HT causes were: dilated cardiomyopathy (47%), coronaryopathy (29%), valvulopar, hypertrophic or restrictive cardiomyopathy (9%), congenital or retransplantation (2%) each. 11.8% (n = 44) patients died during waiting time. Among the 323 HT patients, 98 (30%) died before month 3. In univariate analysis, M3 death was associated with (data expressed as median for continuous variable): total bilirubinemia (27.5 vs. 17 µmol/l, p = 0.001), creatininemia (129.5 vs. 101.5 µmol/l, p = 0.0007), AST (37 vs. 33 IU/l, p = 0.04), PAL (113.5 IU/l vs. 88, p = 0.002) or MELD score (161.1 vs. 117, p = 0.001), negative Rhesus group (35.6 vs. 12.5% p = 0.02), right ventricular failure (36 vs. 19%, p = 0.03), clinical ascites (47 vs. 27%, p = 0.006), Child B or C vs. A (39 vs. 20%, p = 0.008) or treatment with ARA II (43 vs. 25%, p = 0.03); no association was found with sex, blood group, age at listing, redux, invasive ventilation, VAD or vasopressive drugs. Logistic regression analysis only found ascitis (OR = 0.26, p = 0.04) and MELD score (OR=0.86, p=0.02) as independent variables at 3 months. Both variables were not associated with waiting list mortality nor 1 year post-transplant survival.

Logistic regression model with an area Under ROC Curve of 0.78 could correctly classify 79% of the patients.

Conclusion: In HT candidates, severe liver impairment characterized by ascitis or MELD score are independently associated with early post-transplant mortality.

1006 AN OVERVIEW OF LIVER TRANSPLANTATION IN ACUTE LIVER FAILURE

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Background: Acute liver failure (ALF) is a slashing syndrome that has high mortality rate. Liver transplantation (LT) is the treatment that can importantly improve survival rates and the prognoses of
those patients. Our aim was to analyze patient survival rate (PSR) and graft survival rate (GSR) between 2001 to 2011 for patients with ALF in different continents.

**Material and Methods:** We selected 25 articles with higher casuistic in different continents published between 2001-2011 (Fig. 1). In all articles the following subjects about LT were gathered: author; number of patients; etiology; types of liver transplantation [orthotopic liver transplantation (OLT) and living donor liver transplantation (LDLT)]; and their PSR and GSR from 1 to 5 years (Fig. 2).

**Results:** 2981 patients underwent LT therapy for ALF: North America (1948), Europe (569), Oceania (193), Asia (192) and South America (79). There were 1021 males and 1735 females (n = 2756). The most frequent etiologies were: Paracetamol intoxication, viral hepatitis and drug or toxic reactions. OLT happened 1979 times and LDLT 174 times (n = 2153). The overall PSR was 76%, 70%, and 67.8% at 1, 3, and 5 years, respectively, whereas GSR was 70.5%, 57.7%, and 59.5% at 1, 3, and 5 years, respectively.

**Conclusion:** LT dramatically improved the prognosis and the outcome of ALF patients, as without transplant survival rates were much lower. OLT with a whole graft from a deceased donor is the most effective therapy for ALF. The keys to an optimal outcome include a short waiting time, a large donor pool, good quality grafts, and a dedicated multidisciplinary transplant team. The mortality on the waiting list for deceased donor LT is high, principally in countries where donation rates are low.

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**PRE-OPERATIVE RIGHT PORTAL VEIN EMBOLIZATION (PRPVE) FOR INDUCING HEPATIC HYPERTROPHY**

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**Aim:** To evaluate effectiveness of PRPVE for inducing contralateral hepatic hypertrophy and decreasing risk of postoperative liver failure in patients with large or multiple resectable hepatic tumors.

**Methods:** Between 1997 and 2011, we performed 52 ultrasound-guided PRPVEs in pts with colorectal liver metastases (n = 30), hepatopancreatobiliary carcinoma (n = 14), large hemangioma (n = 5), breast cancer liver metastases (n = 3). For PRPVE, a mixture of lipiodol with 30–50 mg doxorubicin, gelatine sponge and ethanol was used. Computer tomographic liver volumetric studies were performed before and 4 weeks after PRPVE.

**Results:** No complications were observed after PRPVE. The mean volume of the non-embolized left liver lobe showed a 39.2% increase after PRPVE. Right or extended right hepatectomy was made in 26 pts in 28-50 (mean, 31) days. The remaining 26 pts were not operated because of revealing intra- or extrahepatic metastases. The mean intraoperative blood loss was 1.5L. One patient with colorectal liver metastases died from hepatic insufficiency on the 21st day after liver resection (the volume of remnant liver was 32%). No hepatic insufficiency was seen in 51 pts.

**Conclusion:** PRPVE is a safe and well-tolerated procedure decreasing both the intraoperative blood loss and risk of postoperative liver failure.

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**CHARACTER AND TEMPORAL EVOLUTION OF APOPTOSIS IN ACETAMINOPHEN-INDUCED ACUTE LIVER FAILURE**


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**Introduction:** Acute liver failure (ALF) is characterized by overwhelming hepatocyte death. Controversy exists as to whether apoptotic liver cell death plays a role in ALF. Circulating concentrations of apoptosis and necrosis markers (total, cleaved CK-18) have been proposed as biomarkers of disease severity in acetaminophen-induced ALF (AALF). There are conflicting results regarding the prognostic utility of these markers and the contribution of non-hepatic epithelial tissues require further evaluation. Therefore, we sought to assess the role of apoptosis during the clinical course of acetaminophen-induced ALF (AALF).

**Methods:** 88 patients with AALF were recruited from two liver transplant units. Pathological and healthy controls included patients with non-AALF (NAALF, n = 13), non-hepatic multi-organ failure (MOF, n = 28), chronic liver disease (CLD, n = 19) and healthy controls (HC, n = 11). Total and caspase-cleaved cytokeratin 18 (M65, M30) measured on admission and sequentially on day 3, 5, 10. Levels were also determined from hepatic, portal vein in seven patients undergoing transplantation. Protein arrays of liver homogenates from 6 AALF explants and 6 control liver tissue were assessed for apoptosis-associated proteins. Immunostaining of 5 AALF explants tissue was performed using immunohistochemistry to Ki-67/Hep Par-1 and Ki 67/CK-19.

**Results:** Admission M30 levels were significantly elevated in AALF (3644 pg/ml) and NAALF (3331 pg/ml) patients compared to MOF (1083 pg/ml), CLD (184 pg/ml) and HC (83 pg/ml; all p < 0.001). Admission M30 levels correlated with outcome with AUROC of 0.755 (0.639–0.885, p < 0.001). Peak levels in ALF patients were seen on admission and reduced at day 3–5 (867 vs 276; p < 0.05). A negative gradient of M30 from the portal to hepatic vein was demonstrated in AALF patients. Protein array data demonstrated lower apoptosis-associated proteins (SMAC-Diablo; HIF-1a; cIAP-2; all p < 0.05) and higher catalase (p < 0.05) concentrations in AALF liver compared to controls. Histological analysis revealed evidence of proliferation in biliary epithelial and hepatocytes with no evidence of apoptosis.

**Conclusions:** Hepatocellular apoptosis occurs in the early phases of human AALF, peaking on day 1 of hospital admission and correlates strongly with poor outcome. Hepatic regenerative/tissue repair responses prevail during the later stages of ALF where elevated...
levels of M30 are likely to reflect epithelial cell death in extra-hepatic organs.

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DEXAMETHASONE INDUCED HEPATIC DIFFERENTIATION OF RAT PANCREATIC PROGENITOR CELLS (B-13) IN A 3D MULTICOMPARTMENT BIOREACTOR SYSTEM

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Background and Aims: While primary human hepatocytes are suitable for in vitro research on liver cells, their use for clinical application in liver support systems is problematic due to their limited availability. Within the European project d-LIVER a rat pancreatic cell line (B-13) that has been shown to be able to trans-differentiate into hepatocyte-like cells (B-13/H) when treated with dexamethasone is under investigation. These cells express hepatocyte specific markers and liver-specific CYP enzyme activities in their B-13/H phenotype. However, to apply such a cell source for extracorporeal liver support in patients, cells have to be expanded and processed in a closed environment and in a culture vessel that allows for culturing sufficient cell numbers to support a human liver. In these studies, an experimental 3D multi-compartment bioreactor system offering dynamic 3D culture conditions has been examined for its ability to support B-13/H function.

Methods: Two different approaches were investigated in successive experiments. In the first approach, B-13 cells were inoculated and trans-differentiated in the bioreactor. In the second approach, trans-differentiated cells (B-13/H) were inoculated and maintained over 15 days in the bioreactor. Primary rat hepatocytes were used as a control culture and treated with the dexamethasone as for B-13 cells. Efficacy of trans-differentiation and cell viability were assessed by determination of liver-specific marker expression and function. With respect to the planned clinical use of the bioreactor system, a focus was laid on clinically relevant parameters.

Results: Initial studies showed successful growth and trans-differentiation of B-13 cells in small-scale bioreactors. Typical hepatocyte functions, including CYP enzyme activities were observed. Urea production was comparable to primary hepatocytes. With respect to clinical application in extracorporeal liver support, further optimization of culture conditions and up-scaling of the methodology for larger cell numbers is under investigation.

Conclusions: The results show that the 3D bioreactor system is suitable for culture and hepatic differentiation of the rat pancreatic progenitor cell line B-13. If a cell line of human origin with similar characteristics could be generated, it would be a promising cell source for bio-artificial liver support.

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PSYCHOSOCIAL EVALUATION OF CANDIDATES FOR LIVER TRANSPLANTATION PREDICTS POST TRANSPLANTAION OUTCOME

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Background and Objective: Psychological and social support is a major component in the recovery process following liver transplantation (LT). The pre-transplant psychological evaluation may serve as a tool for predicting the patient's prognosis. The aim of the present study was to determine the correlation between the pre transplantation psychological evaluation and the post transplant outcome; morbidity and mortality.

Methods: In this retrospective study, 93/100 patients (93%) underwent LT at the Hadassah Hebrew University Medical Center had a pre-LT psychosocial evaluation along 2000–2012. Insight, support system and compliance were evaluated by professional psychologist and social workers. Each parameter was scored on a 1 (optimal), 2 (sub-optimal) and 3 (worse) scales. Total score for each patient was a sum of the 3 parameters. Patients were analyzed according to optimal (total score of 3, 80% of cases) and non-optimal (total score<3) study groups. Post transplant outcome was correlated within both groups, focusing on survival, biopsy proven rejection episodes and complications.

Results: There was no significant difference in the mean age (50.3±14.4 vs. 51.5±10.1), gender (Males 67.7 vs. 56%), MELD (21.9±4.9 vs. 21.4±6.6), Prograf based regimen (41.2 vs. 44%) and etiologic distribution between the optimal and non-optimal groups. Incidence of infection episodes (35.3% vs. 52%, P=0.07) and renal complications (19.1% vs. 40%, P=0.02) were lower in the optimal group. However, the occurrence of other complications (rejection episodes, biliary complications, hyperkalemia, diabetes and recurrence of underlying disease) was similar. While post transplant follow up in both groups was similar (5±3.3 vs. 4.3±2.9 years, P=0.179), survival rate was significantly higher (P=0.001) in the optimal group; 85.3% vs. 56%.

Conclusions: In the Hadassah experience, optimal pre-LT psychosocial assessment predicts better long term outcome regarding survival, renal complications and infection episodes.
In contrast, necessity of RRT was associated with significantly higher mortality rates in patients not listed for transplantation (71% vs. 13%, p < 0.05). Multivariate regression revealed SAPSII score as the best independent predictor of 28d-mortality in these patients (HR = 1.055; 95%CI=1.029; 1.083; P < 0.001). SAPSII score >41 predicted 28d-mortality with a specificity of 99%, and sensitivity of 89.5% (AUROC = 0.924).

**Conclusion:** High SAPSII score and HE 3–4 on ICU admission were independent predictors for RRT in ALF. Although mortality in patients with AKI not eligible for LT was high, RRT should not be withheld in these patients a priori. SAPSII score on admission, but not AKI requiring RRT, was the best independent predictor of mortality in patients not eligible for LT.

**1012 SERUM CREATININE AND THE PRESENCE OF ENCEPHALOPATHY AT PRESENTATION MAY PREDICT MORTALITY IN CHILDREN WITH ACUTE LIVER FAILURE**

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**Background and Aims:** Acute liver failure (ALF) requires fast and efficient management, based on accurate prognosis factors. Our aim was to identify common clinical and biological parameters of prognostic value in a pediatric population with ALF.

**Methods:** We performed a cross-sectional study on 38 consecutive patients diagnosed with ALF in the 2nd Pediatrics Clinic, Cluj-Napoca, Romania, between January 2008 and October 2012. The inclusion criteria were: age between 0 and 18 years, no previous cause of encephalopathy, acute hepatic decompensation (including acute-on-chronic liver failure). Commonly available serum biomarkers at presentation were recorded: liver function tests (transaminases, total protein and albumin, coagulation parameters), glycemia, creatinin, sodium, potassium. We also assessed the degree of hepatic encephalopathy and calculated the PELD score in each patient. Overall survival at 45 days was assessed. Non-parametric statistic tests were used for data analysis.

**Results:** We included 38 children with a mean age of 7.43 years, 50% males. The most frequent etiology was mushroom poisoning (73.7%); the other etiologies were acute viral hepatitis (8.7%), drug intoxication (8.7%) and acute-on-chronic liver disease – Wilson’s disease (2.6%). Fifty percent of the patients died within the follow-up period. In univariate analysis, the following factors were found to positively or negatively correlate with short-term mortality: total bilirubin, transaminases, total protein and albumin, prothrombin time, INR, creatinin, sodium, PELD score and the presence of encephalopathy (p < 0.05 in all cases). In multivariate analysis, however, only hepatic encephalopathy and serum creatinin were found to independently predict mortality (R²=0.78, p < 0.001).

**Conclusion:** In our data set, high creatinin level and the presence of encephalopathy seemed to predict mortality in children with ALF. Interestingly, PELD score was not found to independently predict death, maybe because of the heterogeneity of the sets in terms of etiology and age. However, both creatinin and encephalopathy are linked with the severity of liver disease in adults, so the correlation is not circumstantial. Creatinin measurement and encephalopathy assessment are easy to perform in ER, and should always be performed in children with ALF.

**1013 CD163 IS A MECHANISTIC BIOMARKER IN ACUTE LIVER FAILURE REFLECTING A MACROPHAGE ACTIVATION LIKE SYNDROME**

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**Introduction:** CD163 is highly specific marker of macrophage (m) activation and a biomarker of disease severity in m-driven diseases such as haemophagocytic syndromes. m activation is a key determinant of systemic inflammatory responses and clinical outcome in acute liver failure (ALF). We sought to examine the prognostic utility, source and temporal evolution of CD163 expression in ALF.

**Methods:** In ALF patients, soluble CD163 (sCD163), MCP-1, TNF-a, IL-6 and IL-10 (all pg/ml) were determined on admission (n=76; acetaminophen-induced ALF [n=56; AALF]; non-acetaminophen-induced ALF [n=20; NAALF]) and sequentially (n=20), 15 patients with chronic liver disease (CLD) and 22 healthy volunteers (HC). Monocyte (CD14+) CD163 expression was determined in 37 AALF and 15 HC using flow cytometry. Regional levels (hepatic vein [HV], portal vein [PV]) of sCD163 were determined in 3 ALF patients at the time of transplantation. Immunohistochemistry was used to determine the mCD163 expression in 10 ALF (5 AALF/5 seronegative) explants. Using laser capture microdissection (LCM), CD163 concentration was determined in necrotic and non-necrotic areas of 3 AALF explants AALF explants and 3 control liver tissue using LC-SRM proteome analysis.

**Results:** AALF (1925 pg/ml), NAALF (2233 pg/ml), CLD (1408 pg/ml) patients had significantly higher sCD163 levels compared to HC (500 pg/ml; all p < 0.01). NAALF patients had significantly higher levels compared to AALF patients (p < 0.02). AALF patients with an adverse outcome (OLT/death) had significantly higher sCD163 levels compared to those who survived on supportive medical care (2180vs1430; p < 0.001). sCD163 reduced following transplantation (2280vs1067 pg/ml; p < 0.01) in AALF patients. Admission sCD163 levels strongly correlated with outcome (AUROC=0.83 [0.69–0.96; p=0.0002]), INR (r=0.6; p < 0.01), lactate (r=0.6, p < 0.01), pH (r=−0.52; p < 0.01), encephalopathy (r=0.63; p < 0.01), IL-6 (r=0.7; p < 0.01), IL-10 (r=0.47; p=0.02), MCP-1 (r=0.5; p < 0.01) and TNF-a (r=0.46; p=0.03). CD14+CD16-CD163+ expression was significantly reduced in AALF compared to HC (62.55% vs 91%; p < 0.001). Analysis of ALF explants reveals a dense mCD68+ infiltrate and elevated intrahepatic and regional (HV>PV) concentrations CD163.

**Conclusion:** Our data indicate that sCD163 is released from activated hepatic mCD68+ and circulating monocytes in ALF and strongly correlates with disease severity and outcome. Further studies are required to examine whether ALF represents a spectrum of macrophage activation syndrome.

**1014 Withdrawn**
1015 RESOLVIN D1 PROTECTS FROM ENDOTOXIN-INDUCED FULMINANT HEPATIC FAILURE BY REGULATING KUPFFER CELLS POLARIZATION IN D-GALACTOSAMINE-SENSITIZED MICE

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Background and Aims: Kupffer cells activation plays a key role in the pathogenesis of fulminant hepatic failure (FHF). Resolvin D1 (RvD1), an endogenous lipid mediator, display potent properties on switching off and resolving inflammation. In present study, we investigated the role and mechanism of RvD1 in the pathogenesis of FHF induced by Lipopolysaccharide/D-galactosamine (LPS/D-GalN), focusing on Kupffer cell polarization.

Methods: C57BL/6 mice were pretreated with RvD1 followed by LPS/D-GalN challenge to induce FHF. The mortality was assessed within 48 h, hepatic injury was evaluated by serum aminotransferases activities and hepatic pathological analysis, tumor necrosis factor (TNF-α) was measured by enzyme-linked immunosorbent assay (ELISA), classical (M1) and alternative (M2) macrophage markers were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Results: Pretreatment with RvD1 significantly attenuated LPS/D-GalN induced the mortality, hepatic injury, and TNF-α production. Moreover, mice in response to LPS showed an induction of hepatic M1 markers without affecting the M2 markers, RvD1 markedly down-regulated the expression of M1 genes and up-regulated M2 markers. In vitro study with isolated Kupffer cells (KCs) indicated that RvD1 inhibited LPS-induced M1 polarization and promoted Kupffer cells to shift M2 phenotype. Furthermore, KCs depletion by GdCl3 completely prevented LPS/D-GalN-induced FHF, adoptive transfer of KCs restored the hepatic susceptibility to LPS/D-GalN. However, adoptive transfer of RvD1-primed KCs prevented FHF induced by LPS/D-GalN.

Conclusions: These findings indicate that RvD1 has beneficial effects on LPS/D-GalN-induced FHF, which may be mediated by regulating Kupffer cells polarization from M1 to M2, thereby alleviating hepatic pro-inflammatory cytokine production.

1016 CIRCULATING HISTONES INDICATE DISEASE ACTIVITY AND EXACERBATE INFLAMMATION IN ACUTE LIVER FAILURE

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Introduction: Activation of systemic inflammatory responses syndrome (SIRS) may critically promote acute liver failure (ALF), but the key factors that trigger SIRS in this process are unknown. Circulating histones are a newly recognized mediator released and implicated in a variety of inflammatory states including sepsis, sterile organ injuries. It is likely that the release of histones, from dying hepatocytes or inflammatory leukocytes, into the circulation initiates and amplifies inflammation during the course of ALF. In this study we evaluated a murine model of ALF caused by D-galactosamine (GalN) plus lipopolysaccharide (LPS), and human plasma samples in patients with liver failure/injury to investigate a putative pathogenic role of circulating histones.

Methods: C57BL/6 male mice were given lethal doses of GalN/LPS to induce ALF. Hepatic function and histological indexes, myeloperoxidase (MPO) activity, and hepatocyte apoptosis as well as the concentrations of circulating histones were measured at predetermined time point. To further assess the role of circulating histones related to ALF, exogenous histones and anti-histone neutralizing antibody were administered, respectively. Meanwhile, human plasma samples from ALF patients were analyzed for the levels of circulating histones and the possible mechanisms by which histones mediate cytotoxicity.

Results: GalN/LPS caused severe liver damage in mice, as evidenced by the increased ALT levels and extensive hepatocyte necrosis or apoptosis, as well as a significant neutrophil/macrophage infiltration. Concomitantly, circulating histones were increased notably in the plasma of GalN/LPS-treated mice and correlated with the severity of liver injury. Exogenous histones aggravated GalN/LPS-induced hepatotoxicity remarkably, whereas anti-histone antibody significantly protected mice from death. Similarly, circulating histones were elevated considerably in the plasma of patients with ALF compared to healthy controls. It was found that plasma in ALF patients was cytotoxic to mouse primary hepatocytes and activated mouse nonparenchymal cells (NPCs), whereas anti-histone antibody reversed these effects.

Conclusion: Circulating histones may function as a marker indicating the activity of ALF. Besides, circulating histones contribute remarkably to ALF by exacerbating systemic inflammation, whereas blockade of circulating histones shows potent protective effects, thus suggesting a potentially therapeutic strategy in the future.
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URINARY KIDNEY INJURY MOLECULE-1 (KIM-1) IN THE ASSESSMENT OF ACUTE KIDNEY INJURY IN PATIENTS WITH CIRRHOSIS

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KIM-1 is a protein that is expressed in the kidney in the presence of tubular damage. Studies in the general population of hospitalised patients have shown that measurement of urine KIM-1 concentration (u-KIM-1) is useful to differentiate between acute kidney injury (AKI) caused by tubular damage and kidney injury due to pre-renal causes. So far, there have been no studies reported assessing the potential usefulness of u-KIM-1 in the assessment of AKI in patients with cirrhosis.

**Aim:** To investigate the usefulness of urinary KIM-1 levels in the differential diagnosis of acute kidney injury in cirrhosis.

**Patients and Methods:** 265 patients consecutively admitted for complications of cirrhosis were prospectively evaluated. AKI was defined using the Acute Kidney Injury Network criteria and patients were classified into 4 groups according to the cause of impairment of kidney function: pre-renal (pAKI), Hepatorenal Syndrome (HRS), intrinsic tubular damage (iAKI), and miscellaneous causes. KIM-1 was measured in urine using ELISA (Quantikine Human KIM-1, R&D Systems).

**Results:** One-hundred of the 265 patients (38%) developed AKI. Contrary to the expected results, u-KIM-1 values in patients with AKI were not significantly different from those in patients without AKI (median and interquartile range, p=0.342). In addition, u-KIM-1 was not useful in differentiating the cause of AKI (pAKI (n=25): 3.9 (1.3–5.0); HRS (n=36): 4.3 (2.9–8.0); iAKI (n=15): 2.4 (1.5–3.8) and miscellaneous (n=24): 4.3 (1.6–11) ng/mL, p=0.229). The urinary concentration of KIM-1 in patients with compensated cirrhosis (without AKI) was much higher than that in a group of healthy volunteers matched by age and sex (3.6 ng/mL (1.7–6.7) vs 0.8 ng/mL (0.6–11), respectively; p<0.001), which suggests the existence of an overexpression of KIM-1 in the kidneys of patients with cirrhosis independent of the presence of tubular damage.

**Conclusions:** Contrary to what has already been reported in the general population, measurement of urinary KIM-1 levels in patients with cirrhosis is not useful in either determining the presence or the cause of acute kidney injury. Physicians caring for patients with cirrhosis should be aware that cirrhosis increases the urinary levels of KIM-1.

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URINARY NGAL IS USEFUL TO PREDICT CAUSE AND SEVERITY OF KIDNEY FUNCTION IMPAIRMENT AND IS A PROGNOSTIC MARKER IN PATIENTS HOSPITALIZED FOR COMPLICATIONS OF CIRRHOSIS

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NGAL is expressed in the kidney as a consequence of tubular damage. Measurement of NGAL in urine (u-NGAL) in the general population of patients at hospital admission is useful to determine the cause of acute kidney injury and in-hospital mortality. Limited information exists regarding u-NGAL in cirrhosis.

**Aim:** To investigate whether u-NGAL is useful in determining the cause and severity of impairment in kidney function and prognosis in hospitalized patients with cirrhosis.

**Methods:** Prospective study of 265 consecutive patients admitted for complications of cirrhosis. Impairment of kidney function was evaluated with the Acute Kidney Injury (AKI) criteria and classified into 4 groups according to the cause: pre-renal (pAKI), Hepatorenal Syndrome (HRS), tubular damage or intrinsic (iAKI), and miscellaneous. NGAL was measured in urine at admission using ELISA (Bioporto, DK).

**Results:** One-hundred of the 265 patients (38%) had AKI at admission or developed it early after admission. Overall, patients with AKI showed higher levels of u-NGAL compared with those without AKI (73 (33–203) vs 32 (15–82) μg/g creatinine [median and interquartile range]; p=0.001). Patients with i-AKI had the highest levels, followed by patients with HRS, while patients with p-AKI and those with miscellaneous causes had values similar to those of patients without AKI. The best cut-off value to distinguish i-AKI from other causes was 195 [AUC: 0.86 (0.75–0.98)]. Using the median value in the whole series (44 μg/g creatinine) as cut-off value, the majority of patients with HRS or i-AKI (80%) belonged to this group. A high level of u-NGAL (>44 μg/g creatinine) was associated with a more frequent progression of AKI, greater need for dialysis, and, most importantly, higher mortality or transplantation rate both in hospital and at 3 months (23% and 51%, respectively, compared with 8% and 24% in patients with low levels of uNGAL, p<0.01). u-NGAL was an independent predictive factor of mortality together with serum bilirubin, serum sodium, and hepatic encephalopathy.

**Conclusions:** Measurement of urine NGAL in patients admitted for complications of cirrhosis is useful not only to help establish the cause and severity of impairment of kidney function but also as prognostic marker.
1020 POOR AGREEMENT OF DIFFERENT APPROACHES TO ASSESS BACTERIAL TRANSLOCATION IN PATIENTS WITH DECOMPENSATED CIRRHOSIS

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Background: The detection of bacterial DNA fragments (bactDNA) in sterile non-neutrocytic ascitic fluid (AF) has been interpreted as a correlate of bacterial translocation (BT) and indicates poor survival in non-infected patients with cirrhosis. Furthermore, identification of bactDNA in spontaneous bacterial peritonitis (SBP) may allow targeted therapy in the absence of positive bacterial cultures. However, owing to different methods, contradicting results regarding incidence, identified pathogen spectra and clinical relevance of bactDNA have been reported hampering translation into clinical practice.

Aims: To compare sensitivity, accuracy and concordance of different methods for bactDNA detection.

Methods: AF from 52 patients with decompensated cirrhosis and clinically suspected infection was analyzed for bactDNA and Lipopolysaccharide-binding protein (LBP). BactDNA was identified using three different approaches: using multiplex PCR and subsequent hybridization to reporter probes (method A), in-house 16s rRNA gene PCR and gel electrophoresis (B) and 16s rRNA gene PCR and subsequent mass spectrometry (C).

Results: 13 (25%) patients presented with SBP, 2 (4%) with monomicrobial bacterascites and 37 (71%) had sterile non-neutrocytic ascites. BactDNA was detected in 7 (78%) culture-positive AF samples by method A, 5 (56%) samples by method B and 2 (22%) samples by method C. Moreover, bactDNA was identified in 3 (A), 0 (B) and 2 (C) of 6 culture-negative SBP samples using the respective methods. Bacterial species were correctly identified in 6 cases by method A and 2 cases by method C. In patients with sterile non-neutrocytic ascites, the prevalence of bactDNA was 32% using method A, 30% using method B and 10% using method C. The degree of agreement between the methods was poor (A/B: Cohen’s kappa 0.18; A/C: \( k = -0.19; B/C: k = 0.13 \)). Only the detection of bactDNA by method A demonstrated a fair concordance with the highest quartile of AF LBP concentration as an alternative marker of BT (\( k = 0.38 \)), whereas concordance was poor for any of the other methods (B: \( k = 0.13; C: k = -0.16 \)).

Conclusions: We observed a poor agreement of different surrogate markers to assess BT in cirrhosis. A gold standard for detecting bacterial DNA fragments has to be defined and thoroughly validated before recommending translation into clinical practice.

1021 CARDIAC DIASTOLIC DYSFUNCTION IS ASSOCIATED WITH IMPAIRED NATRIURESIS IN PATIENTS WITH DECOMPENSATED CIRRHOSIS AND ASCITES

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Background: Although several factors have been associated with the pathogenesis of diastolic dysfunction (DD) in patients with decompensated cirrhosis, the relationship of DD with the degree of liver and renal dysfunction has not been fully elucidated.

Aim: To investigate the factors significantly associated with the presence of DD in patients with decompensated cirrhosis.

Methods: In the study we included patients with decompensated cirrhosis and ascites who were admitted in our Department and had complete clinical and laboratory data including glomerular filtration rate (GFR) measured by 51Cr-EDTA. We assessed the independent factors associated with the presence of DD, while their discriminative ability was evaluated by AUC curve. The diagnosis of DD was based on Doppler echocardiography and classified into three categories according to the current guidelines.

Results: We evaluated 100 patients (70 men, mean age 54±11 years, systolic blood pressure: 112±13 mmHg) with decompensated cirrhosis and ascites. Sixty nine patients (69%-group 1) had DD (44 patients grade I, 13 patients grade II, 12 patients with grade III), and 31 (31%-group 2) had no DD. At baseline, group 1 patients, compared to group 2, were older (55±12 vs 47±14 years, \( p = 0.005 \)) with higher body weight (76±14Kg vs 69±10Kg, \( p = 0.021 \)) and heart rate (68/min vs 64/min, \( p = 0.033 \)), and they had higher Child–Pugh score (10±4 vs 7±3, \( p = 0.03 \)). In addition, group 1 patients, compared to group 2, had significantly lower serum albumin (2.9±0.6 vs 3.2±0.5 g/dL, \( p = 0.04 \)) and 24-hour urine sodium excretion [24UNa: 52 (range: 2–408) vs 83 (range: 3–247) mmol/day, \( p = 0.031 \)]. In multivariate logistic regression analysis, 24UNa (OR: 0.98, 95%C.I.: 0.97–0.99, \( p = 0.007 \)) and heart rate (OR: 1.2, 95 C.I.: 1.1–1.3, \( p = 0.02 \)) were the only factors significantly associated with the presence of DD, while both of them had good discriminative ability (AUC: 0.72 and 0.70, respectively) for the presence of DD. Group 1 patients, compared to group 2, had similar GFR (73±22 mL/min vs 79±23 mL/min, \( p = 0.38 \)). At the end of follow up [9 months (range: 6–26)], survival was similar between the 2 groups of patients (85% vs 90%, \( p = 0.51 \)).

Conclusions: In our cohort of patients with decompensated cirrhosis and ascites, DD had no adverse effect on the mid-term outcome and it was independently associated with lower 24UNa.

1022 HEPATIC ENCEPHALOPATHY IS A SIGNIFICANT PREDICTOR OF MORTALITY FOLLOWING TIPS INSERTION FOR REFRACTORY ASCITES

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Background and Aims: Transjugular intrahepatic portosystemic shunt (TIPS) insertion has been used for over twenty years to treat the complications of portal hypertension. TIPS insertion provides better control of refractory ascites than large volume paracentesis but with a higher risk of developing hepatic encephalopathy (HE). However, a survival benefit has only been found in carefully selected patients. The aims of this study were to review the use of TIPS for the treatment of refractory ascites, over a twenty-year period, with the aim of identifying factors predictive of the development of HE and of survival.

Methods: All patients who underwent TIPS for refractory ascites between 1992 and 2012 at the Royal Free Hospital, London, were reviewed. All patients alive in 2012 who had not undergone liver transplantation were recalled for assessment of their neuropsychiatric status using clinical, neuropsychometric and neuropsychological variables. Factors associated with the development of post-TIPS HE and with survival were determined by multivariate analysis using Cox proportional regression model.

Results: Of the 169 patients identified, 96 (56.8%) had died, 22 (13.1%) had been transplanted while the remaining 51 were alive. The median survival time was 18.8 mo (95% CI 12.2:5.4). The
factors predictive of death were a higher serum AST $(p < 0.04)$, ALP $(p < 0.02)$, sodium $(p < 0.02)$, and INR $(p < 0.01)$ and the development of HE $(p < 0.000)$. Of the 51 patients known to be alive, 27 were available for review. Of these, 21 (78%) had some grade of HE; less than 30% were on anti-encephalopathy treatment. The factors predictive of HE were older age $(p < 0.01)$, with the risk of developing HE increasing by 6.5% for every year of age, and non-British white ethnicity $(p < 0.03)$.

**Conclusions:** HE is one of the most important predictors of mortality post-TIPS. Patients are often unaware of the risk of HE development and are rarely monitored beyond the immediate post-TIPS period. Better assessment of the risk of developing HE pre-TIPS as well as closer and longer term follow-up and treatment may help preventing the development of HE and hence improve survival.

### 1023 ACTIVATION OF THE AIM2 INFLAMMASOME IS ASSOCIATED WITH THE SERIOUSITY OF LIVER DISEASE AND THE INFLAMMATORY RESPONSE IN CIRRHOTIC PATIENTS WITH STERILE ASCITES

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**Background:** The inflammasome is a cytosolic multiprotein complex that triggers the activation of Caspase-1 and the maturation of IL-1β and IL-18. Different PRRs (e.g. NLRPs) are able to form inflammasomes upon recognition of different PAMPs and/or DAMPs. Absent in melanoma 2 (AIM2) triggers inflammasome formation in response to cytosolic dsDNA irrespective of its origin. Here, we investigate the role of different inflammasomes in the inflammatory response and the severity of liver disease in cirrhotic patients with sterile ascites.

**Methods:** Ten healthy controls and 60 patients with cirrhosis and non-neutrocytic ascites were included in the study. Ascitic fluid (AF)- and PBMC-derived macrophages were isolated and transfected with polydA:dT to activate the AIM2 inflammasome. Inflammasome activation was evaluated by immunoblot (caspase-1-p20) and ELISA (IL-1β/IL-18) in culture supernatants and AF.

**Results:** AF-macrophages showed an exacerbated inflammasome activation when compared to PBMC-macrophages isolated from the same patients or healthy controls. qPCR and Immunoblot analysis showed a marked increase in the basal expression of AIM2 (but not NLRPs or NLRC4) in both PBMC- and AF-macrophages from the ascitic patients. Moreover, stimulation with bacterial DNA or LPS further enhanced the expression of AIM2 as well as the mRNA levels of Caspase-1 and IL-1β in PBMC-macrophages from both patients and controls. Functional activation of the AIM2 inflammasome in PBMC-macrophages required previous stimulation with TLR ligands, with TLR9 having a much higher effect than the other TLRs tested. Unlike PBMC-macrophages, AF-macrophages did not require TLR pre-stimulation to mount a robust AIM2-inflammasome response, demonstrating the highly pre-activated state of these cells. Accordingly, positive detection of bacterial DNA in the AF of a subgroup of patients (31.6%) was associated with higher inflammasome activation. Noteworthy, Child–Pugh class C patients showed significantly higher inflammasome activation and IL-1β levels in blood and AF than those with a Child–Pugh class B.

**Conclusions:** The activation of the AIM2-inflammasome in response to dsDNA triggers an exacerbated inflammatory response in sterile AF. Notably, inflammasome activation also correlates with a **higher degree of liver disease**. These results lay the foundation to investigate novel therapeutic options aimed at blocking the activity of this inflammatory pathway in ascitic patients.

### 1024 RELAXIN IS A RENAL VASODILATOR IN EXPERIMENTAL MODELS OF CIRRHOSIS AND A POTENTIAL NOVEL THERAPY FOR HEPATORENAL SYNDROME (HRS) IN HUMANS

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**Background:** HRS is a feared complication of cirrhosis with a high mortality rate and limited treatment options. The hallmark of HRS is profound renal vasconstriction, resulting in functional renal failure but normal kidney histology. The peptide hormone relaxin (RLN) mediates maternal haemodynamic adaptations to pregnancy, including increased renal blood flow (RBF) and glomerular filtration rate (GFR). We hypothesised that RLN could modulate RBF in cirrhosis.

**Methods:** Cirrhosis was induced in rats by 16 weeks i.p. carbon tetrachloride (CCl4) and decompensated biliary cirrhosis by 3 weeks bile duct ligation (BDL). We measured the effect of acute i.v. and extended (72hr) s.c. RLN on systemic haemodynamics, RBF, GFR and organ histology. Subgroups of rats were co-treated with the nitric oxide (NO) synthase inhibitor L-NAME. Blood oxygen dependent-magnetic resonance imaging (BOLD-MRI) was used to quantify changes in renal oxygenation. Tissue expression and distribution of RLN receptor (RXFP1) was determined by qPCR and immunohistochemistry. Expression of vasoconstrictor genes was quantified by qPCR array.

**Results:** RXFP1 was detected in glomerular podocytes, renal pericytes, renal, segmental and interlobar arteries of cirrhotic rats. In CCl4 cirrhosis, acute i.v. RLN (4μg) induced a 50% increase in RBF after 60 minutes $(p < 0.01$ vs. placebo, $n=6)$. BOLD-MRI showed increased tissue oxygenation at the same timepoint in renal cortex and medulla. Extended s.c. RLN increased RBF by 54% in CCl4 $(p < 0.01$ vs. placebo, $n=8)$ and 87% in BDL $(p < 0.05$ vs. placebo, $n=3)$ and increased GFR by 138% in CCl4 $(p < 0.01$ vs. placebo, $n=8)$ and 70% in BDL $(p < 0.05$ vs. placebo, $n=3)$. Mean arterial pressure was unaffected by RLN. L-NAME (250 mg/L) p.o. abrogated the effect of RLN on RBF and GFR. Relative expression of vasoconstrictor genes in kidney was markedly reduced by RLN treatment.

**Conclusion:** RLN increased RBF in experimental cirrhosis. Critically, RLN also improved renal function and oxygenation but did not induce systemic hypotension even in decompensated disease. The effects of RLN were mediated via augmentation of NO and downregulation of vasoconstrictor genes known to be important in the pathogenesis of HRS. RLN has potential as a treatment for HRS and further translational studies are warranted.

### 1025 MARKERS OF ENDOTHELIAL DYSFUNCTION AS PREDICTORS OF ASCITIC DECOMPENSATION IN PATIENTS WITH LIVER CIRRHOSIS

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**Background and Aims:** Natural history of liver cirrhosis (LC) is accompanied by an intra- and extrahepatic endothelial dysfunction (ED), which is considered to have a pivotal role in the development of portal hypertension (PH). Serum levels of markers of ED (MED) are increased in LC patients, and they correlate with the stage of liver disease. Aims of the present study were to assess, (1) differences between MED serum levels in patients with compensated and decompensated LC; (2) possible prognostic
role of MED in the development of ascites in patients with compensated LC.

Methods: 90 consecutive LC patients (mean age 65±9 years, 24 female) underwent a complete clinical, radiological and biochemical evaluation in order to assess clinical characteristics and the stage of disease; all subjects were assessed for MED [P-selectin, von Willebrand factor (vWF), endothelin-1 (ET-1), thrombomodulin (TM) and nitric oxide (NO)] serum levels. The 70 patients (mean age 65±9 years, 19 female) with compensated LC (no ascites, cLC) underwent a 2 years-follow-up for ascites development; their data were also compared with those of 20 (mean age 63±10 years, 5 female) LC patients with decompensated LC (presence of ascites, dLC) and those of 11 healthy controls (mean age 26±6.6 female).

Results: ET-1, P-selectin and TM serum levels were significant higher in LC in and dLC patients with respect to controls. NO e vWF serum levels were higher in dLC patients, whereas no difference was observed in cLC with respect to controls. 33/70 (47.1%) of cLC patients developed ascites during follow-up. At univariate analysis, predictors of ascites development in cLC patients were serum concentrations of ET-1 (OR=3.56, p=0.000), TM (OR=1.95, p=0.000) and P-selectin (OR=1.03, p=0.004) and Child–Pugh score (OR=1.05, p=0.041). At multivariate analysis (Cox regression), serum ET-1 and diabetes were independent predictors of early development of ascites during the two-years follow-up period (HR=2.631, p=0.004) in cLC patients. Efficiency (measured by ROC method) of high levels (cut-off value=6 pg/ml) of ET-1 in predicting the ascites development was good (AUC=0.803).

Conclusions: Among serum MED, vWF, ET-1, NO, P-selectin and TM resulted significantly higher in dLC patients as compared to cLC patients. Plasma ET-1 resulted as an independent predictor of ascites development in cLC patients. Considering also the possible pathophysiological role of ET-1 in PH development, this result may have important implications in early detection and prevention of ascites in cLC patients.

1026 CULTURE-INDEPENDENT CHARACTERISATION OF VIABLE BACTERIA IN ASCITES REVEALS A BROAD RANGE OF SPECIES INCLUDING THOSE OF NON GUT ORIGIN

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Introduction: Identification of pathogenic bacteria in ascites correlates with poor clinical outcomes. Ascites samples are commonly reported culture-negative by traditional microbiology, even when frank infection is evident. Culture-independent methods have previously reported bacterial DNA in ascites, however, whether this represents viable bacterial populations has not been determined. We report the first application of 16S rRNA gene pyrosequencing in conjunction with propidium monoazide sample treatment to characterise the viable bacterial composition of ascites.

Methods: Twenty five cirrhotic patients undergoing paracentesis provided ascites. Samples were treated with propidium monoazide to exclude non-viable bacterial DNA. Total bacterial load was quantified by 16S rRNA Q-PCR with species identity and relative abundance determined by 16S rRNA gene pyrosequencing. Clinical measures and diagnostic microbiology were recorded as routine. The composition of the microbiota revealed was correlated with clinical parameters.

Results: Viable bacterial signal was detected in 84% of ascites samples, both by Q-PCR and pyrosequencing (mean bacterial densities of 1.5 x 10⁶ cfu/ml equiv., std dev 1.8 x 10⁵, n=21). Bacteria were also detected in patients with normal ascetic WCC and no clinical evidence of SBP. Approximately 190,000 ribosomal pyrosequences were obtained, representing 236 species across diverse array of primarily opportunistic pathogens including both gut and non gut-associated species. There was high species variation in the ascites microbiota between patients with high relative abundance species commonly unique to one patient. Statistically significant relationships were identified between the composition of the bacterial communities detected and clinical measures, including ascitic white cell count (clinical evidence of SBP) and Child-Pugh score.

Conclusions: Viable bacteria are present in the ascites of a majority of patients with cirrhosis with those with no clinical signs of infection. Entry of bacteria into ascites is not limited to translocation from the gut, raising fundamental questions about the processes that underlie the development of spontaneous bacterial peritonitis. The ascitic microbiota composition correlates with clinical status.

1027 COMPARTMENTAL REGULATION OF suPAR IN PATIENTS WITH DECOMPENSATED CIRRHOSIS: EVALUATION OF ORIGIN, REGULATION AND PROGNOSTIC RELEVANCE

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Background: Patients with decompensated cirrhosis are susceptible to bacterial infections that are associated with organ failure and a high mortality. Reliable biomarkers that predict unfavorable outcomes are essential to identify high-risk patients who require intensified treatment. In non-cirrhotic patients with infections and sepsis, elevated serum levels of soluble urokinase plasminogen activator receptor (suPAR) predict mortality but little is known about the regulation and short-term prognostic relevance of suPAR in patients with advanced cirrhosis.

Patients and Methods: To study suPAR in serum and ascitic fluid (AF) samples from 162 consecutive patients with decompensated cirrhosis undergoing paracentesis for suspected bacterial infection were enrolled in this study. suPAR levels were assessed by ELISA and prognostic value was calculated by cox-regression and Kaplan–Meier curve analysis. Ex vivo, immune cell subsets were stimulated with varying concentrations of TNF-alpha and various Toll-like-Receptor Agonists and suAPR release was measured. Surface-bound uPAR was determined by flow cytometry. Monocytic uPAR expression was quantified by RT-PCR.

Results: Circulating suPAR levels were increased in decompensated compared with compensated cirrhosis, correlated with the severity of liver dysfunction and surrogate markers of systemic inflammation and liver-related mortality but were not indicative of bacterial infection. Circulating suPAR equaled MELD in 28-days mortality prediction (AUC 0.711 for suPAR and 0.705 for MELD) and circulating suPAR levels above 14.4 ng/ml predicted 28-day mortality even after adjustment for MELD score and confounders (hazard ratio 3.0), whereas cut-off levels derived from non-liver cohorts were not applicable due to low specificity. AF suPAR levels were elevated during SBP but not during bacterascites or bacterial translocation. They correlated poorly with systemic suPAR but were associated with a more severe course of SBP and worse outcome.

In vitro experiments revealed that monocytes and to a lesser extent
neutrophils secrete suPAR after Toll-like-receptor ligation, which led to rapid uPAR (CD87) cleavage followed by increased synthesis. **Conclusions:** Blood and ascitic suPAR levels provide distinct but relevant prognostic information on the severity of infectious complications in end-stage liver disease. suPAR hence represents a useful biomarker to detect disease severity and to predict outcome in patients with decompensated liver cirrhosis and might individualise disease management.

03a. LIVER TUMOURS: EXPERIMENTAL

1028 EARLY FIBROSIS INHIBITS HEPATOCELLULAR CARCINOMA MODELS, ASSOCIATED WITH OXIDATIVE STATUS ALTERATIONS

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**Background:** Hepatic-stellate-cells (HSCs) activations are the key of hepatic-fibrosis and cirrhosis progressions. Cirrhosis is a main risk factor for hepatocellular-carcinoma (HCC) development. However, direct HSCs role in HCC-progression is not fully understood.

**Aims:** To study the in-vitro and in-vivo interactions between HCC and HSCs in an early and advanced fibrosis models.

**Methods and Results:** in-vitro; cultured HCC cell-line (Hep3B) secreted high levels of alpha-feto-protein (αFP) in medium. Human HSCs (LX2 cell-line) co-cultured with Hep3B-cells significantly decreased αFP secretions and increased their apoptosis. Activated LX2 (with leptin) co-cultured with Hep3B-cells further suppressed αFP levels. These effects were associated with increase in the reactive oxygen species and decreased in reduced glutathione levels (p < 0.05). Confocal microscopy demonstrated Hep3B-phagocytosis inside the LX2-cells suggesting a direct cellular-contact mediating anti-tumor effect. In-vivo; the HCC/HSCs interactions were studied in nude-nu mice through models of intra-hepatic injections. Hepatic tumor sizes and serum αFP were then assessed. In these models, mice with “advanced liver-fibrosis at time of tumor growth” had higher tumor size and serum αFP compared to non-fibrotic livers. However, mice with “early liver-fibrosis at time of tumor growth” had a significant decrease in tumor and high Malondialdehyde serum levels compared to advanced-fibrosis animals. Although serum αFP levels maintained same pattern at all tested time points of liver tumors; significance in their levels were noticed only at end of the first week.

**Conclusions:** Cirrhosis, as end-result of HSCs-activation with advanced fibrosis and angiogenesis, is an indirect pro-HCC condition. However, at early fibrosis stages, activated-HSCs express direct anti-tumor effects by phagocytosis and apoptosis of tumor-cells mediated by oxidative status imbalance. These findings support the growing evidence that HSCs play an integral role in promoting HCC progression through their oxidative status and reinforces the importance of HSCs as a therapeutic target for HCC.

1029 POTENT ANTI-CANCER EFFECT OF CROCIN AGAINST HEPATOCELLULAR CARCINOMA: A PRECLINICAL STUDY

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**Background and Aims:** This is a follow up of our study published in September issue of Hepatology and featured in October issue of Science News that documented the anticancer potential of saffron. Here, we show the chemopreventive actions and mechanisms of saffron-based bioactive ingredient; crocin, against diethylnitrosamine (DEN)-induced liver cancer in rats.

**Methods:** Administration of crocin at two doses of 100 and 200mg/kg body wt per day was started two weeks prior to the DEN injection and was continued for 22 weeks.

**Results:** Crocin decreased the number and the area of placent al glutathione-S-transferase positive foci in livers of DEN-treated rats. Furthermore, crocin counteracted DEN-induced oxidative stress in rats as assessed by restoration of superoxide dismutase, catalase, and glutathione-S-transferase levels and diminishing of myeloperoxidase activity, malondialdehyde and protein carbonyl formation in liver. The results of immunohistochemical staining of rat liver showed that crocin inhibited the DEN-mediated elevations in numbers of cells positive for Ki-67, cyclin, DNA synthesis, 2, inducible nitric oxide synthase, nuclear factor-kappa Bp-65 and the phosphorylated tumor necrosis factor receptor. Crocin also blocked the depletion in the number of cells positive for TUNEL and M30 CytoDeath in liver tissues of DEN-treated rats. In vitro experiments carried out using HepG2 cells also confirmed these findings and showed inhibition of NFkB activation, increased cleavage of caspase-3, as well as DNA damage and cell cycle arrest upon saffron treatment.

**Conclusions:** The present study provides evidence that crocin exerts a significant chemopreventive effect against liver cancer through inhibition of cell proliferation and induction of apoptosis. This report also shows some evidence that crocin protects rat liver from cancer via modulating oxidative damage and suppressing inflammatory response. This study is funded by Emirates Foundation.

1030 STEM CELLS COMPARTMENT ACTIVATION DURING HEPATOCARCINOGENESIS IN A HBV-TRANSGENIC MOUSE MODEL

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**Background and Aims:** Hepatocellular Carcinoma (HCC) represents more than 85% of primary liver tumor. Several studies had demonstrated the up-regulation of stem cell (SC) markers in HCC supporting the cancer stem cell theory, but there is limited data on preneoplastic stages. We employed male HBV-transgenic mice C57BL/6J-TG(ALB1HBV)44BRI/J (TG) which mimic the natural history of HCC and their normal counterpart C57BL/6j mice (CTRL) as controls to follow the hepatocarcinogenesis process from early inflammation to HCC. We followed the expression of the SC markers CD34, CD90, CD133, cytokeratin-19 (CK19), Sca1, and alpha fetoprotein (AFP) in liver tissue together with serum ALT and AST, at several stages of injury: 3 (inflammation), 6 and 9 (preneoplastic lesion), and 12 (HCC) months of age.

**Methods:** A total of 423 tissue samples from TG and CTRL were analyzed using RT-qPCR and IHC, FACS, and WB for gene and protein expression, respectively. The isolation of hepatic SC and cancerous SC from each indicated ages was evaluated by the expression of the SC markers together with hepatocytes markers (albumin and CK18) and fibroblast (FSP1) markers. The clonogenic capability was evaluated by 3D matrigel assay.

**Results:** In the TG model, starting from 3 months ALT and AST level was higher compared to CTRL (p < 0.001 and p < 0.05, respectively). Gene analysis showed an interesting pattern of SC markers in its correlation with disease progression. CD34, CD133, CK19, Sca1 and AFP expression was significantly up-regulated in TG compared to CTRL and increased moving from inflammation to HCC. No dysregulation of CD90 was observed. The up-regulation and spread
of CD34 cells in hepatic parenchyma in HCC tissues was confirmed by IHC analysis. Cells isolated from TG (cancerous SC) and CTRL (SC) showed up-regulation of the SC markers CD34, CD90, CD133 and CK19 compared to total tissue and low or no expression of hepatocytes markers. IHC and FACS confirmed the positivity of CD34, CD133 and CD90, mainly in small cells subpopulation. These cells were able to form 3D clones in matrigel after 7–10 days plating.

**Conclusion:** These data indicate that during hepatocarcinogenesis the activation and the dysregulation of SC compartment occur.

### 1031 FUNCTIONAL ROLE OF B CELLS DURING DEVELOPMENT AND PROGRESSION OF HEPATOCELLULAR CARCINOMA


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**Background:** B cells have been implicated to play a pivotal role during chronic inflammation and carcinogenesis in various tissues. While it has been appreciated that B cells promote hepatic fibrogenesis resulting in a pro-tumorigenic environment, the impact of B cells during hepatic carcinogenesis has yet not been systematically investigated. Recently, our group revealed that intrahepatic expression of the B cell attracting chemokine CXCL13 is significantly increased in patients with hepatocellular carcinoma (HCC) but not at earlier stages of chronic liver diseases (n = 132, unpublished).

**Methods:** Intrahepatic CXCL13 expression was visualized by immunohistochemistry (IHC) and serum CXCL13 levels were assessed in patients with chronic liver diseases with or without HCC. Moreover, infiltration of B cells and plasma cells was quantified by immunofluorescence staining. The hepatoma cell-line Hepa129 was stimulated with B cell conditioned medium and proliferation was assessed by BrdU incorporation. HCC was induced in B cell deficient mice (lghm−/−) and corresponding wildtype mice by application of DEN at 15 days of age followed by tumor growth promotion through weekly CCl4 application. Mice were sacrificed at 19, 22 and 26 weeks of age and tumor burden was analyzed.

**Results:** Patients with HCC display higher levels of serum CXCL13 as compared to patients with mild or advanced fibrosis without malignant lesions. By IHC, carcinoma cells could be identified as major sources of CXCL13. Immunofluorescence staining revealed a significant increase of infiltrating CD20+ B cells and CD38+ plasma cells (both P < 0.001) within HCC stroma as compared to fibrotic liver tissue. Stimulation of Hepa129 cells with conditioned medium of B cells augments proliferation as compared to control medium. Constitutively B cell deficient have a substantially delayed HCC progression in an experimental HCC model as demonstrated by significantly decreased liver/body weight ratio and total tumor number compared to wildtype mice in sequential analysis (all P < 0.05).

**Conclusion:** During hepatic carcinogenesis, high intratumoral CXCL13 expression is associated with an enhanced accumulation of B cell and plasma cell in HCC lesions, which promotes carcinoma cell proliferation and subsequent tumor progression. These results set the stage for further anti-B cell directed strategies in the treatment of HCC.

### 1032 DISSECTING THE ROLE OF Chk2, p53 AND p21 FOR LIVER REGENERATION AND TUMOR DEVELOPMENT DURING CHRONIC LIVER INJURY

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Hepatocellular carcinoma (HCC) is the most common primary liver tumor and most lethal cancer worldwide. The presence of specific risk factors promotes DNA damage and chromosomal aberrations, which lead to a cascade of molecular deregulations resulting in transformation of hepatocytes. ATM/Chk2 and ATR/Chk1 are kinases, which sense DNA breaks and trigger DNA damage response pathways including p53/p21. Our aim was to delineate the role of Chk2, p53 and p21 during acute and chronic liver injury and in HCC initiation and progression.

For this aim, mice with a targeted genetic deletion of Chk2, p53 or p21 were crossed into a murine model of human disease hereditary tyrosinemia type-1 (HT1). HT1 is an autosomal-recessive disease, caused by a genetic inactivation of fumarylacetoacetate hydrolase (FAH), which is characterized by an extremely high susceptibility for liver cancer. Our data show that p53 plays a central role in liver homeostasis during acute and chronic injury. Loss of p53 did not only accelerate tumor development, but also dramatically increased the mortality of Fah-deficient mice. In contrast, loss of Chk2 did not affect the mortality and tumor initiation in livers of Fah-deficient mice, but significantly accelerated tumor progression. Deletion of Chk2 and p53 in Fah-deficient mice revealed that p21 induction following DNA damage occurs independently of Chk2 and only partly dependent on p53. Surprisingly, p21 appears to play a dual role. Loss of p21 results in continuous liver regeneration in mice with severe liver injury thereby allowing the survival at the price of rapid tumor development. Unexpectedly, liver regeneration was significantly impaired in p21-deficient mice with moderate liver injury and tumor development was markedly delayed. Mechanistically, we evidence that the p53 target genes Sestrin1 and Sestrin2 suppress mTOR activity in mice with a moderate hepatitis suppressing hepatocyte proliferation and subsequent tumor development. Together, the Chk2/p53/p21 pathway is not a simple linear unidirectional, but a complex dynamic network. Our data uncover a molecular link in the complex mTOR and p53/p21-signaling network through activation of Sestrin-1/2, which is essential for the regulation of liver regeneration and tumor development following chronic injury.

### 1033 SERPIN-B3 INDUCES HIF2α NUCLEAR TRANSLLOCATION IN HEPATIC CANCER CELLS: A PARACINEME LOOP ABLE TO AFFECT CANCER CELL BEHAVIOUR

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**Background and Aims:** SERPIN-B3 (S-B3) is a serine protease inhibitor over-expressed in hepatocellular carcinoma (HCC) and up-regulated by hypoxia through a redox- and HIF2α-dependent mechanism. Moreover, S-B3 and hypoxia, as independent stimuli, can trigger epithelial-to-mesenchymal transition (EMT) and increased invasiveness in hepatic cancer cells. In the present study...
we have further investigated the complex relationships between S-B3 up-regulation, hypoxia and HIF2α-mediated mechanisms in hepatic cancer cells.

**Methods:** S-B3 and HIF2α expression as well as related events have been investigated by employing morphological, molecular and cell biology techniques in the following models or experimental conditions:

- a. transgenic mice overexpressing S-B3 in the liver;
- b. normal HepG2 exposed to human recombinant S-B3 (rS-B3);
- c. normal HepG2 and HepG2 stably transfected to over-express S-B3 (HepG2/SB3) in both normoxic and hypoxic conditions.

**Results:** The possible existence of a complex cross-talk between S-B3 and HIF2α was first suggested by immunohistochemistry performed in the liver of S-B3 transgenic mice revealing that S-B3 overexpression was associated to an impressive scenario of HIF2α positive nuclear staining in hepatocytes throughout the entire parenchyma. This scenario, with HIF2α increased expression and nuclear translocation, was confirmed by western blot analysis on total, cytosolic and nuclear liver extracts. Experimental manipulations provided further evidence for: (a) rS-B3 as well as conditioned medium containing S-B3 released by either cells exposed to hypoxia or by HepG2/SB3 results in a very significant HIF2α nuclear translocation, which is functional, being followed by up-regulation of the target gene Cyclin D1; (b) S-B3 does not up-regulate transcription of HIF2α but rather acts at post-transcriptional level by negatively affecting its ubiquitination and proteasome degradation.

**Conclusions:** The already described hypoxia- and HIF2α-dependent up-regulation of S-B3 by liver cancer cells can switch on a paracrine loop that, through the release of S-B3 in the extracellular environment, can amplify in a hypoxia-independent manner changes in cancer cell behaviour, including EMT and increased invasiveness, by inducing in neighbouring cells a hypoxia-independent increase of HIF2α nuclear translocation and then up-regulation of HIF2α-related target genes.

**1034 ABNORMALITIES IN LIPID METABOLISM AND SIGNALING IN HBV-ASSOCIATED HEPATOCELLULAR CARCINOMA**

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**Background and Aims:** Steatosis increases the risk of developing cirrhosis and hepatocellular carcinoma (HCC) in the setting of chronic hepatitis C virus (HCV) infection and nonalcoholic fatty liver disease (NAFLD). However, the relationship of lipid metabolism and signaling in hepatitis B virus (HBV)-related HCC is not completely understood. We aimed to evaluate the evidence of aberrant lipid metabolism in HBV-related HCC patients.

**Methods:** Hepatic steatosis were examined in the tissue samples from 335 HBV-related HCC patients and 1181 chronic hepatitis B (CHB) patients. The serum levels of lipid were tested among inactive hepatitis B carriers, HBV cirrhosis patients, and HBV-related HCC patients. cDNA microarray was used to detect the lipid signaling gene regulation in HBV-related HCC cancerous tissue samples and noncancerous samples. The serum liver type fatty acid binding protein (L-FABP) levels of 96 HCC patients and 68 CHB patients were measured by ELISA, meanwhile, immunohistochemistry was used to evaluate L-FABP expression in the liver tissue of those patients.

**Results:** The prevalence of hepatic steatosis in the tissue samples of HBV-related HCC patients were significantly higher than in the samples from CHB patients (60% vs. 39.04%, p < 0.01). Significant differences were demonstrated in serum lipid profile among inactive hepatitis B carriers, HBV cirrhosis patients, and HBV-related HCC patients. The cDNA microarray showed 17 lipid signaling genes upregulated and 44 lipid signaling genes downregulated in HBV-related HCC cancerous tissue samples compared to noncancerous samples. In addition, 15 lipid signaling pathways changed significantly in the setting of HCC. The serum liver type fatty acid binding protein (L-FABP) level in HCC patients were significantly higher than in CHB patients (p < 0.01). The expression of L-FABP in the liver tissues of HCC patients was significantly higher than in CHB patients (75% vs. 20.6%, p < 0.01).

**Conclusions:** Our results demonstrated an association between abnormal lipid metabolism and signaling in HBV-related HCC.

**1035 HEAT SHOCK FACTOR 1 ACCELERATES HEPATOCELLULAR CARCINOMA DEVELOPMENT BY ACTIVATING NUCLEAR FACTOR κB/MITOGEN-ACTIVATED PROTEIN KINASE**

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**Background and Aims:** Heat shock factor 1 (HSF1), a major transactivator of stress responses, has been implicated in carcinogenesis in various organisms. The aim of this study was to clarify the functional role of HSF1 in the development of hepatocellular carcinoma (HCC).

**Methods:** HSF1−knockdown cells were established by stably expressing either small hairpin RNA (shRNA) against HSF1 (HSF1−KD). The cells were analyzed by orthotopic transplantaion in vivo and proliferation and anti-apoptosis in vitro. Clinicopathological features in human HCCs were also characterized.

**Results:** Tumorigenicity was significantly reduced in orthotopic mice with HSF1−KD cells than in those with HSF1−control cells. Reduced tumorigenesis in HSF1−KD cells appeared attributable to increased apoptosis and decreased proliferation. Tumor necrosis factor κ-induced apoptosis was increased in HSF1−KD cells and HSF1−/− mouse hepatocytes compared with controls. Decreased expression of IκB kinase (IKK) γ, an essential modulator of nuclear factor κB, was also observed in HSF1−KD cells and HSF1−/− mouse hepatocytes, and might have been associated with the increased apoptosis. Furthermore, expression of bcl-2-associated athanogene domain 3 (BAG3), which may inhibit proteasomal degradation of IκKγ, was dramatically reduced in HSF1−KD cells and HSF1−/− mouse hepatocytes. We also found that epidermal growth factor-stimulated mitogen-activated protein kinase signaling was impaired in HSF1−KD cells. Clinicopathological analysis demonstrated frequent overexpression of HSF1 in human HCCs. Significant correlations between HSF1 and BAG3 protein levels and prognosis were also observed.

**Conclusion:** These results identify a mechanistic link between HSF1 and liver tumorigenesis and may provide as a potential molecular target for the development of anti-HCC therapies.

**1036 SERPINB3 INCREASES RESISTANCE TO CHEMOThERAPEUTIC AGENTS INHIBITING ROS PRODUCTION AND THE PERMEABILITY TRANSITION Pore**

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**Background and Aims:** Resistance to chemotherapeutic agents is well known in patients with hepatocarcinoma. Inhibition of the mitochondrial permeability transition pore (PTP) is a crucial step in tumor cell resistance to apoptosis induced by anticancer drugs.
Since SERPINB3 (SB3) is overexpressed in hepatocellular carcinoma and has an anti-apoptotic activity, aim of the study was to assess the role of this serpin on PTM modulation during treatment with chemotherapeutic agents.

**Material and Methods:** HepG2 cells stably transfected with SB3 where assayed for cell death induced by Cisplatin, Doxorubicin, Etoposide and 5-Fluorine Uracil. Reactive Oxygen Species (ROS) were detected with dichlorofluorescein. Threshold of PTP opening was evaluated by CRC assay and Complex I activity was determined by spectrophotometric assay.

**Results:** After cell death induction by Cisplatin and Doxorubicin, HepG2 cells expressing SB3 showed a significant increase in viability compared to controls, while there was no difference in cell death after Etoposide and 5-FU treatments. The addition of the antioxidant N-acetyl cysteine abrogated cell death induction by Cisplatin and Doxorubicin, suggesting that SB3 protects from death through an antioxidant activity. Since Cisplatin and Doxorubicin induce mitochondrial oxidative stress and favor PTP opening, cells were treated with the PTP inducer EM20–25 that acts at mitochondrial level. In presence of SB3 the effect of EM20–25 resulted in PTP opening inhibition and decreased ROS formation. Subcellular localization analysis revealed that a fraction of SB3 was located in mitochondria and that it increased after death-promoting treatments. Mitochondrial SB3 was found associated to respiratory chain Complex I by immunoprecipitation experiments. In vitro analysis confirmed the inhibition by SB3 of Complex I activity, known as one of the main sources of mitochondrial ROS.

**Conclusions:** SB3 acts at mitochondrial level protecting cells from chemotherapeutic-induced oxidative stress and consequent cell death. This antioxidant function could represent a relevant advantage for SB3-expressing cells exposed to anticancer treatments.

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**ROLE OF GENE AND microRNAs EXPRESSION RELATED TO HEPATOCELLULAR CARCINOMA DEVELOPMENT IN A HCV-INDUCED MODEL IN VITRO. EFFECT OF METFORMIN**

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**Background:** Complications of fibrosis results in hepatocellular carcinoma (HCC), the fifth most common solid cancer. The multifaceted molecular pathogenesis of HCC has been linked with alterations in multiple cellular signaling pathways. Among them, the interplay between the regulatory subunit IKK\(/NEMO of the nuclear factor-a, b, c) and the c-Jun terminal kinases (JNK) can be crucial in the regulation of hepatocyte fate towards survival or death.

**Aim:** We evaluated the interaction between the Jnk genes and the IKK\(/NEMO signaling pathway during the progression of chronic liver disease and HCC.

**Methods:** Hepatocyte-specific NEMO knockout mice (NEMO\(^{+/+}\)) were crossed with Jnk1\(^{-/-}\) and Jnk2\(^{-/-}\) yielding Jnk1\(^{-/-}\)/NEMO\(^{+/+}\) and Jnk2\(^{-/-}\)/NEMO\(^{+/+}\) mice, respectively, and were characterized for the relevance of Jnk and Jnk2 in the progression of chronic liver disease into HCC.

**Results:** Alternating the crosstalk between Jnk1 and IKK\(/NEMO increased liver injury as assessed by serum markers – ALT and alkaline phosphatase (AP) – at 1 year of age. Jnk1\(^{-/-}\)/NEMO\(^{+/+}\) mice exhibited significantly increased liver size, liver weight vs body weight ratio, number of nodules bigger than 5.0 mm, tumor size and number. Furthermore, markers of compensatory proliferation and tumor progression such as cyclin D1, PCNA, c-myc, AFP, glutamine synthetase and CK-19 were found overexpressed in Jnk1\(^{-/-}\)/NEMO\(^{+/+}\), associated with deregulation of the MAPK signaling pathway (pERK1/2/pAKT/p38). In contrast, Jnk2\(^{-/-}\)/NEMO\(^{+/+}\) elicited strong activation of the IL-6/GP-130/STAT3 pathway which resulted in increased collagen IAI deposition and expression of cSMA, TIMP1, MMP13 and BMMI but lower tumorigenic potential related with lower TGFI levels and down-regulation of cancer pathways such as Shh and Wnt.

**Conclusion:** Disruption of the crosstalk between IKK\(/NEMO and Jnk1 increases chronic liver damage, compensatory proliferation and the development of HCC through alteration of the MAPK pathway, whereas changes in the interaction between IKK\(/NEMO and Jnk2 promote liver fibrosis. These findings further extend the possibility of gene-directed therapy against HCC.

**Background and Aims:** The key cellular regulator p53 is a common target of viral oncoproteins. miRNAs are negative regulators of gene expression and can work as tumor suppressors. Several miRNAs are associated with the development of hepatocellular carcinoma (HCC). Use of metformin is associated with a decreased risk of HCC in diabetic patients in a dose-dependent manner. Patients harboring T allele in IL28B rs12979860 polymorphism have an increased risk of developing HCC. In order to identify key genes in HCC development upon HCV infection, we have investigated the expression of several genes and miRNAs in vitro and the antineoplastic role of metformin.

**Methods:** Huh7.5 (IL28B rs12979860-CT genotype) and Huh7 (CC) cells were grown and infected with the full-genome JFH1 replicon (1-particle/cell). Metformin (2mM) was added to the cells. Total RNA extraction was performed 48/72 hours after the media was changed. The expression of the different genes was quantified using the qRT-PCR Quantace (Bioline) kit. miRNA expression was quantified using the miScriptReverse-Transcription and miScript SYBR® Green commercial kits (Qiagen). Primary human hepatocyte isolation was based on the two-step collagenase method.

**Results:** TP53 gene expression was upregulated in both Huh7.5 and Huh7 cells infected with JFH1 and treated with metformin (1.9±0.3 vs. 3.7±0.3). In human hepatocytes, TP53 was found to be inducible (2.9±0.4). PTEN, a tumor suppressor that negatively regulates AKT/PKB signaling pathway, is highly induced in Huh7 infected cells (7.2±2.5) compared to Huh7 (1.6±0.1). Metformin increased PTEN expression in primary hepatocytes (2.2±0.05). PTPIB is a negative regulator of insulin signaling and it is implicated in cell growth. This gene is highly induced in Huh7.5 (3.7±0.7) and Huh7 (4.7±0.7) cells infected with JFH1 and treated with metformin. Huh7.5 cells were infected with JFH1 and screened for miRNAs expression: mir150, mir125a, mir125b and mir302c were significantly inhibited (>5fold).

**Conclusion:** Differential gene expression induction in Huh7.5 (CT-genotype) vs. Huh7 (CC) cells supports the role for the T allele in HCC development induced by HCV infection. Cancer related miRNAs inhibition in Huh7.5 cells indicated putative targets for HCV infection and HCC development, being useful as molecular
markers. Metformin induces PTEN expression, which may explain the positive therapeutic effect of metformin in several tumors.

1039 ACTIVATION OF THE MITOGEN-ACTIVATED PROTEIN KINASE ERK5 REGULATES THE DEVELOPMENT AND GROWTH OF HEPATOCELLULAR CARCINOMA

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Background and Aims: Current treatment options for hepatocellular carcinoma (HCC) are limited. The interference with signaling pathways deregulated in hepatocarcinogenesis could be considered a promising therapeutic strategy. ERK5 is the least studied member of the MAPK family and has been implicated in several biologic actions relevant for tumor development. Moreover, deregulation of the ERK5 pathway has been shown to be associated with cancer. Aim of this study was to understand the role of ERK5 in HCC in vitro and in vivo.

Methods: Huh-7 and HepG2 were cultured by standard methods. Liver tissue was obtained from HCC and peritumoral areas. ERK5 was silenced by siRNA transfection or with shRNA and lentiviral vectors. The specific ERK5 inhibitor XM8D–92was also used. In vivo development of HCC was evaluated using the Huh-7 xenograft model in athymic nude mice.

Results: Analysis of ERK5 by IHC in human tissues showed more abundant nuclear localization in patients with HCC or cirrhosis than in normal liver, indicating ERK activation. ERK5 silencing in HCC cells or exposure to XM8D–92 blocked the increase in migration and invasion induced by EGF or serum. Similar results were observed in response to hypoxia. Immunofluorescence experiments demonstrated that ERK5 silencing or inhibition caused cytoskeletal remodeling and rearrangement of focal adhesions, consistent with a decrease in cell motility. In addition, ERK5 activation was necessary for the growth of HCC cells, affecting the G1/S transition. In a mouse model of HCC xenograft, administration of XM8D–92 significantly decreased tumor volume by approximately 40% compared to vehicle, 13 days after starting the treatment. Moreover, in mice injected with Huh-7 cells silenced for ERK5 using a lentiviral shRNA vector, the rate of tumor appearance was significantly lower (4/16 mice, 25%) than in animals inoculated with cells transduced with non targeting shRNA (9/15, 60%). In addition, at the end of the experiment, tumor volume was smaller in the presence of ERK5 silencing.

Conclusions: The ERK5 pathway plays a critical role in HCC tumor development and growth in vivo. Blocking the ERK5 pathway should be further investigated as a novel approach for the treatment of HCC.

1040 INTERACTION OF THE INNATE AND ADAPTIVE IMMUNE SYSTEM CONTRIBUTE TO LIVER INJURY AND HEPATOCARCINOCESIS

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Background: Activation of the immune system by tumor cells might result in eradication of malignant cells. Modulation of the immune system has been shown to increase carcinogenesis in several knock-out models. There is increasing evidence that chronic activation of the immune system in pre-malignant tissues might promote tumor development. To characterize the role of the immune system in chronic liver injury and hepatocarcinogenesis mouse model of hereditary tyrosinemia type 1 (HT1) was used. HT1 is a genetic defect of tyrosine catabolism and is characterized by an extremely high susceptibility for liver cancer. The only effective therapy is 2-(2-nitro-4-trifluoromethylphenyl)amino-3-quinizarin hydrochloride (NTBC)-treatment.

Methods: To induce flares of liver injury NTBC was repeatedly withdrawn for 21 days followed by 5-day-NTBC-treatment to allow partial liver regeneration. Liver injury was analyzed by immunohistochemistry and biochemistry. Liver-progenitor-cells and inflammatory cells were analyzed and quantified by FACS and immunohistochemistry. Gene expression arrays and RT-PCR arrays have been performed.

Results: Flares of liver injury induced a severe hepatitis and significantly accelerated liver tumor development. Liver regeneration occurred by proliferation of differentiated hepatocytes, increase in size of hepatocytes and activation of liver-progenitor-cells. Liver injury was accompanied by infiltration of T-cells, Monocytes and Macrophages. To determine the role of the immune system, Fah−/− Rag-2−/− IL-2−/− mice (FCR) were treated in the same way. Interestingly, survival of alymphoid Fah−/− mice was dramatically shorter than that of Fah−/− mice. Almost 70% of the FCR mice died within the first 2 months whereas over 90% of Fah−/− mice survived the FAA-induced hepatitis suggesting that the immune system plays an important pro-survival role during chronic liver injury. Liver tumor development was completely suppressed in surviving FCR mice. To better understand the role of the immune system in the Fah−/− model, extensive molecular and immunological profiling, hepatocyte and hematopoietic stem cell transplantations, adoptive transfers of specific immune cell populations were performed and additional immune-suppressed mice were generated.

Conclusions: Our analysis identifies distinct functions of the immune system, which are required for liver regeneration/survival and for hepatocarcinogenesis on the other hand. Targeting specifically the tumor-promoting pathways may be an attractive chemopreventive strategy for patients at risk.
and DNA copy number alterations (CNA) were identified after normalization, GC correction and smooth segmentation.

Results: Approximately 2 million, 76bp reads were randomly generated per sample, providing 5% coverage of the human genome. CNAs were identified and showed no significant aberrations in Macroregenerative nodules (MRN) (n=8). Dysplastic nodules (DN) (n=5) showed development of spikes of amplifications and deletions, which became increasingly complex and involved larger areas of the chromosome as HCC developed (n=22). This progression was demonstrated on chromosomes 1, 5, 7, 14, 22 for amplifications and 1, 4, 6, 14, 16, 22 for deletions (figure 1).

Conclusion: NGS can provide CNA data from FFPE material with reasonable costs ($50/sample). Genetic profiles of pre-malignant nodules revealed no changes within MRNs. However lesions start to occur with the onset of dysplasia and become increasingly complex. Initial amplifications and deletions expanded in size with progression. More work is needed to expand cohorts and investigate heterogeneity within individual lesions.

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HEPATOCELLULAR CARCINOMA REPLICATING HEPATITIS B VIRUS HAVE TRANSCRIPTOMIC, HISTOLOGICAL AND CLINICAL CHARACTERISTICS
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Background and Aims: Hepatocellular carcinoma (HCC) is a heterogeneous tumor both in a clinical and molecular perspective. Gene expression studies have contributed to its molecular classification. Expression profiles of microRNAs (miRNAs) have been widely used to classify cancers. The deregulated expression of a miRNA cluster located in DLK1-DIO3 region has been reported in human malignancies. High levels of this miRNA cluster have been associated with aggressive HCCs characterized by a stem-like phenotype, high alpha-fetoprotein (AFP) levels and poor survival. The aim of this study is to investigate the role of DLK1-DIO3 miRNA cluster in HCCs and to characterize epigenetic mechanisms involved in its aberrant expression.

Methods: Real Time RT-PCR analysis was used to investigate the expression of miR-494, a member of DLK1-DIO3 miRNA cluster, in surgically resected HCC tissues. Functional analysis and reporter gene assays were performed to determine the regulation of p27, PTEN and PUMA by miR-494 in HCC cells. Methylation specific PCR (MSP) was used to investigate the methylation status of DLK1-DIO3 region in HCCs. Treatments with DNA methylation agents and inhibitors of histone deacetylases was performed in HCC cells.

Results: An up-regulation of miR-494 expression was observed in 40% of HCCs with respect to cirrhotic tissues. The MSP analysis revealed an association between DNA methylation profiles and miR-494 expression. Modulation of miR-494 expression was observed following treatments targeting DNA methylation machinery and histone modifications in HCC cells. Functional analysis and reporter assays showed a regulation of tested targets by miR-494 in HCC cells.

Conclusions: MiR-494 is aberrantly expressed in a subset of HCCs and it is regulated by epigenetic mechanisms. MiR-494 participates in the regulation of key biological processes, such as proliferation,
cell cycle progression and apoptosis through the inhibition of p27, PTEN and PUMA.

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LYSOPHOSPHATIDIC ACID RECEPTOR 6 (LPA6) PROMOTES HEPATOCELLULAR CARCINOMA GROWTH AND PROGRESSION THROUGH ACTIVATION OF PIM-3 PROTO-ONCogene KINASE
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Lysophosphatidic acid (LPA) acts as a mediator of tumor progression in hepatocellular carcinoma (HCC) by binding G protein-coupled LPA receptors. The role of LPA receptor 6 (LPA6), originally referred to as the purinergic receptor P2Y5, is currently unknown in the liver. Using a combination of biochemistry and genetic approaches, we demonstrate that LPA6 contributes to HCC progression by promoting cell proliferation and enhancing tumor growth. We found that stable knockdown of LPA6 by a lentiviral-based RNAi system dramatically impaired tumor growth and progression in a xenograft model of human HCC. Conversely, ectopic expression of LPA6 in non-aggressive HLE cells led to a more aggressive-tumor phenotypes, by increasing tumor growth. Using a digital gene expression profiling by MACE in LPA6 knocked-down Huh7 cells, we also found that expression of over 500 genes were altered, including cytoskeleton, ECM and tissue development genes. Amongst the latest class of genes, we focus our attention on pim-3 and found that its expression is finely regulated by LPA6 both in vitro and in vivo. Finally, we found that LPA6 is significantly overexpressed in patients with HCC tumors compared to peritumoral cirrhotic liver and that its expression has high prognostic significance. Also, a significant correlation between the tissue expression levels of LPA6 and PIM-3 was found in these patients. Our results show that LPA6 is a critical regulator of tumor growth and progression in HCC.

1045
ALPHA-1-ANTITRYPSIN DEFICIENCY: FROM GENOMA TO LIVER DISEASE. PIZ MOUSE AS MODEL FOR THE DEVELOPMENT OF LIVER PATHOLOGY IN HUMANS
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Background: The homozygous individuals with alpha-1-antitrypsin deficiency (AATD) type PiZ are associated with an increased risk of chronic liver disease and hepatocellular carcinoma (HCC). It is noteworthy that HCCs arises specifically from AAT-negative hepatocytes. However the reason for this remains unclear. The aim of this study was to determine liver pathology in PiZ mice, focusing the attention on distribution of PAS diastase resistant AAT globules in normal liver as well as in regenerative and neoplastic liver.

Methods: Liver specimens from 77 PiZ and 17 wild type (Wt) mice were histologically analyzed for hyperplasia and neoplasia. The status of AAT (human and murine) in neoplastic/hyperplastic nodules (rich in globule-devoid hepatocytes) and in non-neoplastic liver (rich in globule-containing hepatocytes) was verified by qPCR and qRT-PCR. The differentiation degree of hepatocytes in neoplastic/hyperplastic nodules was determined, with RT-PCR, analyzing RNA expression of liver-specific genes: albumin, α-fetoprotein (AFP), transthyretin, AAT, glucose-6-phosphate (G6P), tyrosine aminotransferase and β-actin.

Results: Liver pathology was observed in older PiZ (17–24 months) and consisted in malignant tumors (15/40), 8 of which HCC, nodular hyperplasia (18/40), nonspecific changes (33/40), whereas only 7/40 were normal. In Wt mice, 5/17 showed no specific changes and no tumors. Human AATZ showed no difference either in gene copy number or in mRNA expression. Murine mRNA AAT was reduced in pooled tumor and nodules. AFP (early hepatic marker) was present in all but one HCC. G6P (late hepatic marker) was expressed in all but two HCC; one AFP positive and one AFP negative.

Figure 1. Hepatocellular carcinoma in PiZ mouse.

Figure 2. Scheme of hepatocellular carcinogenesis.

Conclusion: Accumulation of AAT is associated with an increased risk of liver changes. The presence of globule-devoid hepatocytes, the reduced expression of murine mRNA AAT and the re-expression of AFP in the hyperplastic/neoplastic nodules would suggest that the hepatic lesions in AATD originate from proliferating precursors cells lacking AATZ accumulation.

1046
miR-34a & beta-CATENIN SIGNALING IN MOUSE LIVER: A COMPLEX NETWORK REGULATING LIVER ZONATION, METABOLISM ... AND CANCER
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Context: microRNAs (miRNAs) are small non-coding RNAs, which finely regulate many cellular processes and probably the liver...
HEPATOCELLULAR CARCINOMAS decreased the proliferation of hepatocytes isolated from the Apc capacity of HNF-4 zonation and its oncogenicity. We are now testing a locked b

Conclusions: activating mutation of Apc in hepatocellular carcinoma samples from patients, presenting an

loss required for biliary lineage. We also observed a miR-34a induction in β-catenin activated tumors from the oncogenic Apc KO model, as compared to normal livers. Interestingly, we noticed an inverse correlation between miR-34a and HNF-4α expression in these tumors. We finally confirmed miR-34a overexpression in hepatocellular carcinoma samples from patients, presenting an activating mutation of β-catenin (25 samples) as compared to non-mutated tumors (22).

Conclusions: These data suggest a close relationship between β-catenin, HNF-4α and miR-34a for the control of liver metabolic zonation and its oncogenicity. We are now testing a locked nucleic acid against miR-34a in the two mouse models of β-catenin overactivation. Promisingly, this inhibitor was efficient in vitro and decreased the proliferation of hepatocytes isolated from the Apc KO model.

1047 KERATIN 19: A KEY ROLE PLAYER IN THE INVASION OF HUMAN HEPATOCELLULAR CARCINOMAS O. Govaere1, M. Komuta1, J. Berkers1, B. Spee1, C. Janssen1, F. de Luca2, A. Katoonzadeh2, J. Wouters1, L.C. van Kemen2, A. Durnez1, C. Verslype2, J. De Kock3, V. Rogiers4, L.A. van Grunsven5, B. Topal6, J. Pirenne9, H. Vankelecom3, J. Berkers1, B. Spee1, C. Janssen1, F.de Decker6, J. Pirenne9, H. Vankelecom3, F. De Neven5, J.J. van den Oord1, G. Lendvai1, É. Végh2, G. Bodoky2, B. Járav1, E. Székely1, Z. Schaff2, A. Kiss1, 2nd Department of Pathology, Semmelweis University,1 Department of Liver and Digestive Health, Royal Free Hospital, London, UK; 3Department of Development and Regeneration, KU Leuven, Leuven, Belgium; 4Department of Pathology, McGill University, Jewish General Hospital, Montreal, QC, Canada; 5Department of Hepatology, KU Leuven and University Hospitals Leuven, Leuven, Belgium; 6UCL Institute for Liver and Digestive Health, Royal Free Hospital, London, UK; 7Department of Development and Regeneration, KU Leuven, Leuven, Belgium; 8Department of Pathology, McGill University, Jewish General Hospital, Montreal, QC, Canada; 9Department of Hepatology, KU Leuven and University Hospitals Leuven, Leuven, Belgium; 10Department of In Vitro Toxicology and Dermato-Cosmetology (IVTD/FAY), 11Department of Cell Biology, Vrije Universiteit Brussel, Brussels, 12Department of Abdominal Surgery, 13Department of Abdominal Transplant Surgery, KU Leuven and University Hospitals Leuven, Leuven, Belgium E-mail: olivier.govaere@uzleuven.be

Background and Aims: Keratin(K)19, a biliary/hepatic progenitor cell (HPC) marker, is expressed in a subset of hepatocellular carcinomas (HCCs) with poor prognosis. The underlying mechanisms driving the highly aggressive phenotype of K19-positive HCC remain elusive.

Methods: Clinicopathological value of K19 was assessed in comparison with EpCAM, and AFP, in a Caucasian cohort of 407 HCCs with different aetiologies. Keratin(KRT)19-associated genes and microRNAs were identified using transcriptome data of 139 samples and miRNA PCR Arrays. Clinical HCC samples were submitted to in vitro invasion assays and to Side Population analysis using the fluorescent dye Hoechst33342. HCC cell lines were transfected with synthetic siRNAs against KRT19, and synthetic microRNAs or hairpins.

Results: In comparison with EpCAM and AFP, K19 expression was most strongly correlated with clinicopathological parameters, including increased tumor size (p = 0.03), decreased tumor differentiation (p = 0.0001), metastasis (p = 0.0005) and microvascular invasion (p = 0.0001). Network analysis linked KRT19-associated genes with other malignant HCC subtypes, pointed to a role for K19 in cytoskeletal dynamics and cell motility, and underlined the importance of the extracellular matrix. K19-positive HCCs showed high expression of invasion-/metastasis-related markers (e.g. VASP, TACSTD2, LAMC2, PDGFRA) and biliary/HPC markers (e.g. CD133, GSTP1, NOTCH2, JAG1). miR-141, miR-200c and miR-885-p were shown to regulate the K19-positive phenotype in vitro. Additionally, we showed that K19 regulates primary HCC invasiveness in vitro by stimulating invadopodia formation, and that K19-positive tumour cells reside in the chemoresistant Side Population.

Conclusions: Not only the clinicopathological relevance of K19 was confirmed in a large prospective Caucasian series with different aetiologies, but we unravelled the molecular profile and distinct invasive properties of K19-positive HCCs.
treated patients with low pretreatment miR-223 expression is longer in comparison to those with high miR-223 expression. This suggest that miR-223 might predict the success of Sorafenib treatment.

Acknowledgement: This work was supported by grants from the Hungarian Scientific Research Found OTKA K101435 and OTKA T75468.

1049 microRNA EXPRESSION DIFFERS IN EMBRYONAL AND FETAL SUBTYPES IN HUMAN HEPATOBLASTOMA

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Background and Aims: Hepatoblastoma (HB) is the most common primary liver cancer in childhood, which has epithelial, mixed (epithelial and mesenchymal) and non specified types, based on tissue components. Since the prognosis of the two epithelial subtypes, embryonal and fetal, is different, we aimed to examine whether these differences were present at microRNA (miRNA) expression level as well.

Methods: Total RNA was isolated from 56 formalin fixed paraffin-embedded samples consisting of 15 embryonal, 22 fetal and 19 non-tumorous surrounding liver samples taken from 26 patients. Following DNase treatment, the expression of 14 microRNAs was determined using TaqMan MicroRNA Assays. Relative expression was calculated applying the average of miR-140 and miR-328 expressions as the reference. Statistical analysis was performed using Wilcoxon matched pairs, Kruskal-Wallis and Log-Rank tests.

Results: In 56 samples, elevated expression levels of miR-18a, miR-96 and miR-224 were found in the embryonal component compared to fetal. As compared to non-tumorous surrounding liver samples, decreased miR-17–5p, miR-122, miR-195, miR-210, miR-214 levels and increased miR-221 level were detected in fetal subtypes, furthermore decreased miR-122 and miR-214 levels were found in the embryonal components. Survival analysis revealed low expression level and better Overall-Survival (OS) and Event Free Survival (EFS) in case of miR-224. These results were statistically significant (p < 0.05).

Conclusion: The results indicate that different miRNA expression patterns exist in the epithelial hepatoblastoma subtypes. Interestingly, the up- and downregulation of miR-17–5p and miR-210 in HB differed from that reported in hepatocellular carcinoma. Acknowledgement: This study was supported by grant OTKA T75468 from the National Scientific Research Foundation.

1050 HUMAN LIVER CARCINOMAS RECRUIT MESENCHYMAL STEM/STROMAL CELLS THAT CAN PROMOTE TUMOR GROWTH VIA PARACRINE SIGNALING

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Background and Aims: Bone marrow mesenchymal stem/stromal cells (MSCs) can migrate to tumor sites and contribute to the tumor microenvironment. However, it is still hotly debated whether MSCs have a positive or negative effect on tumor growth. This study aims to investigate whether human liver carcinomas contain MSCs and whether MSCs may affect tumor growth.

Methods: MSCs were cultured from surgical resected hepatocellular carcinoma (HCC) (n=6) and liver metastatic colorectal tumor (LM-CRC) (n=7). Immunohistochemical staining of STRO-1 (the best-known MSCs marker for in vivo detection) was performed in paraffin-embedded patient HCC and LM-CRC tissues (n=24). The effects of MSCs on tumor growth were evaluated in immune-deficient mice.

Results: Solid tumors formed in mice by subcutaneous engraftment of human hepatoma Huh7 cells were able to recruit MSCs. MSCs were also found in patient liver tumors (successfully cultured from 11 out of 13 liver tumors). Their MSC properties were characterized by adipocyte and osteocyte differentiation and common mesenchymal markers. Notably, in situ staining showed that STRO-1 positive cells are significantly enriched in the tumor, in particular the tumor-stromal region, compared with the adjacent area in HCC and LM-CRC tissues (n=24, p<0.01). In mice, co-engraftment of Huh7 and MSCs resulted in significant larger tumors than engraftment of Huh7 alone (tumor weight 1.56±0.27 g Vs 0.44±0.19 g, Mean±SEM, n=8, p<0.01). Consistently, co-culturing Huh7 with irradiated MSCs significantly increased the number (196±29 Vs 123±36 clones/5000 Huh7, Mean±SD, n=5, p<0.01) and the size (1329±258 Vs 570±155 pixels, n=5, p<0.01) of formed colonies. This effect was also observed by treatment of MSCs conditioned medium (MSC-CM), suggesting secreted tropic factors contributing to the tumor promoting effect. Genome-wide gene expression array and pathway analysis confirmed the up-regulation of cell growth and proliferation-related processes and down-regulation of cell death-related pathways by treatment of MSC-CM in Huh7 cells.

Conclusion: Human liver carcinomas recruit MSCs, which in turn can promote tumor growth. These results shed new light on the crosstalk between MSCs with liver cancer cells but also caution stem cell therapy of using MSCs for liver cancer and other liver diseases with high risk of developing malignancy.

1051 THE MAGNITUDE OF THE RESPONSE OF HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA TO SORAFENIB IS AFFECTED BY THE EXPRESSION OF INACTIVATING VARIANTS IN THE SLC22A1 GENE

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Background and Aims: Reduced efficacy of pharmacological treatment of cancer is due in part to decreased intracellular content of active drugs. Thus, lowering uptake is an important mechanism of tumour chemoresistance. In this respect, downregulation of SLC22A1 encoding the organic cation transporter-1 (OCT1) may affect the response of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCG) to sorafenib, which is taken up in part by OCT1. The aim of the present study was to investigate whether SLC22A1 variants may contribute to chemoresistance of the primary liver tumours HCC and CCG to sorafenib.

Methods: Gel-electrophoresis-based complete sequencing and selective variant identification by RT-PCR was performed to detect SNPs in SLC22A1 cDNA. Modifications in the wild-type sequence of the OCT1 ORF were mimicked by directed mutagenesis and used
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for cell transfection, immunofluorescence, transport assays and the determination of sorafenib antitumour activity in vitro.

**Results:** In addition to 4 previously described alternative spliced variants and 11 SNPs, 2 novel alternative spliced variants and 3 SNPs were identified in HCC and CGC. To study their functional consequences, SLC22A1 cDNA containing one of the identified SNPs was expressed in human hepatoma Alexander cells. Both c.181deICGinsT and c.262deIT resulted in a reduction of tetraethylammonium uptake, which was due to the absence of OCTI targeting to the plasma membrane. This was consistent with a reduced sensitivity to sorafenib. Screening of these SNPs in 23 HCC and 17 CGC revealed that c.181deICGinsT was present in both HCC (17%) and CGC (13%), whereas c.262deIT was only found in HCC (17%). Considering all SLC22A1 variants, at least one inactivating SNP was found in >50% HCC and >30% CGC.

**Conclusions:** Liver carcinogenesis is accompanied by the appearance of aberrant variants of OCTI that may dramatically affect the ability of HCC and CGC to take up and hence respond to sorafenib.

**1052 SOCS1 PROTECTS AGAINST THE ONCOGENIC POTENTIAL OF p21(WAF1/CIP1) IN THE LIVER**


1Pediatrics, Immunology Division, 2Anatomy and Cell Biology, Functional Consequences, variants and SNPs, 2 novel alternative spliced variants and determination of sorafenib antitumour activity in vitro.

**Methods:** SOCS1 on the DEN-induced model of HCC.

**Results:** We found elevated transcript levels of p21Cip1 activation in the livers of DEN-treated SOCS1 deficient mice, senescence. Although we did not observe any change in p53 following DEN treatment was not elevated in SOCS1 deficient null primary hepatocytes showed increased stability of p21, whereas HepG2 cells overexpressing SOCS1 showed lesser p21 protein levels after Cisplatin treatment. SOCS1 deficient livers also showed increased p21 expression after partial hepatectomy. While nuclear p21 inhibits cell proliferation by inhibiting CDKs, aberrant cytoplasmic localization of p21 may play an oncogenic role by inhibiting apoptosis pathways. In the absence of SOCS1, more p21 is found in the cytoplasm, which is prevented by inhibiting AKT signaling. SOCS1 deficient hepatocytes resist Cisplatin-induced apoptosis, which was reversed by re-introduction of SOCS1.

**Conclusion:** SOCS1 is an important modulator of p21 expression at the transcriptional and post-transcriptional level, and also regulates its potential oncogenic activity. These activities of SOCS1 may contribute to its tumor suppressor function in the liver.

**1053 A NOVEL SYNTHETIC SULFOGLYCOLIPID SULFOQUINOVOSYLACYL PROPANEDIOL (SQAP) SWITCHES OFF TUMOR ANGIOGENIC POTENTIAL OF HEPATOCELLULAR CARCINOMA THROUGH THE UPREGULATION OF VON HIPPEL–LINDAU PROTEIN**

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**Background and Aims:** Hypoxic microenvironments are a common feature of solid tumors. Hypoxia inducible factor alpha (HIFα) plays a central role in tumor hypoxia. Sulfoglycosylglycerols (SQAG) are sulfoglycolipids that were originally derived from sea urchins. We investigated the effect of a novel synthetic SQAG derivative sulfoglycosylacyl propanediol (SQAP) for hepatocellular carcinoma (HCC).

**Methods:** We evaluated the therapeutic effects of SQAP using three HCC cell lines (HAK1-B, Huh-7 and KYN-2) xenograft mouse model and examined the antitumor mechanisms of SQAP. We generated a HCC cell line using a retroviral transduction system that expressed SOCS1, which is a known inhibitor of the HIF-1α transcriptional activity. The effects of SQAP were evaluated in this cell line and in the parental cell line. We also examined the antitumor effects of SQAP in a mouse model of HCC.

**Results:** SQAP significantly inhibited tumor growth by inhibiting tumor angiogenesis in HAK1-B and Huh-7, but not in KYN-2. These SQAP sensitive HCCs showed the multiple decreased tumor angiogenic factors, e.g. vascular endothelial growth factor, angioptoiitin-2 and fibroblast growth factor 2 and the increased endogenous antiangiogenic factor, Thrombospondin-1. These negative changes of tumor angiogenic potential were contributed by downregulation of HIFα and HIF2α protein via upregulation of pVHL. In contrast, HIFα proteins were not changed in SQAP resistant HCC KYN-2 because of decreased pVHL degradation. Multiple mechanisms for the pVHL-dependent decrease in HIFα protein levels were identified: (a) increasing pVHL-dependent HIFα protein degradation, which was confirmed by the inhibition assay of pVHL degradation using a proteasome inhibitor; and (b) decreasing HIFα protein synthesis with downregulation of NFκB activity; and (c) improving hypoxic conditions in tumors by vascular normalization, which was confirmed by pimonidazole staining and increased pericyte coverage. All effects of SQAP have been clearly demonstrated in SQAP sensitive HCC cell lines and all clinical tissues showed wild-type VHL gene profile but the only SQAP resistant HCC cell line showed VHL gene mutation.

**Conclusions:** SQAP is a novel antiangiogenic drug which can switch off tumor angiogenic potential. The effects of SQAP depended on tumor VHL gene profile. Our results suggest that SQAP therapies targeting pVHL and HIFα proteins could be a promising strategy for HCC.

**1054 CIRCULATING MICROVESICLES’ MICRONRNAS EXPRESSION PROFILES IN HEPATOCELLULAR CARCINOMA**

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**Background and Aims:** Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Microvesicles (MVs), as a
secretory particles, packaged with microRNAs (miRNAs) have been shown to be released mainly from tumor cells. We explored the circulating MVs' miRNAs expression profiles from HCC patients.

**Methods:** The presence and levels of 888 miRNAs in MVs derived from the peripheral blood of HCC patients and normal controls were performed by Agilent miRNA microarray analysis. Bioinformatics tools were used to analyze function of the altered miRNAs, including targeted genes prediction, gene ontology (GO) and pathway annotation.

**Results:** A total of 242 aberrant expression miRNAs were identified in HCC-MVs, compared with the control group. Among of them, 115 miRNAs were overexpressed with up to 31 fold changes (hsa-miR-671–5p) and 127 were underexpressed with up to 0.041 fold changes (hsa-miR-432) in HCC group. Bioinformatics analysis were applied to the top ten dysregulated miRNAs. By software miRror2.0, NOL3 was found to be the core player among the 300 target genes of top ten up-regulated miRNAs and SRRM1 was central among the 219 targets of the top down-regulated miRNAs. We also analyzed GO categories for these predicted genes: cellular component including 153 subclasses, biological processes including 175 subclasses, and molecular function including 152 subclasses, as well as biochemical function and cellular role. The dysregulation MVs' miRNAs and their target genes were closely involved in the pathways of cancer, such as MAPK signaling pathway, Wnt/catenin-TCF signaling pathway, Ras and Jak/Stat pathways in HCC.

**Conclusions:** Our study revealed for the first time that miRNAs differentially expressed in MVs derived from HCC when compared to the control. Our result also indicated that the aberrant HCC-MVs miRNAs are important for the development of HCC through the genes and their functional profile and signaling pathways.

**References:**


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**1056 THE INSULIN-LIKE GROWTH FACTOR 2 (IGF2) mRNA BINDING PROTEIN (IMP) p62 PROMOTES HEPATOCARCINOGENESIS IN A TRANSGENIC MOUSE MODEL.**

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**Background and Aims:** Liver-specific overexpression of the oncofetal insulin-like growth factor 2 (IGF2) mRNA binding protein p62 (IMP2)–2 induces the development of fatty liver. Aim of our study was to investigate the role of p62 in hepatocarcinogenesis.

**Methods:** p62 transgenic mice were treated with diethylnitrosamine (DEN) in order to induce an early liver damage or hepatocellular carcinomas (HCCs) in these mice. Histological and immunohistochemical analysis of the liver tissues was performed.

**Results:** In a short term experiment modeling early liver cell damage p62 transgenic mice were more susceptible to the carcinogen DEN than wild-type animals. Eosinophilic degenerated hepatocytes were much more prominent in transgenic livers (p=0.0021). The inflammatory response was increased in transgenic animals as determined by histological analysis of lobular lymphocytic infiltration (p=0.071) as well as granulocytic infiltrations (p=0.0276). Immunofluorescent staining revealed increased nuclear translocation of p65, representing an activated NF-xB signalling. In order to clarify whether an inflammatory environment renders these mice more susceptible towards carcinogenesis HCCs were induced by DEN. Indeed, a 24–28 weeks time point revealed a significantly earlier induction time of HCCs in p62 transgenic mice compared to wild-types (p=0.031). At later stages both tumor incidence (p=0.000003) and tumor multiplicity (p=0.0002) were significantly higher in the transgenics than in wild-type animals. In highly p62 expressing animals the tumor number per liver was even higher than in low expressing mice (p=0.0004). Both wild-types as well as transgenic animals displayed lung metastases, but no difference in the incidence could be observed between these genotypes. Immunostaining for the tumor markers glutamine synthetase and Gp73 revealed tumors in wild-type mice being Gp73 positive, whereas HCCs of the transgenic mice were either Gp73 positive (70.31%) or glutamine synthetase positive (14.06%) or positive for both markers (15.63%) indicating a specific molecular expression pattern of transgenic HCCs. HCC cells in transgenic mice were more mitotically active (granulocytic infiltrations (p=0.0137) and were more mitotically active (p=0.0477). CK19 positive oval cells were only seen in HCCs of transgenic mice. These results indicate an aggressive tumor type in p62 transgenic animals.

**Conclusion:** p62 enhances tumorigenesis and aggravates the HCC phenotype, which might be reflected in a particular human HCC subtype.
Intrahepatic cholangiocarcinoma (ICC) is a lethal disease that affects patients with advanced disease. There is growing evidence indicating that KRAS activity in the PI3K/AKT/mTOR pathway plays an important role in ICC. KRAS mutation status and the efficacy of cetuximab were investigated in different types of ICCs, with the aim of identifying new therapeutic possibilities for ICCs.

Methods: We examined KRAS, BRAF, and EGFR mutation status in 63 resected ICCs (29 muc-ICCs, 34 mixed-ICCs), and compared their mutation status with the different ICC phenotypes. Histological diagnoses were made according to the WHO classification and our publication (ref). KRAS mutation status and the efficacy of cetuximab were assessed by Western blot and flow cytometry.

Results: Among the 63 ICCs, KRAS mutations were found in 12 (19%) [p.G12D (n=7), p.G12V (n=4), p.G12R (n=1)]. EGFR mutation was seen in one (1.6%) ICC, and BRAF mutation was not detected. KRAS mutations were only observed in muc-ICCs, and represented 41.4% of the muc-ICCs. Among the ICC cell lines, KRAS mutation was seen in KMCH-1 and -2 (corresponding to muc-ICCs), but was not seen in KMC-1 and -2 (corresponding to mixed-ICCs). Western blotting showed EGFR phosphorylated (p)-EGFR, and p-ERK1/2 in all cell lines, and p-EGFR and p-ERK1/2 were markedly suppressed in all cell lines after cetuximab treatment. Cell proliferation was significantly suppressed in KMCH-1, and -2, compared with KMC-1 and -2.

Conclusion: KRAS mutations were only observed in muc-ICCs, and not in mixed-ICCs. This confers the validity of the classification and provides new therapeutic possibilities for ICCs. The ICC cell line data suggests the possible efficacy of cetuximab.

Reference(s)

The Interaction of Human Hepatocellular Carcinoma With Dendritic Cells Is Able to Induce a Novel Subset of CD8+ Regulatory T Cells
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Introduction: Tumour specific effector T-cells can be detected in the blood and tumours of patients with hepatocellular carcinoma (HCC) but fail to mount effective immune responses. Attempts to amplify anti-tumour immune responses using immunotherapy show promise, but are hampered by the presence of suppressive regulatory T-cells (Treg) that inhibit anti-tumour immune responses. Treg are crucial to the maintenance of immune homeostasis and prevention of auto-reactive immune responses but in the context of cancer, they can suppress beneficial anti-tumour immunity, leading to tumour progression. We have previously demonstrated CD8+ expressing Treg in HCC. However, little is known about their development. We now report a possible mechanism for the induction of CD8+ Treg by HCC.

Methods: Tissue conditioned media were produced by incubating freshly obtained HCC tumour or matched non-involved distal liver sections in RPMI and stored at −80 degrees until use. Monocyte-derived dendritic cells (moDC) were isolated by cell sorting for downstream functional assays.

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Results: Following culture of MoDC in tumour tissue conditioned media, expression of CD80, CD86 and MHC II molecules was reduced compared to moDCs cultured in distal tissue conditioned media. The co-culture of naïve CD8+ T-cells with tumor conditioned media derived moDC induces a population of CD8+CD25highCD127lowFoxP3+ T-cell (CD8/Treg). This induced population of T-cells secreted interleukin-10 (IL-10) and was able to suppress alloengeneic effector cells proliferation in vitro. In contrast, T-cells cultured in either media alone or distal tissue conditioned media induced less CD8+CD25highCD127lowFoxP3+ T-cells which lacked regulatory activities.

Conclusion: MoDC matured in HCC conditioned media are less activated and are able to induce a novel subset of functional CD8/Treg. This novel pathway of CD8/Treg induction may offer potential for future immunotherapeutic targets in HCC.

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UCN-01 INDUCES S AND G2/M CELL CYCLE ARREST THROUGH THE p53/p21or CHK2/CDC25C PATHWAYS AND CAN SUPPRESS INVASION IN HUMAN HEPATOMA CELL LINES

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Aims: UCN-01 (7-hydroxyxaurastrorpine), a protein kinase inhibitor, has attracted a great deal of attention as a potent antitumor agent. Several clinical trials of UCN-01 treatments alone, or in combination with another agent, for different kinds of tumors are currently underway, and some of these trials have had positive results. Hepatocellular carcinoma has high-incidence rates and is associated with poor prognosis and high mortality rates.

Methods: Three different hepatoma cell lines (Huh7, HepG2, and Hep3B) were treated with different concentrations of UCN-01, and the anti-tumor effects of UCN-01 have been evaluated. Following UCN-01 treatment, cell growth was measured using the MTT assay, cell cycle arrest was assayed using flow cytometry, and the mechanism of cell cycle arrest and invasion inhibition were investigated by western blotting and the Matrigel invasion assay.

Results: After 72 h of UCN-01 treatment, the growth of different hepatoma cell lines was significantly inhibited in a dose-dependent manner, with IC50 values ranging from 69.76 to 222.74 nM. Flow cytometry results suggest that UCN-01 inhibits proliferation in the hepatoma cell lines by inducing S phase and G2/M arrest, but not G1/S arrest, which is very different from previous reports in other tumor cell lines. Western blot results illustrate that UCN-01 can induce G2/M cell cycle arrest, regardless of status of the p53/p21 or CHK2/CDC25C pathway, whereas the CHK2/CDC25C pathway and the p53/p21 pathway are involved in UCN-01-induced cell cycle arrest. UCN-01 remarkably inhibits Huh7 cell invasion in a time-dependent manner. Suppression of Huh7 cell invasion may be due to the down-regulation of phosphorylated β-catenin by UCN-01.

Conclusion: These findings suggest that UCN-01 induces hepatoma cell growth inhibition by regulating the p53/p21 and CHK2/CDC25 pathways. Suppression of Huh7 cell invasion by UCN-01 may be due to the down-regulation of phosphorylated β-catenin. These data lend support to the fact that further studies on UCN-01 as an anti-HCC candidate may be promising.
(HSC) activation plays a key role in fibrogenesis and extracellular matrix deposition. Better knowledge of the interlink among HCC, adjacent cells and their derived components, that integrate the peri-tumoral stroma, will provide rationale for future therapies.

**Methods:** Primary mouse hepatic stellate cells (HSCs), obtained after collagenase digestion, and activated human HSC cell line (LX2) were exposed to conditioned medium from HCC cell lines (HepsG2 and Hep3B), recombinant proteins (e.g. angiogenin) and inhibitors (e.g. neomycin). Western blots and qPCRs were performed to follow HSC phenotypic changes. Protein microarray was analyzed to search for specific HSC activators in medium from HCC cells. Angiogenin nuclear translocation was visualized in LX2 cells by immunofluorescence. Tumor growth was determined subcutaneously and orthotopically after co-injection of LX2 and HepG2 cells on the flanks and liver of nude mice, respectively. Angiogenin and serum levels were detected by western blot and ELISA.

**Results:** HSC activation was observed after exposure to conditioned media from HepG2 and Hep3B cells. Release of angiogenic factors was analyzed in these media and several HCC-derived proteins identified. Among them, angiogenin, a protein secreted by several tumor types with ribonuclease activity and translational control, was able to induce HSC activation in primary mouse and human cell line LX2 after its extracellular caption and nuclear interaction. Blockade of nuclear translocation with neomycin diminished HSC activation by recombinant angiogenin or by HCC-conditioned medium. In vivo, increased angiogenin serum levels were detected in nude mice orthotopically co-injected with LX2 and HepG2 cells. In accordance, higher alpha-SMA mRNA levels were observed in subcutaneous tumors with upper angiogenin levels; while reduced angiogenin signaling, as after neomycin administration, was associated with decreased tumor growth and vascularization.

**Conclusion:** Angiogenin release by HCC promotes a profibrogenic environment by activating surrounding HSCs, pointing to strategies directed against angiogenin production, internalization or nuclear interaction as of potential interest in HCC management.

**1063 microRNAs in the p53 network: epithelial–mesenchymal transition in functional tumor-initiating cells of human gallbladder cancer**

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Epithelial mesenchymal transition (EMT) is a process that is reminiscent of tumor-initiating cell characteristics. Loss of p53 is also associated with the altered key regulatory genes/miRNAs during cancer and tumor-initiating cell development. Our aim was to characterize the functional role of p53 regulated EMT through specific miRNAs in human gall bladder tumor-initiating cells.

**Methods:** miRNA expression in human gall bladder cancer tissues, human gall bladder tumor-initiating cells (GB-TICs), Mz-ChA-1 gall bladder cancer cells and H69 non-malignant cholangiocytes was assessed by hybridization-based microarray. Expression of selected miRNA was further evaluated by Taqman real-time PCR analysis. Chromatin immunoprecipitation assay was modified from the EZ-ChIP protocol using a p53 antibody. The mRNA and protein expression of p53, cancer stem cell marker CD133 and ALDH, epithelial marker cytokeratin 19 and mesenchymal marker S100A4 was quantitated by real-time PCR and/or immunoblot analysis.

**Results:** We identified miR-200 family, including miR-200a, miR-200b and miR-200c that are differentially expressed in doxorubicin-enriched cell fractions in a mouse xenograft tumor model of gall bladder cancer. Members of the miR-200 family were notably more silenced in GB-TICs compared with their parental gall bladder cancer cells (GBCs) and Mz-ChA-1 cells. We found that p53 plays a role in regulating both EMT and EMT-associated tumor-initiating cell properties through transcriptional activation of miR-200b and miR-200c. p53 transactivates miR-200b and miR-200c through direct binding to their promoter region. Loss of p53 in gallbladder cancer cells leads to decreased expression of miR-200b/c and activates EMT program, accompanied by increased mesenchymal tumor-initiating cell population. Re-expressing miR-200b/c suppresses genes that mediate EMT and tumor-initiating properties and thereby reverts mesenchymal and stem cell-like phenotype caused by loss of p53 to differentiated epithelial cell phenotype. Furthermore, loss of p53 negatively correlates with miR-200b/200c level but positively with increased expression of EMT and tumor-initiating markers in GB-TIC xenograft tumor in vivo.

**Conclusion:** p53 can regulate tumor-initiating cell by modulating the expression of miR-200 family, the key regulators of EMT pathway.

**1064 Targeting TLR7 and 9: a novel strategy for treatment of cholangiocarcinoma**

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Background: Cholangiocarcinoma (CC) is a primary liver cancer with a dismal outcome. We have shown that inhibition TLR7 and TLR9 reduced the development and growth of HCC in animal models and cell-systems. The aims of the study were to determine whether TLR7 and TLR9 are upregulated in human CC and whether inhibition of the TLR7 and TLR9 signalling in CC cell lines and animal models reduces cellular proliferation and tumour growth.

**Method:** Expression of TLR7 and TLR9 in human CC: Immunohistochemistry of TLR7 and TLR9 antibody on (tissue microarray). TLR7 and TLR9 inhibition: This was achieved using chloroquine and a selective inhibitory oligonucleotide (IRS, Dynavax). In vitro cellular proliferation studies: Stimulation TLR7 and TLR9 using (Imiquimod) IMQ and CpG respectively and, inhibition using Chloroquine or IRS.

**Results:** In vivo studies in a xenograft model of CC: 5x106 HuCCT cells were injected in NOD-SCID mice for 60 days divided into 3 groups: Control without treatment, Chloroquine in drinking water 25mg/kg, IRS, 100µg IP once per week.

**Histology:** TLR7 staining: Normal bile duct epithelium: negative. 20% of CC cases were negative while 30% had positive nuclear staining (+) and 50% of cases were positive (++). TLR9 staining: Normal bile duct epithelium: negative; 20% of CC were negative, 30% were positive (+) 20% (++) and 30% (+++).

**Conclusion:** The results of our study suggests for the first time that TLR7 and TLR9 are expressed in CC and its inhibition both in vitro
and in vivo inhibits CC proliferation suggesting that these TLRs can be a therapeutic target.

Figure: Image and graph for TLR7&9 in cc.

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MK2, AN EBP50 INTERACTING KINASE, CONFRONTS SURVIVAL BENEFIT IN LIVER CANCER CELLS EXPOSED TO AN OXIDATIVE STRESS

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Background and Aims: MK2 is a kinase of the p38 MAPK pathway that possesses a PDZ-interacting motif. MK2 is activated by oxidative stress to phosphorylate proteins such as AKT or the heat shock protein, HSP27, involved in cell protection. MK2 is also a major regulator of the expression of interleukins (i.e. IL-1b, IL-8). MK2 defect causes a dramatic depletion of the liver in antioxidant (i.e. glutathione). The generation of ROS causing oxidative stress occurs in liver diseases and their progression to liver cancer. Therefore, the aim of the study was to investigate the potential function of MK2 in liver carcinogenesis.

Methods: MK2 pathway was evaluated by immunoblotting in human biliary (Mz-ChA-1) and hepatocellular carcinoma (PLC/PRF/5) cell lines. To induce oxidative stress, hydrogen peroxide at various doses was added in cell cultures. MK2 was inactivated by an inhibitor (MK2iIII). Cell survival and apoptosis were analyzed by MTT assay, flow cytometry and immunoblotting. Expression of interleukins was determined by RT-qPCR. Interaction of MK2 with the major liver PDZ-protein, EBP50, was investigated by co-immunoprecipitation and GST-pull down.

Results: In liver tumor cells, oxidative stress decreased cell viability assessed by MTT assay and increased apoptosis assessed by sub-G1 cell population and caspase-3/PARP cleavage. These cellular responses were amplified with MK2iIII, suggesting a survival function of MK2. Upon oxidative stress, MK2 was activated in a dose and time dependent manner along with the phosphorylation of AKT and HSP27 in these cells. Oxidative stress increased mRNA levels of IL-1b and IL-8 that was not abrogated by actinomycinD, but inhibited by MK2iIII. These data demonstrated that MK2 regulates the expression of interleukins through a post-transcriptional mechanism. We showed that MK2 interacts with PDZ domains of EBP50. Down-regulation of EBP50 by siRNA in cells caused a decrease in the phosphorylation of HSP27 and AKT, and a diminution of interleukins mRNA levels upon oxidative stress.

Conclusion: The MK2 pathway is activated in liver tumor cells in response to oxidative stress and contributes to cell survival. EBP50 links and regulates the activity of MK2 in these cells. These data suggest a contribution of the EBP50-MK2 pathway in liver cancer.
findings indicate that ASM in the liver inhibits tumor growth through cytotoxic macrophage accumulation in response to S1P. In addition, B16C2M melanoma cells formed small metastatic lesions in the liver of ASM− mice (incidence: 44.4%), whereas none of the ASM+ mice developed metastases, suggesting that the anti-tumor effect of ASM is not specific for colon cancer cells. In conclusion, ASM in hepatocytes inhibited tumor growth via S1P formation and subsequent cytotoxic macrophage accumulation. Thus, targeting ASM may represent a new therapeutic strategy for treating metastatic liver tumor.

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A PROTEOMIC STUDY OF CHOLANGIOCELLULAR CARCINOMA FOR DETECTION OF NOVEL BIOMARKERS IN THE BILE
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Background and Aims: Cholangiocellular carcinoma (CCC) is a major subtype of liver cancer that arises in the bile ducts. It is known to have poor patient outcomes, with a 5-year survival rate of about 5%. The incidence of, and mortality from, CCC are increasing worldwide. At present, extensive surgical resection or transplantation remain the only potentially curative treatments, although most patients are considered inoperable at the time of diagnosis. Specific and sensitive biomarkers for the early detection of CCC are therefore needed.

Methods: In order to identify such biomarkers, malignant and healthy tissue from 8 CCC patients was analysed using two techniques from quantitative proteomics. 2D-DIGE (two-dimensional differential in-gel electrophoresis) was performed with minimal labelling of disrupted tissue. Differential spots were detected using DeCyder Software (GE Healthcare) and proteins of interest were identified by MALDI-TOF mass spectrometry on an Ultraflex II (Bruker). Parallel to this, a mass spectrometry-based label-free approach was adopted. For this, an RP-HPLC-MS/MS method was used to analyse the samples, with an Ultimate 3000 RSLCnano system (Dionex) online coupled to an LTQ Orbitrap Elite (Thermo Scientific). Progenesis software (Nonlinear) was used to detect differentially expressed proteins. Additionally, the proteome of bile from five CCC patients was analysed to check for the presence of proteins found to be regulated in the tissue. This was done using RP-HPLC-MS/MS, as described above.

Results: As expected, both strategies show complementary results, which indicates that a combination of both techniques may be very promising in the identification of biomarker candidates for CCC. More than 1000 proteins have been found to be regulated significantly in the tissue based studies, 85 of them have been found using both these techniques. We compared this list with differential proteins in the bile; altogether 30 proteins were differentially expressed in the three studies.

Conclusions: These overlapping results, from the tissue and the bile, make them especially promising candidates for minimally invasive biomarkers. Some of the proteins have been validated using western-blots and immunohistochemistry.

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DOWNREGULATION OF DISCOIDIN DOMAIN RECEPTOR 2 DECREASES TUMOR GROWTH OF HEPATOCELLULAR CARCINOMA
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Background and Aims: Cancer development is essentially a tissue remodeling process in which proteolytic degradation of the extracellular matrix (EMC) plays a crucial role. Discoidin domain receptors (DDRs) have recently been identified as tyrosine kinase receptors for collagen, a key constituent of ECM. The overexpression of DDR1 was reported to be correlated with hepatocellular carcinoma (HCC) progression in vitro. However, little is currently known about the mechanism and functional effects of DDR2 on HCC cells. Therefore we investigated the expression and function of DDR2 in human HCC cells.

Methods: Expression of DDR2 in human HCC cell lines and patient tissues was observed. The suppression of DDR2 by siRNA against DDR2 was performed in vitro and in vivo study.

Results: All of the examined human HCC cell lines expressed DDR2 mRNA in a cell line-dependent manner. All HCC tissues from the five patients with HCC demonstrated DDR2 mRNA expression. Transfection of DDR2 siRNA significantly inhibits cell growth of Hep3B, SNU387, SNU182, and PLC/PRF5 compared to cells with non-target siRNA transfection (P < 0.001). SNU182, Hep3B, and HeLa cell xenograft models were established in nude mice. There was a significant difference in average tumor volumes between the DDR2 siRNA-treated group and the non-target siRNA-treated group at 12 days and at 14 days after the injection (P < 0.05) in SNU182 xenograft mice. DDR2 siRNA injection decreased the mean tumor volume by 65.6% compared to that of the control. The apoptosis analysis demonstrated that DDR2 siRNA treatment significantly increased apoptotic cells (P < 0.01). Cell migration (P < 0.05) and cell invasion (P < 0.01) was dramatically decreased by DDR2 siRNA treatment.

Conclusions: Our study provides the first evidence that human HCC cell lines and HCC tumor tissues of patients express DDR2; that the suppression of DDR2 inhibits in vitro and in vivo growth of HCC cells in a cell type-dependent manner; and that the anti-tumor growth effect of DDR2 siRNA is associated with apoptosis, the inhibition of migration and invasion. Our results strongly support the use of DDR2 as a novel target of HCC treatment.

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DISCRIMINATING CIRRHOSIS AND HEPATOCELLULAR CARCINOMA BY IN SITU CHEMICAL ANALYSIS USING INFRARED MICROSCOPY
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Background and Aims: Liver is subject to various chronic pathologies progressively leading to cirrhosis which is associated with an increased risk of hepatocellular carcinoma (HCC). There is an urgent need of diagnostic or prognostic markers of chronic liver diseases and cancer. Fourier transform-infrared spectroscopy (FTIR) has been established as a unique spectroscopic method for biochemical analysis of cells and tissues. The use of synchrotron radiation (SR) allows investigating at cellular level the subtle chemical changes associated with pathological states. The aim of
this study was to define markers of cirrhosis and HCC using SR-FTIR microspectroscopy.

Methods: The study has been focused on liver samples obtained from surgical specimens including normal livers (n = 7) and patients with HCC on cirrhosis from alcohol (n = 6) or HCV (n = 6) aetiology. Frozen tissue sections stained with H&E were performed for histological examination. Adjacent tissue sections were investigated using SR-FTIR microspectroscopy experiments. The variance was addressed by multivariate statistical methods such as principal component analysis (PCA).

Results: We strived firstly to discriminate the spectroscopic profile of healthy and pathological liver. The infrared spectral pattern of cirrhosis or HCC exhibited significant differences in the composition of lipids, proteins and sugars when compared with tissue from normal liver. Then, we investigated the spectral differences that discriminate tumor tissue from cirrhosis. Detailed analysis demonstrates a frequency shift in proteins suggesting changes in the proteome but the major discrimination was observed on sugars. Furthermore, SR-FTIR experiments followed by PCA analysis allowed the discrimination between hyperplastic and dysplastic cirrhotic nodules on sugars. Infrared signatures of dysplastic nodules superimposed with HCC suggesting that FTIR microspectroscopy allows diagnosing tissue changes associated with early stages of cancer. Finally, experiments were performed on a conventional IR microscope using an internal IR source. Despite the lower spatial resolution of such a device, it was possible to reproduce the results obtained in the exploratory phase using synchrotron radiations.

Conclusions: Infrared microspectroscopy allows discriminating various grades of cirrhosis and HCC. This approach that can be easily implemented into hospitals may open new avenues for clinical applications and personalized medicine.

1071 CONVENTIONAL AND DC-Beads-MEDIATED TRANSARTERIAL CHEMOEMBOLIZATION: ANY DIFFERENCE IN NEOANGIOGENESIS?

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Background and Aims: Transcatheter arterial chemoembolization (TACE) is the conventional palliative treatment for hepatocellular carcinoma in BCLC intermediate stage. Factors potentially interfering with effectiveness include a neo-angiogenic reaction due to ischemia, reflected by a rise in circulating vascular endothelial growth factor (VEGF) levels after TACE. Primary end point of our study was to investigate any significant difference in neoangiogenesis after conventional TACE (C-TACE), compared to DC-Beads mediated TACE (DEB-TACE), measuring serum VEGF.

Methods: VEGF-A (ELISA) was determined in the sera of 88 consecutive HCC intermediate (65%) or early (35%) stage patients, before TACE (t0) and after 4 weeks (t1). C-TACE was administered to the first 60, DEB-TACE to the following 28. Tumor vascularization before TACE (t0) and after 4 weeks (t1). C-TACE was administered to the first 60, DEB-TACE to the following 28. Tumor vascularization before TACE (t0) and after 4 weeks (t1). Complete response was recorded in 25% C-TACE and 29% DEB-TACE patients; mean survival was 32 months (CI 27–36). Side-effects and objective response rates were comparable, the DEB-TACE subgroup showing only a non-significantly better response rate. VEGF t0 levels were higher in multifocal disease (p = 0.015) and non-responders (p = 0.03). At t1 VEGF levels rose significantly overall (p = 0.003), in C-TACE (p = 0.04) and DEB-TACE (p = 0.02). The rise was more significant in partial responders (p = 0.005), especially with DEB-TACE (p = 0.002); in complete responders the rise was significant only in DEB-TACE (p = 0.05). The percentage of patients with VEGF rising at t1 was higher in DEB-TACE (p = 0.007). Tumor size (p = 0.0001), t0 vascularization (p = 0.003) and t0 VEGF levels (p = 0.009) inversely correlated with survival. VEGF t0 levels, tumor size and vascularization were singled out in the Cox multivariate analysis as independent predictors of survival.

Conclusions: DEB-TACE and C-TACE seem equally effective as regards impact on survival, response to treatment and side effects. DEB-TACE tends to cause higher tissue ischemia, as reflected by a more significant rise at t1 VEGF levels, in partial and even in complete responders, and by a higher percentage of DEB-TACE patients with VEGF rising at t1. High t0 VEGF levels predict worse response to treatment and shorter survival. The prognostic value of baseline VEGF, tumor vascularization and tumor burden are equally relevant.

1072 TELOMERIC DYSFUNCTION IN RELATION TO OXIDATIVE DNA DAMAGE IN LIVER CARCINOGENESIS

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Background and Aims: Induction of chronic oxidative stress by hepatitis C (HCV) and B (HBV) virus is one of the molecular events leading to hepatocellular carcinoma (HCC) development. Telomeres are prone to oxidative modifications, that induce progressive telomere shortening and chromosomal instability. Telomerase activity plays a crucial role in telomeres maintenance and cell immortalization. TERT, the rate limiting factor for telomerase transcription, is regulated by epigenetic mechanism and under oxidative stress migrates to mitochondria, where it ameliorates the membrane stability and exerts an anti-apoptotic role. The aim of the study was to investigate the complex molecular network underlying virus-related liver carcinogenesis, evaluating: 8-hydroxydeoxyguanosine (8-OHdG), marker of oxidative DNA damage, telomere length, telomerase activity, TERT promoter methylation and mitochondrial TERT translocation.

Methods: One hundred sixty-two patients were investigated: 21 with HCC (in tumor and peritumoral tissue samples); 71 with HCV and 35 with HBV and 15 controls. Eight-OHdG was quantified through HPLC-EC, telomerase activity and telomeres’ length by Real Time PCR, TERT promoter methylation status by Quantitative Methylation Specific PCR and mitochondrial TERT translocation by Western Blot.

Results: Overall, 8-OHdG levels were significantly higher in tumor tissues than in controls (p = 0.02), telomeres were significantly shorter in HCC compared to the less advanced stages of disease (p = 0.01), whereas telomerase activity was significantly higher in tumor tissues than in controls (p = 0.01). 8-OHdG levels inversely correlated with a telomere length in HCC. Overall, TERT promoter was hypermethylated in HCC and peritumoral tissue samples (p = 0.0001). When the patients were subgrouped on the basis of etiology, HBV-related liver damage progression was characterized by later 8OhdG accumulation and a pronounced telomerase activation limited to HCC tissue. TERT was localized in mitochondria in all investigated HCC samples and in mitochondria DNA 8-OHdG levels were significantly lower than in genomic DNA (p = 0.0003).

Conclusions: These data describe a complex network in which oxidative DNA damage is linked to changes in telomeres length, extent of telomerase activation and higher methylation of TERT promoter in HBV/HCV related carcinogenesis. The role of mitochondrial TERT in HCC is open to debate but an intriguing work hypothesis is that it could downregulate apoptosis.
INTEGRATED GENOMIC ANALYSIS IN HEPATOCELLULAR ADENOMA

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Background and Aim: Hepatocellular adenomas (HCA) are rare benign tumors developed in normal liver predominantly in young women using oral contraception. HCA lead to diagnostic pitfalls and several difficulties to assess the risk of malignant transformation in these young patients. Recent advances in basic knowledge have revealed a molecular classification related to risk factors, pathological features and risk of transformation in hepatocellular carcinoma. Three major molecular pathways have been identified altered in specific HCA subgroups that are define by either (1) inactivation of hepatocyte nuclear factor 1A (HNF1A) transcription factor, (2) activation of the WNT/β-catenin or (3) activation of the IL6/STAT3 pathway by somatic mutation of IL6ST, GNAS or STAT3. Here we performed an integrated genomic approach to analyze a large series of HCA related to the different major subtypes.

Methods: 250 hepatocellular adenomas were collected, pathologically reviewed and classified according to the molecular classification. We performed CGH-SNP, methylose 450K, whole exome sequencing and transcriptome analyses in 50 to 120 of the cases and the results were validated in the second set of tumors.

Results: First, we focused our analysis in multiple tumors. We showed that in most of the cases, all the nodules in a patient were of the same molecular subtype but presented different genomic alterations demonstrating their independent origin. We also analyzed 5 cases with a malignant transformation in hepatocellular carcinoma. Comparison of the benign and malignant part of the tumor enabled to identify the timeframe of accumulation of the genomic alterations. Then, with the transcriptome and sequencing approaches, we were able to identify and validate 2 new subtypes of adenomas. Finally, CGH-SNP and methylose profiling were closely related to the major molecular groups of HCA.

Conclusion: In conclusion, this approach enabled to refine the molecular classification of hepatocellular adenoma with close relationship between the genotype and the phenotype of the tumors, and provides new insights on understanding malignant transformation process.

EVALUATION OF EFFICACY OF ANTIANGIOGENIC TREATMENT IN A MURINE MODEL OF HEPATOCELLULAR CARCINOMA UNDER DIFFERENT TREATMENT SCHEDULES

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Background and Aim: Development of escape pathways from antiangiogenic treatments was reported associated with enhanced tumor aggressiveness. Aim of the study was to evaluate tumor response simulating different conditions of administration of antiangiogenic treatment in a hepatocellular carcinoma animal model.

Methods: Subcutaneous tumors were created by inoculating 5x106 Huh7 cells into the right flank of nude mice. When dimensions reached 5–10mm in diameter, 14 mice were divided in 3 groups according to the following 13-days protocol: group 1 was treated with placebo, group 2 was treated until day +5 with sorafenib until day +5 and then the treatment was definitively stopped. At day +13 all mice were sacrificed, collecting masses for Western-Blot analyses. Volume was calculated with B-mode ultrasonography at day 0, +5, +9, +11 and +13. VEGF2-targeted contrast-enhanced ultrasound using BR55 (Bracco Imaging) was performed at day +5 and +13 and elastosonography (Esaote) at day +9 and +11 to assess tumor stiffness.

Results: Median growth percentage delta at day +13 was 197% (115–329) in group 1, 81% (48–144) in group 2 and 111% (27–167) in group 3. Median growth delta at day +13 with respect to day +5 (when therapy was stopped in group 2 and 3) was 79% (48–127), 37% (−14–128) and 81% (15–87) in group 1, 2 and 3, respectively. Quantification of targeted-CEUS showed difference among the 3 groups at day +13 (p = 0.033) with higher value in group 3 (509, range 293–652) than group 1 (275, range 191–494) and group 2 (181, range 63–318). Western-Blot analysis demonstrated slightly higher VEGF2 expression in group 3 with respect to group 1 and 2. Tumor stiffness decreased in group 2 when treatment was restarted (+11%) and increased in the other groups (−5% in group 1 and −8% in group 3).

Conclusions: An early and short interruption of antiangiogenic treatment does not impede restoration of tumor response, while a final interruption tends to stimulate a rebound of angiogenesis to higher level than without treatment.

IDENTIFICATION BY IN SITU PROTEOMIC IMAGING OF MODIFIED FORMS OF HISTONE H4 AS NEW MOLECULAR MARKERS OF MICROVASCULAR INVASION IN HEPATOCELLULAR CARCINOMAS


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Background and Aim: Microvascular invasion (MVI) is a major risk factor of post-operative tumor recurrence and mortality in Hepatocellular carcinomas (HCC). To date, no reliable tool is available to detect MVI prior to surgical procedures. MALDI imaging mass spectrometry (MALDI IMS) represents an innovative proteomic in situ approach for characterizing the spectrum of peptides/proteins expressed in tissue sections.

The aim of this study was to identify tissue surrogate biomarkers of MVI in HCC using MALDI IMS technology.

Methods: 56 HCC (26 HCC/MVI−; 30 HCC/MVI+) obtained from surgical specimens, for which clinico-pathological data and frozen samples were available, were retrospectively collected and subjected to MALDI IMS (train cohort). A comparative statistical analysis of acquired mass spectra, using a cross classification model, was performed in order to determine protein peaks differentially expressed between the two groups. Molecular identification of differential peaks was performed on protein tissue extracts. Tissue expression of identified proteins was then evaluated by immunohistochemistry in the train cohort and in an independent validation set of 23 HCC.

Results: MALDI IMS analysis yielded 30 discriminating protein peaks. Of these, two modified forms of histone H4 were identified: a form acetylated on Serine 1, di-methylated on Lysine 20 (m/z 5544) and a form acetylated on Serine 1, di-methylated on Lysine 20 and acetylated on Lysine 16 (m/z 3790). Tissue expression of these two
Background and Aims: The majority of hepatocellular carcinoma (HCC) is caused by chronic hepatitis B and C virus infection. A proportion arises from cancer stem cells (CSCs) that are responsible for tumor initiation and progression. Interleukin 6 (IL-6), a pro-inflammatory cytokine involved in liver regeneration and the inflammatory response, is also thought to play a role in hepatocarcinogenesis, though the mechanisms are less clear. We sought to explore the regulatory role of IL-6 on hepatic CSCs.

Methods: CD133+/CD44+ was used to isolate putative CSCs from HuH7 cells by FACSscan and magnetic bead separation. Isolated CSCs were maintained in an undifferentiation state. The expression of commonly used CSC markers (Epcam, CD133, CD44, and AFP), stemness markers (Oct3/4, Nanog), as well as IL-6 signalling genes (CD133, IL-6R, and STAT3) was examined by qPCR and Western blot. The tumour forming ability of the CD133+/CD44+ cells was examined by tumour sphere assays. Expression of CSC markers was also examined in mouse liver tissue up to 144 hours after administration of the carcinogen DEN.

Results: Significantly higher level of IL-6, IL-6R, and pSTAT3 were observed in CD133+/CD44+ cells compared to CD133−/CD44− cells. CD133+/CD44+ cells demonstrated typical characteristics of CSCs, including enhanced expression of common CSC markers (CD133, CD44, Epcam, and AFP), stemness markers (Oct3/4, Nanog), and most importantly, an enhanced ability to form tumour spheres. In DEN-treated C57 mice, there was a significant up-regulation of IL-6 signalling at 24–48h demonstrated by increased circulating IL-6 and pSTAT3 up to 48h. A temporospatial increase in the expression of Notch signalling genes (DLL4, Hes1, Hey1, NICD) was observed. In contrast, IL-6 KO mice did not respond to DEN with the above changes. Regression analysis of Western blot data demonstrated a correlation between IL-6 and Hes1, as well as IL-6 and DLL4 mRNA expression. In nude mice, IL-6 stable cell lines induced tumours that grew faster and at harvest were ~5-fold larger than xenografts from control cells. A significant increase in Notch target genes such as Hes1 and NICD was observed in IL-6 stable cell line-derived xenograft tumours.

Conclusions: IL-6 may exert its oncogenic effect on liver, in part, through an up-regulation of Notch signalling.

1078 MECHANIC-BASED REPROGRAMMING OF HEPATIC SELF-RENEWING TUMOR-INITIATING CELLS THROUGH DNMT1-INHIBITION
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Background and Aims: Modulation of cellular fate in solid tumors is defined to a large extent by DNMT1-regulated epigenetic machinery and cellular-non-cellular constituents in the tumor-initiating cell (TIC) niche. Current study examines the significance of the DNMT1-cellular interactions in reprogramming of TICs properties.

Methods: Seven HCC cell lines were plated in 2D culture at various cell densities and exposed to a transient nontoxic dose of a DNMT1-inhibitor Zebularine (ZEB). After a 3-day treatment, cells were cultured in 3D non-adherent condition in ZEB- and serum-free media to generate primary spheres (G1) which were then passaged through generation G5. Differences in long-term self-renewal, gene expression, tumorigenicity and metastatic potential of G1–G5 spheres were examined.

Results: Transient exposure to ZEB produced the differential cell density-dependent responses in 5/7 tested HCC cell lines. In cells grown at low density (LD), ZEB caused a remarkable increase in G1 sphere formation. This effect persisted through G5. In striking contrast, untreated LD cells failed to form primary spheres while the sphere forming potential of high density (HD) and HD ZEB-treated (HDZ) cells rapidly decreased over the first 3 generations. Likewise DNMT1 depletion by shRNA promoted acquisition of self-renewal potential in LD cells. The increase in sphere forming potential of LDZ cells strongly correlated with a stable overexpression of cancer stem cell-related markers and key genes involved in self-renewal and epithelial–mesenchymal transition. Moreover, when dissociated LDZ, HD and HDZ spheres were injected subcutaneously into NOD/SCID mice, LDZ cells generated tumors more rapidly and were more metastatic. Both
gene reactivation and tumorigenicity progressively increased from G1 to G4. Tumors derived from G1–G4 LDZs were also increasingly more vascular. Global transcriptome analysis of LDZ spheres at G1–G4 confirmed that a LDZ signature was enriched in genes associated with oncogenic signaling pathways and could predict clinical outcome of liver cancer patients.

Conclusions: DNMT1 inhibition combined with cellular context-dependent cues results in reprogramming of hepatic TICs which persists long after the drug removal and affects their fate. These findings may provide a new venue for therapeutic strategy in HCC patients.

1079 HEDGEHOG SIGNALING CONTRIBUTES TO CHEMOTAXIS IN CHOLANGIOCARCINOMA CELLS

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Background and Aims: Hedgehog (Hh) signaling pathway is activated in cholangiocarcinoma (CCA). Hh signaling occurs when Hh ligands [Sonic Hh, Indian Hh, and Desert Hh] bind Patched1 derepressing Smoothened, a G-protein-coupled receptor. Smoothened further activates: a) a cilia-dependent transcriptional pathway involving Gli proteins; and b) a cilia-independent pathway involving G-proteins and chemotaxis. Our aim was to determine which Hh signaling pathway(s) contributes to CCA biology.

Methods: Human [normal cholangiocytes (H69), CCA (KMCH, HuCCT-1, Mz-ChA-1)] and rat [normal cholangiocytes (NRC), CCA (BDEneu)] cell lines were employed. A stable rat CCA BDEneu cell line with a dominant-negative Patched1 was generated using the Sleeping Beauty transposon transfection system; this cell line does not respond to Hh ligand stimulation. Cells were assessed for a cilia expression; transcriptional and G-protein-dependent pathways activation; and migration. Intrahepatic implantation of wild-type or dominant-negative Patched1 BDEneu cells was performed in male Fischer 344 rats.

Results: Although normal cholangiocytes abundantly express cilia, these organelles were absent in human and rat CCA cells (p < 0.01). Consistent with the absence of cilia, canonical Hh signaling with increased Gli1 expression was not universally observed in all CCA cell lines. In contrast, evidence for noncanonical Hh signaling was observed in the human and rat CCA cell lines (i.e., translocation of Smoothened to the plasma membrane, and chemotaxis in Boyden chambers). The chemotaxis in response to Sonic Hh ligand was abrogated by the G-protein inhibitor, pertussis toxin. In vivo, none of the animals implanted with BDEneu cells with dominant-negative Patched1 (n = 9) developed tumors, unlike animals with wild-type CCA cells (n = 9), which formed aggressive tumors (100% of animals) with metastases (89% of animals). In addition, only 54.5% of animals treated with the Smoothened inhibitor, GDC-0449, (n = 11) developed tumors, as compared with 100% of vehicle treated animals (n = 10).

Conclusions: Cancer cells lacking cilia respond to Hh signaling with chemotaxis via a G-protein-coupled pathway. In vivo, genetic or pharmacological inhibition of Hh signaling suppressed tumor engraftment and metastasis likely by blocking CCA chemotaxis.
NEW DIAGNOSTIC AND PROGNOSTIC PLASMA BIOMARKERS FOR PATIENTS WITH HEPATOCELLULAR CARCINOMA IDENTIFIED BY PROTEIN PROFILING

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Background and Aims: Hepatocellular carcinoma (HCC) is the main cause of death in cirrhotic patients. For that reason, these patients are included in a surveillance program with abdominal ultrasonography twice per year to detect HCC at early tumor stages, when cure is highly likely. Nevertheless, only 40% of the cases are diagnosed at early stages and the HCC recurrence rate after curative treatment – surgical resection or percutaneous ablation – is very high. Therefore, the identification of biomarkers is needed to use them as complementary tools for surveillance and for predicting tumor recurrence after treatment. The aim of this study was to perform proteomic profiling of the plasma of cirrhotic patients with and without HCC to identify diagnostic and invasiveness biomarkers.

Methods: We analyzed the plasma protein profile of 17 selected cirrhotic patients (15 men, median age 70yr, 94% HCV, 88% ChildA), 10 of them with HCC (BCLC 0/A, n = 5; BCLC C/D with portal thrombosis and/or metastasis, n = 5). Plasma samples were depleted of abundant interfering proteins (ProteoPrep-20 kit, Sigma-Aldrich), fractionated by electrophoresis and enzymatically digested. The resulting peptides were analyzed by liquid chromatography coupled with mass spectrometry (HPLC/LTQ-VELOS-Orbitrap, Thermo Fisher) using SEQUEST as search engine and Trans-Proteomic Pipeline (v4.6.1) to generate probabilities for protein identifications. Quantitative analysis was done using Spectral Count algorithms.

Results: A panel of 29 diagnostic markers was identified by comparing the plasma proteome of cirrhotic patients with HCC versus those without HCC (p value <0.01, number of peptides >1, 80% protein probability). Functional annotation analysis revealed an enrichment of proteins related to the wound healing response as well as the complement and blood coagulation cascades (p <0.00001). Furthermore, a panel of 12 biomarkers related to tumor invasiveness was found in disseminated HCC as compared to early cases. Interestingly, 8 out of 12 have been already reported as metastasis-related genes in other cancers.

Conclusions: We have been able to identify a panel of diagnostic and prognostic biomarkers of HCC by plasma protein analysis. Once validated in an independent cohort, our plasma-based biomarker panels could be used as complementary tools to improve the clinical management of HCC patients.

A FUNCTIONAL POLYMORPHISM IN THE OSTEOPONTIN PROMOTER IS ASSOCIATED WITH HCC IN PATIENTS WITH HBV INFECTION

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Background and Aims: Osteopontin (OPN) is an extracellular matrix protein in hard tissues. The polymorphism in the promoter region of OPN gene have recently been shown to be associated with tumor progression of various types of cancer, including cancer of the gastric, glioma and oral carcinogenesis. However, their association with hepatocellular carcinoma (HCC) is unknown. The aim of this study was to evaluate the association of Osteopontin polymorphisms with risk of HCC in patients with hepatitis B virus (HBV) infection in south China.

Methods: A total of 225 cases diagnosed with hepatitis B virus (HBV)-related HCC and 200 age-matched patients with HBV infection without HCC were collected. Three polymorphisms (~156delG/G, ~443T/C and ~616T/G) in the Osteopontin promoter were genotyped using direct sequencing. The difference in the allele distribution and genotype distribution was statistically analyzed using the chi-square test or Fisher’s exact probability analysis. Logistic regression was used to test the associations between each polymorphism with HCC.

Results: The genotype frequencies of ~156 were 27.5% (G/G type), 42.5% (delG/G), and 30% (delG/delG) in HCC group, and 15.6% (G/G type), 42.2% (delG/G), and 42.2% (delG/delG) in controls. The frequency of ~156delG/G and ~156 del(delG) genotype in the HCC group was higher than that in the control group (p = 0.003). Compared with the controls, there was a significantly increased frequency of the alleles ~156G (p < 0.001) in HCC patients. Logistic regression analysis was performed to show an increase HCC risk associated with the delG variant genotypes, (OR 1.64; 95% confidence interval, 95%CI 1.25–2.16). There were no differences between the groups in the genotype distribution and allele frequencies of SNPs ~443T/C and ~616T/G.

Conclusion: Our findings suggest that allele ~156delG in the Osteopontin promoter may be a marker for risk of HCC with HBV infection in Chinese populations.

PROGNOSTIC SIGNIFICANCE OF HISTOLOGIC LOGISTIC EXPRESSION OF HEPATOCYTE NUCLEAR FACTORS 4α AND 1α IN RESECTABLE HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocyte nuclear factors (HNF) 4α and HNF1α have been found to be potent tumor inhibitor proteins for hepatocellular carcinoma (HCC) in multiple animal and tumor tissue studies. HNF1α, a protein homologous to HNF1α, is essential for alpha-fetoprotein (AFP) promoter activity. HCCs in which AFP is up-regulated in serum or tissue have been frequently shown to be more malignant. This study aimed to investigate the prognostic value of expression of these HNFs in patients undergoing curative resection for HCC.

Methods: We performed immunohistochemical analyses on microarrays of the tumors and matched adjacent tissue using antibodies against HNF1α, HNF1α, HNF4α, and alpha-fetoprotein (AFP). We evaluated the prognostic value of biomarker expression using Cox regression and the Kaplan–Meier method in a training
cohort of 220 patients, and conducted an independent validation in 232 patients. We also determined whether measurement of HNFs improved risk prediction beyond the use of established factors, using net reclassification improvement (NRI).

**Results:** Post-surgical recurrence and hepatic death were predicted by intratumoral HNF4α undereexpression in both cohorts (P < 0.05). In the training cohort they were also predicted by peritumoral HNF1α positivity (P < 0.05). A pooled cohort analysis showed that these predictors were independently associated with early but not late phase recurrence, and resultant mortality (P < 0.05). Intratumoral expression levels of HNF4α were significantly correlated with those of HNF1α, HNF1β, and AFP (P < 0.05). Similarly HNF1α expression in peritumoral tissue was correlated with that of other markers (P < 0.05). There was no significant correlation between expression of HNF4α in tumors and HNF1α in peritumoral tissue (P > 0.05). Adding combinations of intratumoral HNF4α and peritumoral HNF1α to 2-year recurrence and 5-year mortality models including known clinicopathological prognostic factors significantly improved the NRI indexes (39% and 44%, respectively; P < 0.05).

**Conclusions:** Immunohistological activation of intratumoral HNF4α and depletion of peritumoral HNF1α have prognostic significance for delayed recurrence and death after HCC resection, and these proteins may be promising therapeutic agents or targets for HCC.

**1084 PIN1 FACILITATES NF-κB ACTIVATION AND PROMOTES TUMOR PROGRESSION IN HUMAN HEPATOCELLULAR CARCINOMA: USEFULNESS OF PIN1 AS A PROGNOSTIC PREDICTOR AND THERAPEUTIC TARGET**


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**Background and Aims:** The prognosis of advanced hepatocellular carcinoma (HCC) is relatively poor because of its rapid progression. NF-κB plays a potent role in HCC progression; however, therapies targeting NF-κB are not widely used at present. Therefore, the development of a new therapeutic target is essential. Recently, we have reported that Pin1 specifically binds to the pThr254-Pro motif in p65, inhibits its binding to IκBα, facilitates its nuclear translocation and enhance protein stability, leading to constitutive NF-κB activation during hepatic ischemia/reperfusion injury (Kuboki, et al. J Hepatol 2009). However, the role of Pin1 in HCC progression is unclear. Therefore, we sought to determine whether Pin1-mediated NF-κB activation was a relevant factor in HCC progression.

**Materials and Methods:** Tumor specimens were prospectively collected from 100 patients undergoing surgical resection of HCC. Pin1, phosphorylated NF-κB-p65, Ki-67, and CD34 expression in HCC was evaluated by immunohistochemistry. NF-κB activation was assessed by EMSA. Expression of Pin1-NF-κB-p65 complex was detected by western blot. Moreover, HCC cells were treated with Pin1 inhibitor, Juglone. Cell proliferation and NF-κB activation was evaluated.

**Results:** Pin1 expression and NF-κB activation was increased in HCC compared with adjacent liver tissue. HCC patients were divided into two groups based on the expression levels of Pin1. Expression of phosphorylated NF-κB-p65 and Pin1-NF-κB-p65 complex was higher in HCC with high Pin1 expression. Accordingly, NF-κB activation was increased in HCC with high Pin1 expression. Cell proliferation determined by Ki-67 and microvessel density determined by CD34 was increased in HCC with high Pin1 expression. Moreover, high Pin1 expression was an independent predictor of poor prognosis. When HCC cells were treated with Juglone, cell proliferation was significantly inhibited in a dose dependent manner. In brief, Juglone inhibited NF-κB activation through decreased expression of Pin1-NF-κB-p65 complex.

**Conclusion:** Pin1 promotes HCC progression by inducing NF-κB activation, through increased expression of Pin1-NF-κB-p65 complex. High expression of Pin1 in HCC was an independent prognostic factor. Pin1 inhibitor suppressed HCC cell proliferation in vitro by inhibiting NF-κB activation. Therefore, Pin1 is an important endogenous regulator of HCC progression, and is a potential therapeutic target for HCC.

**1085 ABROGATION OF TUMOR-ASSOCIATED IMMUNOSUPPRESSION BY TARGETING TUMOR-INFILTRATING REGULATORY T-CELLS RESTORES IMPAIRED T CELL RESPONSES IN PATIENTS WITH LIVER CANCER**

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Growing evidence shows that regulatory T cells (Treg) hamper the development of effective anti-tumor immunity in patients with cancer, and present a major hurdle for tumor immunotherapy. We recently described accumulation of activated CD4+FoxP3+ Treg at the tumor site in patients with liver cancer (hepatocellular carcinoma or metastasized colorectal cancer). These tumor-infiltrating Treg are potent suppressors of the local anti-tumor T cell responses, and are characterized by the expression of higher levels of CTLA-4 and GITR than Treg in normal liver tissue or blood from the same patients. Now we show that treatment with a soluble form of the natural ligand of GITR (GITRL), or with blocking antibodies to CTLA-4, reduces the suppression mediated by tumor-derived Treg in ex vivo assays, restoring proliferation and cytokine production by effector T cells. These results suggest that modulation of intra-tumoral Treg function by either GITR-ligation or blocking CTLA-4 may be a promising strategy for alleviation of intra-tumoral immunosuppression, thereby contributing to immunotherapy induced effective immune responses in liver cancer patients.

**1086 TUMOR-INFILTRATING IL-10 PRODUCING CELLS ARE POTENT SUPPRESSORS OF THE LOCAL ANTI-TUMOR IMMUNITY IN PATIENTS WITH LIVER CANCER**

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Hepatocellular carcinoma (HCC) and liver metastases from colorectal cancer (LM-CRC) are the main malignancies affecting the liver and they are among the most common cancers and leading causes of cancer mortality. Curative treatment options are limited to very early stages, and these current treatments cannot prevent the recurrence of the disease. Several attempts to design alternative immunotherapeutic strategies have only shown limited efficacy in clinical trials. The failure of immunotherapy may be related to suppressive mechanisms in the tumor environment. In support of this, we recently described the accumulation of highly activated FoxP3+ regulatory T cells (Tregs) that are potent...
suppressors of anti-tumor immunity at the tumor site of patients with liver cancer. Now, we observed that in addition to Treg, tumor-infiltrating lymphocytes (TILs) contain a subset of CD4+ T cells that is concentrated at the tumor site, produces IL-10, and does not express FoxP3. These cells do not produce the Th2-like cytokine IL-13, but the majority can produce IFNg. Tumor-derived IL-10 producing CD4+ T cells have a potent capacity to suppress proliferation and cytokine production by autologous and allogenic effector T cells. These results indicate for the first time to our knowledge that besides CD4+FoxP3+ Tregs, liver tumors contain another subset of CD4+ T cells that is able to suppress the local immune response. It’s phenotype and function resemble that of type 1 regulatory T (Tr1) cells, and it may also interfere with immunotherapeutic efforts designed to treat patients with liver cancer.

1087 THERAPEUTIC EFFECT OF S-ADENOSYLMETHIONINE ON LIVER TUMOR DEVELOPMENT IN MURINE INFLAMMATION-MEDIATED MODEL IS ASSOCIATED WITH INCREASED EXPRESSION OF TUMOR SUPPRESSOR GENES AND CELL CYCLE ARREST
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Background and Aims: Chronic liver inflammation precedes the majority of hepatocellular carcinoma (HCC) cases. Previously, we demonstrated a decreased expression of Methionine-Adenosyltransferase 1a (Mat1a) and hypermethylation of the 3′ CpG islands specifically at the late precancerous stage in the liver of Mdr2-knockout (Mdr2-KO) mice, a model of inflammation-mediated HCC development. Now, we explore the therapeutic potential of the S-adenosylmethionine (SAM) in this model, and investigate potential mechanisms of its action.

Methods: SAM was supplied during 17 days by gavage. DNA methylation level of individual genes was determined by methylation-sensitive restriction enzyme (MSRE) digestion followed by PCR amplification. Gene expression was determined by RT-PCR. Protein expression was determined by immunohistochemistry.

Results: SAM supplementation significantly decreased numbers of small tumor nodules, of proliferating and polyplid hepatocytes, and the total DNA methylation level, while it increased expression of the Mat1a, p21 and γH2AX proteins, and of several tumor suppressor gene transcripts, including chemokine (C-X-C motif) ligand 14 (Cxcl14), in the liver of treated Mdr2-KO mice. Among 16 tested genes which were aberrantly expressed in the Mdr2-KO liver specifically at the late precancerous stage, only 5 changed their expression following SAM treatment: expression of only one gene was reversed, while expression of four other genes were even more aberrantly expressed. Most of the genes which were up-regulated by SAM treatment have tumor suppressor properties. Among 10 tested CpG islands which were hypermethylated in the Mdr2-KO liver specifically at the late precancerous stage, only one (in the promoter of the Fam65b gene) was reversed to the normal level following SAM treatment.

Conclusions: SAM supplementation decreased liver tumor development most probably due to an increased expression of tumor suppressor genes, DNA damage and cell cycle arrest and not due to a reversion of the aberrantly hypermethylated or aberrantly expressed genes. SAM should be further investigated as a potential preventive treatment for HCC.

1088 HCC DEVELOPMENT IS SUPPORTED BY PROTEASOMAL DEGRADATION OF THE TUMOR SUPPRESSOR PML
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Background: Hepatocellular carcinoma (HCC), which accounts for 80%-90% of primary liver tumors, is characterised by a very poor prognosis and is associated with high mortality. The molecular mechanisms responsible for development of this tumor are only partially understood. It could previously show that the promyelocytic leukaemia (PML) protein, a nuclear phosphoprotein which has been shown to inhibit cell growth and transformation of tumor cells, plays a role in the regulation of apoptotic factors in hepatocellular carcinoma (HCC). To clarify the clinical implication of PML in HCC, the expression of PML was analysed in a large series of human HCCs.

Methods: 90 patients undergoing partial liver resection or LTx because of HCC were included. Liver tissue was macrodissected and analysed for expression of PML (immunohistochemistry, qrt-PCR, western blot), proliferation- and apoptosismarkers. The same was done in several hepatoma cell lines. In addition, hepatoma cell lines were investigated for PML expression before and after treatment with proteasome inhibitors.

Results: Expression of the PML protein was reduced or abolished in all tumors compared to normal liver tissue and correlated inversely with tumor grade. Both mRNA and DNA was present in HCC and hepatoma cells as well as in normal liver tissue, suggesting that the decreased protein expression was due to either a defect in translation or protein instability, rather than the consequence of decreased transcription or gene deletion. Double staining showed that PML expression was inversely correlated with the proliferation marker Ki67 and is positively correlated with levels of apoptotic cells in these tumors. Upon treatment of hepatoma cells with proteasome inhibitors MG132 and bortezomib, we receive a considerable increase of PML protein expression up to levels in normal liver tissue.

Conclusions: Our results suggest that PML protein loss occurs in HCCs during carcinogenesis and progression, what is due to a proteasome-dependent pathway. PML protein expression is lost in advanced HCC which is due to an increased degradation of the protein. Thus, a decrease in PML expression may play an important role in HCC development.

1089 CHARACTERIZATION AND IMAGING OF CD133+ LIVER CANCER STEM CELLS
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Background and Aims: Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver. Although advances in HCC detection and treatment have increased a cure at early stages of the disease, HCC remains largely incurable because of late presentation and tumor recurrence. In the past few years, compelling evidence has emerged in support of the hierarchical Cancer Stem Cell (CSC) model for solid tumors, including HCC. CD133 has drawn significant attention as a critical liver CSC marker. We investigated whether CD133+ cells isolated from human hepatocellular carcinoma cell line possess CSC properties and demonstrated the differential behavior, including malignancy and chemosensitivity, of CSCs and non-CSCs.
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Methods: CD133+ cells were isolated by magnetic bead sorting after Huh-7 cells were genetically labeled with green fluorescent protein (GFP) or red fluorescent protein (RFP). In this scheme, CD133+ cells were labeled with GFP and CD133+ cells were labeled with RFP. The same number of GFP CSCs and the RFP non-CSCs were mixed and injected subcutaneously or in the spleen of nude mice.

Results: CSCs had higher proliferative potential compared to non-CSCs in vitro. CSCs performed a higher in vitro proliferative potential and lower mRNA expressions of mature hepatocyte markers, glutamine synthetase and cytochrome P450 3A4, than non-CSCs. When either CD133+ or CD133− cells were subcutaneously injected into SCID mice, CD133+ cells formed tumors, whereas CD133− cells induced either a very small number of tumors or none at all. GFP-CSCs were highly tumorigenic and metastatic as well as highly resistant to chemotherapy in vivo compared to RFP-non-CSCs.

Conclusions: The identification of CD133+ cells could thus be a potentially powerful tool to investigate the tumorigenic process in the hepatoma system and to also develop effective therapies targeted against hepatocellular carcinoma. The ability to specifically distinguish CSCs in vivo in real time provides a visual target for prevention of metastasis and drug resistance.

1090 ROLE OF TRANSFORMING GROWTH FACTOR-beta IN HEPATOCARCINOGENESIS AND HEPATOCELLULAR CARCINOMA CELL SURVIVAL
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Background and Aims: The development and progression of hepatocellular carcinoma (HCC) have been shown to be regulated by various cytokines including transforming growth factor-beta (TGF-β). However, the role of TGF-β signaling in HCC remains controversial. We aimed to reveal TGF-β signaling status in human and murine tissues of HCC and to assess TGF-β signaling activity and molecular events in various HCC cell lines.

Methods: TGF-β signaling pathway was evaluated by quantitative real-time RT-PCR, Western blotting assay, and promoter report assays. The effect of altered TGF-β signaling activity on the growth of several HCC cell lines was analyzed in vitro with viability, apoptosis, and soft agar assays, and in vivo with xenograft and experimental metastasis assays in nude mice.

Results: Gene expression profiling and quantitative real-time RT-PCR analyses revealed significant downregulation of TGF-β receptor II (TβRII) expression in two different HCC patient cohorts. Consistently, Smad3 phosphorylation was also downregulated in HCC tissues of patients and mice in comparison to that in adjacent normal tissues. Interestingly, many HCC cell lines were sensitive to TGF-β and growth-inhibited by exogenous TGF-β. However, stable knockdown of TβRII inhibited cell growth on plastic and in soft agar, and induced apoptosis resulting in suppressed subcutaneous tumor growth and metastatic potential in vivo. Furthermore, knockdown of Smad4 also led to a significant inhibition of growth on plastic and in soft agar with concomitant increase of PTEN expression and apoptosis.

Conclusions: Our results suggest that TGF-β signaling pathway plays a dichotomous role in hepatocellular carcinogenesis. It appears to inhibit HCC development as its activity is attenuated in HCC tissues in comparison to normal liver tissues. On the other hand, TGF-β signaling activity is retained for HCC cell survival and malignancy. Furthermore, Smad4 can mediate both growth inhibitory activity induced by exogenous TGF-β and the survival activity induced by autocrine TGF-β revealing a delicate selection of the two opposing activities of TGF-β during HCC evolution. These observations suggest that TGF-β/Smad pathway is necessary for the survival of HCC cells and that HCC may be uniquely suited for therapeutic intervention with novel TGF-β inhibitors.
systemic agent demonstrating a survival benefit in subjects with unresectable HCC. The aim of this study was to discover novel promising kinase inhibitors for the treatment of HCC.

Methods: A MTs assay screening of 165 kinase inhibitors was performed in Huh7 and Hep3B cells. Inhibitors were selected on (1) activity similar to sorafenib (cell viability inhibition >60–65%) and (2) efficacy in both cell lines. The IC50 (inhibitory concentration) was calculated (4-parameter logistic regression analysis) and a toxicological screening in mice (100–300 mg/kg) was performed to select candidates. Their activity was evaluated using thymidine incorporation, migration assay, FACS and a subcutaneous HCC xenograft model. The kinase profiling of one compound was performed (Reaction, Biol Corp). The expression of AMPK-related kinase 5 (ARK5), target of one selected drug, was investigated in cirrhosis (n = 13), dysplastic (n = 18) and HCCs (n = 89) using microarray (Affymetrix U133 2.0+).

Results: Eleven inhibitors decreased cell viability >65% in Huh7 and >60% in Hep3B cells. Five of them showed IC50 values comparable to sorafenib (<5μM). Two molecules (ON123300 and ON123890) did not show toxicity in mice, inhibited cell proliferation and migration (p < 0.05) and induced apoptosis. Kinome profiling of ON123300 showed that one of its main targets was ARK5, involved in the mTOR pathway. ON123300 inhibited the phosphorylation of S6. ARK5 was overexpressed in HCC compared to preneoplastic stages (p < 0.0001). ARK5 overexpression was prominent in HCC at advanced stages (p = 0.012), poor differentiation degree (p = 0.013) and was enriched in a molecular subclass characterized by activation of proliferative pathways (p < 0.0001). In vivo, ON123300 and ON123890 decreased tumor growth and prolonged survival compared to controls (p < 0.05), although no synergistic effects were observed in combination with sorafenib.

Conclusion: Two novel kinase inhibitors with IC50 values comparable to sorafenib and low toxicity were identified. The kinase ARK5 was identified as one main targets of ON123300. ON123300 and ON123890 exhibited potent anti-tumor effects in HCC experimental models and might represent an alternative therapeutic strategy for HCC patients who are intolerant or unresponsive to sorafenib.

1093 LIVER CANCER PROGRESSION FROM DYSPLASIA TO NEOPLASIA REVEALED BY TRANSCRIPTOME ANALYSIS: REARRANGEMENT OF ACTIN CYTOSKELETON AS AN EARLY EVENT

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Background and Aims: Our research is focused on characterization of dysplastic nodules and tumors induced experimentally on a rat model of hepatocarcinogenesis through the gene expression analysis. Methods: Liver carcinogenesis was induced with diethylnitrosamine and 2-acetyliminofluorene, dysplastic nodules and tumors were classified according to their expression pattern of the tumor-marker gamma glutamyl transferase (GGT). With this enzymatic staining both nodules and tumors were obtained by laser capture microdissection and processed for DNA microarrays analysis (Rat Gene ST 1.0, Affymetrix). Six types of liver samples were evaluated: Normal Liver (NL, n = 6), Remodeling nodules Negative to GGT (RN, n = 3), Remodeling nodules Positive to GGT (RP, n = 4), Persistent nodules (P, n = 4), these three types of nodules at 4 months, Early Tumors of 9 months (ET, n = 4) and Advanced Tumors of 17 months (AT, n = 6). Differential gene expression profiles were calculated from NL data. Expression changes of 17 selected genes were confirmed by qRT-PCR. Actin cytoskeleton was studied by immunofluorescence microscopy with a high-affinity F-actin probe.

Results: A projection of microarray data using principal component analysis revealed homogeneity in biological replicas in each sample type and proximity among them as follow NL:RN:RP:ET:AT. Also a hierarchical clustering analysis denoting that Persistent type of nodules and early tumors had a similar transcriptome signature. There were 936 differentially expressed genes in which a cluster of 242 genes corresponded to gene overexpression from dysplasia to neoplasia. A pathway analysis using Ingenuity Systems IPA software revealed 12 genes (Actb, Lcp1, Tagln2, Vim, Cfl1, Ezr, Podxl, Cd44, Axl, Gas6, Prkcb, Map3k1) involved in several cellular functions such as organization of cytoskeleton, movement, growth and proliferation. There was a strong correspondence between microarray data and validation by qRT-PCR. Additionally there was abnormal rearrangement of F-actin staining in early tumors compared to their corresponding non-tumoral region.

Conclusion: Differential expression of actin and actin binding protein such as Lcp1, Tagln2, Cfl1 and Podxl as well as differential F-actin staining suggest that the actin-cytoskeleton rearrangement is an early cellular event associated to transition from dysplasia to neoplasia in chemical hepatocarcinogenesis. Acknowledgements to Grant CONACYT 115431 and JETM fellowship CONACYT 98841

1094 URI GENERATES METABOLIC ALTERATIONS-DEPENDENT DNA DAMAGE INDUCING HETEROGENEOUS LIVER TUMOURS K.S. Tummala1, A.L. Gomes1, I.M. Ruppen2, L. Bakiri3, R.C. Olivas4, O. Graña5, M.P. Ximénez de Embún6, M. Cañamero7, D.G. Pisano7, E. Wagner3, M. Rodriguez-Justo3, N. Djouder1, 1Cancer Cell Biology Programme, Growth Factors, Nutrients and Cancer Group, 2Biotechnology Programme, Proteomics Core Unit, 3Cancer Cell Biology Programme, Genes, Development and Disease Group, 4Structural Biology and Biocomputing Programme, Spectroscopy and Nuclear Magnetic Resonance Unit, 5Structural Biology and Biocomputing Programme, Bioinformatics Unit, 6Biotechnology Programme, Comparative Pathology Core Unit, Centro Nacional de Investigaciones Oncológicas, CNIO, Madrid, Spain; 7Department of Pathology, University College London NHS Trust, London, UK E-mail: kstummala@cnio.es

Background and Aims: Hepatocellular carcinoma (HCC), one of most frequent malignancies world-wide, is associated with poor patient’s survival. Among signalling pathways important for liver tumorigenesis, mTORC signaling has been linked to poor prognosis, implying a critical role for this pathway and its downstream effectors in hepato-carcinogenesis. In this regard, URI (Unconventional prefoldin RP55 Interactor), a member of prefoldin molecular chaperones, previously identified as a downstream effector of mTOR/S6K1 pathway, has been reported to increase HCC cell proliferation pointing out URI relevance towards HCC development. URI has also been described as an additve oncogene amplified in human ovarian cancer. Finally, data from our lab show URI is frequently up-regulated in human hepatitis and HCC and correlates with decreased patient’s survival. However in vivo URI oncogenic activity and underlying molecular mechanisms remain unknown. Using mouse models we aimed at deciphering the role of URI in liver carcinogenesis.

Methods: URI knock-in mouse has been generated by targeting FLAG tagged human URI (hURI) in the Col1A1 locus. tTA transactivator, expressed under the LAP promoter, induces hURI expression specifically in hepatocytes. Liver histo-pathological characterization, together with genomic studies (deep RNA sequencing) and biochemical approaches including quantitative proteomics (iTRAQ) and H-1NMR have been employed to better understand molecular mechanisms.
### Results

We report that hURI expression in mouse hepatocytes leads to multistep tumorigenesis resulting in spontaneous and heterogeneous liver tumours, including hepatoma and HCC. We show that even in the absence of rapid proliferation, hepatocyte-specific URI expression induces replicative stress and consequently genomic instability-associated DNA damage. Importantly, during early stage of tumorigenesis and prior to premalignant lesions, metabolic profiling identified alterations in pathways converging to insufficient nucleotide synthesis that fail to support normal replication and genomic stability. Finally, restoring metabolic intermediates in the liver rescued the premalignant phenotype.

### Conclusions

Our results identify URI as an oncogene involved in multistage liver carcinogenesis and point to an unanticipated role of metabolic pathways alteration-dependent DNA damage leading to heterogeneous liver tumours. We finally propose that reinstating corrupted metabolic pathways could be beneficial as a preventive treatment in liver cancers.

These authors contributed equally to the work: Isabel Maria Ruppen, Latifa Bakiri, Ramón Campos-Olivas, Osvaldo Graña.

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### 1095 ROLE OF FXR IN THE ACTIVATION OF HEPATOCYTE CHEMOPROTECTION AND IN THE REFRACTORYNESS OF LIVER TUMORS TO CHEMOTHERAPY

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#### Background:

Several mechanisms of chemoresistance are involved in the multifactorial process accounting for the very poor efficacy of the pharmacological treatment in patients with advanced primary liver tumors. The role of FXR in the high drug refractoriness of liver cancer is unclear because, although in cancer cells incubation with cisplatin is able to up-regulate FXR, the response to anticancer drugs is low in liver tumors despite the FXR expression is reduced.

#### Aim:

The aim of this study was to investigate whether FXR is involved in the development of chemoresistance.

#### Methods and Results:

In human hepatoma Alexander cells, with negligible endogenous FXR expression, transfection with FXR/RXR resulted in a significant protection against cisplatin-induced toxicity. This was further enhanced by FXR activation with GW4026. FXR/RXR expression also protected against doxorubicin, mitomycin C and potassium dichromate, but not against colchicine, paclitaxel, acetaminophen, artesunate or sorafenib. In FXR/RXR expressing cells, both GW4026 and cisplatin up-regulated FXR target genes, such as BSEP, SHP, OSL1 and TCEA2. In primary cultures of human hepatocytes, cisplatin also induced the expression of FXR target genes. In both HepG2 cells, that constitutively express FXR, and in Alexander FXR/RXR transfected cells, cisplatin was able to stimulate luciferase expression driven by an IR1 element. Among 109 genes that were investigated as potential candidates for FXR-dependent chemoresistance, only changes in ABCB4, TCEA2, CCL14, CCL15 and KRT13, both in FXR/RXR expressing hepatoma cells and in human hepatocytes, were found.

#### Conclusions:

Activation of FXR, even in the absence of bile acids, stimulates the expression of genes involved in several mechanisms of chemoresistance, which results in a reduced response of liver tumors to pharmacological treatment and in an enhanced chemoprotection of healthy hepatocytes against potentially toxic compounds.

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### POSTERS

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### 1096 ENHANCED ANTITUMORAL EFFECTS BY COMBINING ADENOVIRUS-MEDIATED ANTI-ANGIOGENESIS WITH Ad-flk1 AND VACCINATION WITH AFP-PULSED DENDRITIC CELLS IN AN ORTHOTOPIC MURINE HCC MODEL

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#### Background and Aims:

Dendritic cells (DC) as professional antigen presenting cells are able to prime T-cells against tumor-associated antigens (TAA) such as α-fetoprotein (AFP). However, their efficacy as a vaccine in patients with hepatocellular carcinoma (HCC) is limited. HCC is a strongly vascularized tumor. Anti-angiogenic therapies, such as the soluble VEGFR-2 (flk-1), showed strong antitumoral effects towards HCC in vivo. The aim of this study was to analyze whether combining a vaccination using AFP-pulsed DC with an adenosin vector-mediated antiangiogenetic treatment improve the antitumoral effect towards HCC in vivo.

#### Methods:

For tumor induction 106 AFP positive Hepa129-cells were injected into the left liver lobe of C3H-mice. 3 days later mice received intravenously (i.v.) Ad-flk1 or Ad-LacZ as control (109 pfu/mouse). Survival time and development of malignant ascites were monitored. In a second experiment the effect of vaccination with AFP-pulsed DC combined with i.v. injection of Ad-flk1 was studied in the same orthotropic model. Briefly, DC were isolated from the bone marrow of C3H-mice and were transduced with Ad-AFP. Mice were then immunized by subcutaneous (s.c.) injection of 106 AFP-DC.

#### Results:

After i.v. injection of Ad-flk1 alone a significant antitumoral effect against orthotopic HCC development was observed. Ad-flk1 treated mice showed a significant reduction of malignant ascites as well as a prolonged survival compared to Ad-LacZ treated animals (p < 0.05). On day 22 after tumor induction 67% of Ad-LacZ treated animals had died whereas 100% of the Ad-flk1 treated animals were still alive. The combination of s.c. vaccination with AFP-pulsed DC and i.v. applied Ad-flk1 enhanced antitumoral effects with significantly prolonged survival time compared to the single treatment regiments. 14% of mice treated with the combination therapy survived more than 30 days while all control animals had died at that time point.

#### Conclusions:

Our data show that antiangiogenic genetransfer decreases orthotopic HCC growth in a mouse model. The antitumoral effect is further enhanced when combined with a s.c. vaccination using AFP-pulsed DC, suggesting that combining antiangiogenetic approaches with DC-based immunotherapy may be a promising strategy to treat HCC.

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### 1097 HYPERMETHYLATION OF THE microRNA LET-7A-3 GENE REPRESSES THE PRIMARY AND MATURE LET-7A-3 WITH AN INVERSE CORRELATION TO IGF-II mRNA IN HCV-INDUCED HEPATOCELLULAR CARCINOMA

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#### Background and Aims:

Ongoing research has been focusing on the impact of microRNAs dysregulation on malignant transformation. MicroRNA let-7a is a member of the archetypal let-7 miRNA gene family. Owing to its known tumor suppressor activity, aberrant
expression of microRNA let-7a was found to deregulate ~200 genes involved in cell proliferation, differentiation and adhesion. Among them is the Insulin-like Growth Factor-II (IGF-II) which is involved in the pathogenesis of Hepatocellular carcinoma (HCC). The embedment of the let-7a-3 gene in a well defined CpG island on chromosome 22q12.31 suggests that its expression might be epigenetically controlled via DNA methylation. The impact of DNA methylation on the expression of the primary and mature let-7a-3 transcripts has never been investigated in HCC. Thus, we aimed at investigating whether DNA methylation is responsible for let-7a downregulation and consequently on IGF-II expression in HCC.

**Methods:** Total RNAs were isolated from 16 HCV-induced HCC tissues and the 3’UTR of the healthy liver tissues. Expression of both Primary and mature microRNA let-7a as well as the IGF-II mRNA was analyzed using RT-qPCR. In addition, DNA extracted from HCC tissues was bisulfite converted and its methylation status was assessed by PCR using two sets of nested primers to amplify the whole CpG Island followed by quantification by SYBR-Green qPCR using methylation and non-methylation specific sets of primers.

**Results:** Bioinformatics analysis revealed three possible hits where let-7a may target the IGF-II 3’UTR with promising scores. Also, the expression of primary and mature microRNA let-7a showed significant downregulation in HCC tissues compared to healthy tissues (p=0.0203 and p=0.0208, respectively). Furthermore, we found a significant inverse correlation between mature let-7a and IGF-II mRNA expression (Pearson R=−0.21). Interestingly, the methylation analysis showed hypermethylation (88%) of the investigated CpG island in the let-7a-3 gene.

**Conclusion:** For the first time, we showed that hypermethylation of micro-RNA let-7a-3 gene is responsible for the downregulation of both the primary and mature transcripts which lead to an exaggerated IGF-II mitogenic signal contributing to malignant transformation.

**1098 TLR4 DEFICIENCY PROTECTS AGAINST HCC INITIATION IN A FIBROTIC LIVER**

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**Background:** The development of hepatocellular carcinoma (HCC) is usually the consequence of advanced liver fibrosis but the mechanisms are still poorly understood. Recently, it has been shown that HCC promotion depends on Toll-like receptor (Tlr) 4 and intestinal microbiota (Dapito et al. Cell 2012). HCC initiation can be modelled in mice by the administration of diethylnitrosamine (DEN) with or without Silibinin (i.e. 6 production (Naugler et al. Science 2007). Mice that lack the hepatocanicular phosphatidylcholine transporter Abcb4 develop biliary fibrosis and can be used as a model to study tumor formation in injured liver. The aim of our study was to investigate HCC initiation in Abcb4-deficient (Abcb4−/− Tlr4−/−) and Abcb4/Tlr4-double-deficient (Abcb4−/− Tlr4−/−) mice.

**Methods:** Abcb4-deficient mice on the FVB/NJ genetic background were crossed to two distinct genetic backgrounds (Tlr4-sufficient C3H/HeN and Tlr4-deficient C3H/HeJ) for at least 10 generations. Hepatic collagen contents were evaluated by hydroxyproline (HyP) assay at the age of 3 weeks (when biliary fibrosis is established). Congenic knockout and wild-type (wt) mice were treated with a single dose of DEN. Phenotypic differences after 48 hours were determined by analyzing hepatic apoptosis (TUNEL) and proliferation (Ki67) rates as well as inflammatory markers including IL6 expression.

**Results:** Hepatic collagen contents were significantly reduced in Abcb4−/− Tlr4−/− as compared to Abcb4+/− Tlr4−/− mice (282.84±18.21 vs. 327.90±13.61 μg HyP/g liver). After DEN challenge, apoptosis, proliferation and inflammation (IL6 expression) were significantly decreased in Abcb4−/− Tlr4−/− mice (6.43±0.61 vs. 10.96±1.05% proliferative cells), IL6 expression was markedly reduced (44.42±9.19 vs. 63.29±7.32-fold expression, untreated C3H/HeN wt was set to 1.00) and apoptosis was slightly elevated (7.07±0.91 vs. 6.00±0.82% apoptotic cells).

**Discussion:** This study demonstrates that HCC initiation upon DEN challenge depends on pre-existing fibrosis and genetic background. Our findings indicate that Tlr4 deficiency protects from HCC initiation in Abcb4/Tlr4-double-deficient mice.

**1099 MESENCHYMAL STEM CELLS MODIFIED TO EXPRESS INTERFERON-β INHIBIT THE GROWTH OF HEPATOCELLULAR CANCER THROUGH INHIBITING AKT/FOXO3a PATHWAY**


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**Objective:** This study aim to investigate using of bone marrow mesenchymal stem cells (BMSC) genetically engineered to produce interferon-β (IFN-b) as a gene delivery system to treat liver cancer in vitro and in vivo.

**Methods:** To measure the effects on tumor cell growth, IFN-b-producing BMSCs (BMSCs/IFN-b) were established. We used ELISA to detect the IFN-b secretion in the BMSC culture condition medium (CM) and measured the effect of BMSCs/IFN-b on hepatoma cells proliferation by MIT and colony formation assay. We used Brdu staining and cell cycle analysis to investigate the effect of BMSC on hepatoma cell cycle. RT-PCR and Western blotting was used to detect cell cycle related proteins and AKT signaling pathway.

**Results:** BMSCs/IFN-b cells can stably secrete high levels of IFN-b. MIT showed hepatoma cell had a lower growth rate from the first three days when cultured in BMSC/IFN-b-CM compared to in BMSC/vector-CM or DMEM culture group. The number of colony forming and clone size detected in BMSC/IFN-b-CM cultured cells was less than the control group. Co-cultured with BMSC/IFN-b-CM dramatically decreased the percentages of cells with incorporated BrdUrd. In BMSC/IFN-b-CM treated liver cancer cells, the proportion of G1 phase cells increased but decreased in S phase of the cell (the ratio for BMSC/vector: BMSC/IFN-b in HepG2: 20.5±1.8%: 11.2±1.2%, t = 7.53, P < 0.01; HuH7: 24.7±1.8%: 12.7±2.0%, t = 7.87, P < 0.01). BMSC/IFN-b inhibited HCC growth in NID/SCID mouse. Compared with the control group, P21 and P27 expression of hepatoma cells increased, while CyclinD1 and phosphorylation of Rb expression decreased when co-cultured with BMSC/IFN-b-CM. It was mechanistically associated with suppression of Akt activity and enhanced transcriptional activity of FOXO3a.

**Conclusion:** IFN-b gene modified BMSC can effectively inhibit the proliferation of hepatoma cells in vitro and in vivo through inhibit AKT/FOXO3a pathway. These results indicate that IFN-b/BMSCs are a powerful anti-cancer cytotoxic therapeutic tool for HCC.

**1100 REGULATION OF RADIATION INDUCED LIVER CANCER STEM CELL MIGRATION AND METASTASIS BY ADAM17**

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**Background and Aims:** Recent studies have described that cancer stem cell plays a key role radioresistance. The cell surface marker CD133 has been known as a cancer stem cell marker expressed in hepatocellular carcinoma (HCC). ADAM17 gene was reported.
to contribute to cancer metastasis. The aim of this study was to investigate the roles of liver cancer stem cell (LCSC) and ADM17 in the molecular and cellular mechanisms underlying the metastatic properties of irradiated hepatoma cells.

**Methods:** After sorting CD133+ and CD133− HuH-7 cells by FACS, both CD133+ and CD133− sorted cells were exposed to γ-irradiation. We next investigated the key gene/pathway responsible for metastasis in post-irradiated LCSCs, CD133+ and CD133− cells using cDNA microarray and Multiplex cytokine analysis. Invasive and metastatic activities in sorted cells were analyzed by cell migration assay. Also, the expressions of the typical genes related metastasis MMP-2 and MMP-9 were also measured by gelatin zymography. We evaluated ADAM17 expressions on time dependent by real-time PCR, Western blot. After suppressing ADAM17 gene expression by transfecting with ADAM17 lentiviral shRNA, the migration activity of CD133+ and CD133− cells were analyzed.

**Results:** In cDNA microarray analysis, eighty nine metastasis-related genes were upregulated. In particular, the ADAM17 gene was more highly expressed in CD133+ cells treated with γ-irradiation than CD133− cells treated with γ-irradiation. In addition, the vascular endothelial growth factors (VEGF) in CD133+ cells after irradiation were consistently expressed in higher levels. Also, irradiated CD133+ cells migrated more actively, and showed an increased invasion rate compared to irradiate CD133− cell. Gelatin zymography and ELISA showed that MMP-2 and MMP-9 protein expression are more significantly higher in CD133+ cells media samples than CD133− cells media samples. However, the suppression of ADAM17 in irradiated CD133+ cells reduced migration activity.

**Conclusions:** These results suggest that CD133− cells have more metastatic capacity than CD133+ cells after irradiation. In addition, the suppression in the migration potential of LCSCs through targeting ADAM17 may be promising therapeutic strategy for ideal radiotherapy for HCC.

“This work was funded by the National Research Foundation of Korea grant funded by the Korea government (No. 2011-0027071).

**1101**

**ANTI-ANGIOGENIC AGENTS MODULATED THE HOST BY SUPPRESSING IL-12b EXPRESSION AND PROMOTED LUNG METASTASIS IN EXPERIMENTAL HEPATOCELLULAR CARCINOMA**

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**Background:** Sorafenib, an anti-angiogenic agent targeting vascular endothelial growth factor receptors (VEGFRs), has become the standard of care for the patients with advanced hepatocellular carcinoma (HCC), however, the improvement in median survival time is far from satisfactory. Previous preclinical studies have found that anti-angiogenic therapy promoted tumor metastasis and the mechanism studies were mainly focus on tumor cells and tumor microenvironment.

**Methods:** In order to treat the host without exposing tumor cells to anti-angiogenic therapies, a pretreatment schedule was used in this study. The significantly changed cytokine in peripheral blood were surveyed by an antibody array and its role in anti-angiogenic therapy mediated lung metastasis was further investigated in mice models.

**Results:** Pretreatment with two VEGFR inhibitors, sunitinib and sorafenib, facilitated tumor cell survival in blood stream and promoted lung metastasis from tumors that were subsequently incubated in immunodeficient and immunocompetent models, indicating that host response joined into the pro-metastatic effects. IL-12b was found to be significantly suppressed by sunitinib and sorafenib. Treatment-induced IL-12b suppression in macrophages and dendritic cells from host organs was found to play a crucial role in treatment-induced metastasis. Supplement with recombinant mouse IL-12b or restoration of IL-12b expression by zoledronic acid, which was previously reported to enhance IL-12 expression in vitro and in vivo, alleviated the metastasis-promoting effects of sunitinib and sorafenib.

**Conclusions:** These findings suggest that host response to anti-angiogenic therapy, in a non-cancer-cell-autonomous manner, facilitates tumor metastasis and restoration of IL-12b expression in patients received sorafenib treatment could translate into clinical benefits.

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**04c. MOLECULAR AND CELLULAR BIOLOGY: HSCS AND FIBROSIS**

**1102**

**NEUROLIGIN-4 RECEPTOR SILENCING INCREASED HUMAN NATURAL KILLER ACTIVITY AND DECREASED HEPATIC STELLATE CELLS ACTIVATION**

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**Background and Aim:** Neuroligin-4 (NLGn4) is involved in the neuronal post-synapse formation and remodeling. NLGn4 interacts with NMDAR (an insulin responding N-Methyl-D-Aspartate Receptor) to encode PSD-95 (Post Synaptic Density 95 proteins) and control vesicular release through F-Actin. NK cells lose their original anti fibrotic properties in cirrhosis; our gene-array analysis of NK cells revealed NLGn4 over expression in cirrhosis. We investigated a potential NLGn4 role to mediate NK responses in NALFD progression via its downstream family proteins.

**Methods:** We have conducted a siRNA silencing of the NLGn4 receptor on NK cells isolated from peripheral blood lymphocytes of patients with F4-score liver fibrosis. NLGn4 silencing or nonsilencing control siRNAs were prepared prior to co-culture with LX2 hepatic stellate cells (HSCs). The Pre-synaptic β-neurexin ligand for the NLGn4 was assessed on HSCs. Following 24 hr of co-cultures, cells were trypsinized, washed and analyzed for adherences/ killing pathways performed by the flow cytometry. Adherence was defined as the double positive signal markers of anti-α-SMA (Smooth Muscle Actin, a HSCs activation marker) and anti-CD56 (NK cell marker). Annexin-V and propidium iodide were used to determine apoptosis and viability, respectively.

**Results:** β-neurexin was found to be 75% in HSCs. The NLGn4 receptor silencing on the NK cells significantly decreased IL-4 secretions as compared to the non-silenced NK cells (p<0.05). In additions, NK cells with NLGn4 showed enhancement in their lyosomal-associated membrane protein-1 (CD107a, NK granzyme activation marker) up to 4-fold (p<0.01). Those NK phenotypic changes were associated with their ability to decrease activations of HSCs (% α-SMA+ from 90% to 45%) and accompanied with increased apoptosis rate.

**Conclusions:** β-neurexin-NLGn4 recognition mediates HSCs-NK immune synapse to control release of NK vesicles. siRNA NLGn4x receptor silencing unleashed NK granzymes and augment HSCs killing.
A 4 SERUM PROTEIN SIGNATURE FOR THE DIAGNOSIS OF MILD FIBROSIS IN CHRONIC HEPATITIS C (HEPACHRONIX STUDY)


Methods: Liver biopsies from 244 untreated CHC patients were studied. Among them, 66% had mild fibrosis (F1, Metavir) and 34% moderate fibrosis (F2). Patients were mainly infected with genotype 1 (55%), 2 (11%), 3 (11%), 4 (20%) and 5–6 (3%) respectively. Real-time quantitative RT-PCR assays were used to analyse the mRNA expression of 51 genes involved in fibrogenesis. The concentration of 6 proteins was assayed in duplicate by ELISA in the serum of 228 patients and reliable data were obtained for 216 (65% with mild fibrosis and 35% with moderate fibrosis).

Results: 28 genes were found to be upregulated in F2 patients. These genes were mainly involved in extracellular matrix production and remodelling, in cell–cell and cell-extracellular matrix interactions, in cell cycle, or encode growth factors/cytokines families.

Conclusion: We demonstrated in a large independent cohort that mild and moderate fibrosis have different liver gene expression. The most notable changes occurred mainly in cell-matrix turn-over. Several genes that are up-regulated in the liver encode molecules detected in the serum and provide a logical functional approach for the development of serum markers of fibrosis progression. A 4-protein signature (A2M, CXCL10, IL8, and SPP1) was identified that demonstrates high value for the diagnosis of early fibrosis and particularly the discrimination of F1/F2 stages.

HEPATITIS C VIRUS NON-STRUCTURAL 3/4A PROTEIN EXPRESSION PROMOTES HEPATIC FIBROGENESIS IN MICE AFTER ADMINISTRATION OF CARBON TETRACHLORIDE

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Background and Aims: Hepatitis C virus (HCV) primarily infects hepatocytes and the infected hepatocytes with ongoing inflammation appear to promote fibrogenesis. To date, the underlying mechanism of HCV-induced fibrogenesis e.g. role of HCV non-structural proteins, hepatic stellate cells and regulatory T cells (Tregs) in the disease progression remains unclear.

Methods: We used transgenic (Tg) mice with liver-specific expression of the HCV non-structural NS3/4A protein complex to accomplish the aims of the study. Both acute and chronic hepatic fibrosis was induced in wild-type (n = 5) and NS3/4A-Tg (n = 5) mice either by single injection of carbon tetrachloride (CCL4) or multiple injections for 4 or 8 weeks. Furthermore, to study the role of Tregs; wild-type (n = 5) and NS3/4A-Tg (n = 5) mice were administered for 2 weeks with either isotype control or mCD25+ mGITR antibodies for Tregs depletion followed by 2 weeks of CCL4 administration. Liver damage, inflammation, fibrotic and HSC markers were examined.

Results and Conclusions: Hepatic expression of NS3/4A did not induce spontaneous liver disease. After acute CCL4 treatment, NS3/4A-Tg mice exhibited enhanced liver fibrogenesis in comparison to wildtype controls as analyzed by collagen staining. Furthermore, there was significantly increased stellate cells proliferation and activation as analyzed by immunostaining and western blot analysis. No significant differences in intra-hepatic inflammation was observed as determined by ALT levels and HE stainings. The long-term CCL4 treatment (4 weeks and 8 weeks) and Tregs depletion studies have been accomplished and analyses are currently ongoing.

Acknowledgements: The study was supported by the Ruth and Richard Julin’s Foundation. Ruchi Bansal is supported by Sheila Sherlock Fellowship from European Association for the Study of the Liver (EASL).

PHARMACOLOGICAL INHIBITION OF CCL2 LIMITS ANGIOGENESIS ASSOCIATED WITH CHRONIC TOXIC LIVER INJURY IN VIVO BY RESTRAINING INFILTRATION OF PROANGIOGENIC INFLAMMATORY MONOCYTES

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Background and Aims: Chronic liver injury and fibrosis are associated with hepatic blood vessel formation, but mechanisms of fibrosis-related angiogenesis and its therapeutic implications are unclear. We reported earlier that pharmacological inhibition of CCL2, a key factor in recruiting inflammatory monocytes into injured liver, is a promising therapeutic strategy in murine steatohepatitis models. Here, we investigated the effects of CCL2 inhibition on the development of liver vessel formation in fibrosis in vivo.

Methods: We developed a novel in vivo imaging approach for visualization and quantification of hepatic blood volume by micro-CT using a blood pool contrast agent. Angiogenesis was studied at 2, 4, 6 and 8 weeks of repetitive carbon tetrachloride (CCL4) injury in c57BL/6 mice, with and without inhibition of CCL2 by a specific antagonist (mNOX-E36). Hepatic macrophage subsets were characterized by FACS and further studied after sorting by qPCR.

Results: Liver fibrosis progression upon chronic hepatic injury in mice was associated with neo-angiogenesis in the liver. Blood vessel formation was associated with the infiltration of inflammatory monocytes into injured liver. The inflammatory monocyte-derived macrophages, but not liver-resident macrophages (Kupffer cells), expressed high levels of proangiogenic factors like Vgfa, alongside inflammatory markers such as IL-1β. Pharmacological inhibition of CCL2-dependent inflammatory macrophage infiltration significantly reduced angiogenesis, both by micro-CT based in vivo quantification of hepatic blood volume as well as by immunohistochemistry for quantification of the CD31 area fraction. However, reduced
angio genesis did not directly impact development of fibrosis in the CCL model.

Conclusions: The CCR2-dependent infiltration of inflammatory monocytes functionally promotes angiogenesis in chronically injured liver, which can be largely blocked by pharmacological inhibition of CCL2. Our data further suggest that fibrosis progression, at least for early stages, is largely independent from hepatic angiogenesis.

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VITAMIN D – SMALL MOLECULE – GRAND ANTIFIBROGENIC EFFECT IN HEPATIC STELLATE CELLS? A. Beilfuss1, J.-P. Sowa1, R.K. Gieseler2, A. Zahn1, M. Schlattjan1, G. Gerken1, A. Canbay1, 1Department of Gastroenterology and Hepatology, University Hospital Essen, Essen, 2Rodos BioTarget GmbH, Hannover, Germany

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Background and Aims: Liver disease is associated with a lack of vitamin D (VD) and activation of primary human hepatic stellate cells (phHSC). The objective of this work was to analyse possible connections between these effects. To this end VD concentration, vitamin D receptor (VDR) expression and activation of HSC were detected and evaluated.

Methods: Metabolism of VD₂ into 25VD₂ was assayed in cell culture supernatants (EIA) of phHSC. Influence of VDR expression (VDR-siRNA knock down) and VD₂ supplementation (1 μM) on TGF-beta mRNA expression (qRT-PCR, WB) in HSC after 24h. To demonstrate binding of VD to VDR cytosolic and nuclear fractions were analyzed separately. In addition effects of VD to TGF-beta signaling pathways were analyzed.

Results: phHSC metabolized VD in a concentration dependent manner. The fractionation study showed that VD was increased in the nuclear fraction when VD was added. In vitro analysis of VD in TGF-beta pre-incubated phHSC led to diminished TGF-beta, alpha-SMA, PDGF mRNA. To further investigate interaction of VD, VDR and TGF-beta, VDR expression was knocked down by siRNA. The knock down resulted in a significant reduction of VDR and in an increase of alpha-SMA mRNA after TGF-beta stimulation compared to wild type phHSC. Inhibition of TGF-beta mRNA expression by VD was only observable in phHSC without VDR knock down. In absence of VD an increase of profibrotic proteins (TGF-beta, alpha-SMA, Col1alpha) was observable compared to wildtype. The same effect was seen for proteins of the TGF-beta signaling pathway. Trypsin degradation of VDR in protein lysates was strongly dimished by VD. Moreover, VD is able to inhibit activation of the TGF-beta signaling pathway (pSmad2 and pSmad3) until 1 hour after parallel addition of VD and TGF-beta.

Conclusion: Lack of VDR expression results in increased expression of profibrogenic genes. Supplementation of VD reduces degradation of VDR and counteracts TGF-beta signaling. VD might ameliorate the advancement of fibrosis in chronic liver diseases.

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THE INFLAMMASOME COMPLEX IS A TARGET OF THE HIV ENVELOPE PROTEIN gp120 IN HUMAN HEPATIC STELLATE CELLS (HSC) AND MONONUCLEAR CELLS (MC) A. Capponi1, R. Bruno2, E. Mingarelli1, S. Gessani1, A. Masotti1, F. Marra3, 1University of Florence, Florence, 2University of Pavia, Pavia, 3Istituto Superiore di Sanità, Rome, 4Ospedale Bambin Gesù, Roma, Italy

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Background and Aims: Patients with HCV/HIV co-infection show a faster progression of hepatic fibrosis and more severe inflammation. The HIV envelope protein gp120 has been previously shown to modulate different aspects of hepatic stellate cells biology, including directional migration and expression of profibrogenic cytokines, at least in part via activation of the chemokine receptor CCR5. Recent work has identified the NALP3 inflammasome as a critical pathway in the generation of proinflammatory signals during liver injury, but no information is available on a possible direct link between the inflammasome and HIV proteins.

Methods: Myofibroblastic HSCs were isolated from normal human liver tissue and cultured on plastic until fully activated. MNC were separated from human whole blood. Gene expression was measured by qPCR. Protein IL-1β protein levels were assayed by ELISA.

Results: HSCs or MNC were exposed to 500 ng/ml recombinant M-tropic gp120 (CN54) for 2, 8 and 24 hours. A time-dependent, significant upregulation of ASC and NALP3, proteins critical for the assembly of NALP3-dependent inflammasome was observed in both cell types. This effect was associated with increased expression of caspase-1, which caused conversion of pro-IL-1β into mature IL-1β, in both HSCs and freshly isolated MCs. Remarkably, a significant increase in gene expression of IL-1β was observed in both cell types, together with increased protein levels in the supernatant. Notably, preincubation of HSCs with TAK779, a CCR5 receptor antagonist, partially reverted gp120-mediated IL-1β expression. Furthermore, gp120 induced a significant upregulation of the expression of the pro-inflammatory chemokine CXCL8 (interleukin-8) in HSC and MNC.

Conclusions: HIV-gp120 significantly increased the expression of components of the NALP3 inflammasome in human HSC and MNC, at least in part through activation of CCR5. These data identify a novel mechanism by which HIV-gp120 may directly influence hepatic necroinflammation and fibrosis during HCV/HIV coinfection, through increased production of IL-1β.

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ANTIFIBROTIC EFFECTS OF A RECOMBINANT ADENO-ASSOCIATED VIRUS CARRYING SMALL INTERFERING RNA TARGETING TIMP-1 IN RAT LIVER FIBROSIS M. Cong, T. Liu, P. Wang, X. Fan, J. Jia, H. You. Beijing Friendship Hospital, Capital Medical University, Beijing, China

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Background and Aims: Elevated tissue inhibitor of metalloprotease (TIMP)-1 expression contributes to excess production of extracellular matrix in liver fibrosis. Here, we constructed a recombinant adeno-associated virus (rAAV) carrying small interfering RNA of TIMP-1 gene (rAAV/siRNA-TIMP-1) and investigated its effects on liver fibrosis in rats.

Methods: Two models of rat liver fibrosis, the carbon tetrachloride (CCL₄) and bile duct ligation (BDL) models, were treated with rAAV/siRNA-TIMP-1. Moreover, hepatic cells were isolated from both model rats to detect the infection efficiency and expression of TIMP-1, matrix metalloproteinase (MMP) 13 and MMP2.

Results: In CCL₄ model, rAAV/siRNA-TIMP-1 administration attenuated fibrosis severity, as determined by histological analysis of hepatic collagen accumulation, hydroxyproline, and concentrations of types 1 collagen in livers. Levels of mRNA and active MMP13 were elevated, while both mRNA and active MMP12 decreased. Moreover, a marked decrease was noticed in the expression of α-SMA, a biomarker of activated hepatic stellate cells (HSCs), and transforming growth factor (TGF)-β1, critical for development of liver fibrosis. Similarly, rAAV/siRNA-TIMP-1 treatment significantly alleviated BDL-induced liver fibrosis as detected by histological examination. mRNA expression of TIMP-1 in HSCs isolated from rAAV/siRNA-TIMP-1 treatment rats showed a 37% reduction compared with the levels in HSCs from rAAV/EGFP treatment rats, and the protein level of TIMP-1 in HSCs isolated from rAAV/siRNA-TIMP-1 treatment rats showed more than 90% down-regulation. MMP13 expression was increased in HSCs from
rAAV/siRNA-TIMP-1 treatment rats compared with those from rAAV/EGFP treatment rats (more than 90% increase in mRNA and about 20% increase in protein). Although MMP13 could also be produced by Kupffer cells, the increased expression in this cell type was not so significant from rAAV/EGFP treatment rats compared with rAAV/siRNA-TIMP-1 treatment rats (47% increase in mRNA and about 10% increase in protein) because of less rAAV infection efficiency in Kupffer cells compared with that in HSCs. These data indicate that administration of rAAV/siRNA-TIMP-1 attenuated liver fibrosis by directly elevating the function of MMP13 and diminishing activated HSCs. It also resulted in indirect decreased expression of type I collagen, MMP2, and TGF-β1.

**Conclusions:** The rAAV/siRNA-TIMP-1 may be an effective antifibrotic gene therapy agent.

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**THE Wnt PATHWAY IN HEPATIC STELLATE CELLS: CANONICAL vs. NON-CANONICAL SIGNALING**

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**Background and Aims:** Hepatic stellate cells (HSCs) are the key cellular regulators in the onset and progression of liver fibrosis. They are activated upon injury, transdifferentiating to a fibrogenic, myofibroblast-like phenotype. Understanding the mechanisms underlying this activation is necessary for advancement of liver fibrosis treatment and therapy. Perturbations in Wnt signalling can predispose to disease as Wnts are critical in regulating cell differentiation as well as regeneration. Recent studies have suggested increased Wnt signalling may contribute to HSC activation. Wnts can signal either through canonical/β-catenin associated or non-canonical pathways. Which type of Wnt signalling is functional in HSCs has yet to be resolved.

**Methods:** The expression of Wnt pathway components and ligands was assessed in activated and quiescent rat HSCs by qRT-PCR and Western Blot. Wnt signalling was then simulated in HSCs either by overexpression in LX2s (a HSC cell line), or by treatment of primary rat HSCs with Wnt containing conditioned medium. A TCF luciferase reporter (TOPFLASH) was used to measure canonical/β-catenin associated activity. Non-canonical activity was assessed by measuring downstream effectors such as JNK and NFAT by Western Blot or luciferase reporter.

**Results:** HSCs express a wide range of Wnt components. Transdifferentiation of HSCs was associated with upregulated expression of noncanonical Wnts (Wnts 4, 5a and 6) and extracellular modulators as well as decreased Frizzled (FZD) receptor expression. Canonical Wnt expression was not detected in quiescent or activated HSCs. Endogenous β-catenin activity was limited with no increase observed under Wnt stimulated conditions, either through treatment with Wnt conditioned medium or transfection with an active β-catenin construct. Increased phosphorylation of JNK, a non canonical downstream effector, was observed with Wnt treatment.

**Conclusions:** Canonical Wnt activity does not appear to be a contributing factor to HSC transdifferentiation. HSCs express non-canonical ligands and display limited β-catenin activity even under Wnt stimulated conditions. Expression of non-canonical ligands and increased expression of non-canonical downstream components of the branches suggests that non-canonical Wnt may be contributing to HSC activation. Further work on the mechanisms by which Wnt is influencing HSC biology now needs to be undertaken.

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**LIVER Bid-SUPPRESSION BY NOVEL RNAi TECHNOLOGY FOR TREATMENT OF FIBROSIS ASSOCIATED WITH NONALCOHOLIC STEATOHEPATITIS**

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Liver fibrosis is the most worrisome feature of Nonalcoholic Steatohepatitis (NASH). Growing evidence supports a link between hepatocyte apoptosis and liver fibrogenesis. The aim of our study was to determine the therapeutic efficacy and safety of liver Bid, a key pro-apoptotic molecule, suppression using RNA interference (RNAi) for treatment of fibrosis.

**Methods:** siRNA system was optimized by screening 10 siRNAs delivered in vivo using two lipid nanoparticles – Invivofectamine®2.0 and a newly developed Formulation401 – that have been designed for high efficacy of accumulation in the liver. C57BL/6 mice were injected with siRNA formulations and Bid mRNA was checked by real time PCR. C57BL/6 mice were placed on choline-deficient L-amino acid defined (CDAA) diet for NASH mouse model. After 19 wks of CDAA diet that results in severe fibrotic-NASH, mice were injected with Bid siRNA-Formulation401 by weekly for three weeks, at 1.5 mg/kg following 0.15 mg/kg twice. Plasma and liver tissue were collected for determination of NASH features by histopathology, cell death assessment including TUNEL assay and immunoblotting. Hepatic stellate cell (HSC) activation was determined by real time PCR and liver fibrosis quantitated by image analysis of Sirius-red stained sections.

**Results:** The best siRNA that lead to a most efficient knockdown was selected from 10 siRNAs. A maximum knockdown was achieved at a siRNA dose of 1.5 mg/kg with Formulation401, whereas it was at 7 mg/kg with Invivofectamine®2.0. In NASH mice, after 3 weeks of treatment, Bid mRNA was suppressed to 50% (p < 0.003) and Bid protein was reduced to 10% (p < 0.002). Liver fibrosis was improved by Bid suppression as assessed by Sirius red quantitation as well as mRNA expression of fibrosis genes such as TIMP-1 (p < 0.03) or CTGF (p < 0.05). These changes were associated with marked reduction on TUNEL-positive cells and reduction on mitochondrial BAX.

**Conclusion:** This study demonstrates that Formulation401 has a better therapeutic index and liver Bid suppression by RNAi technology improves liver fibrosis associated with experimental NASH. These findings are consistent with evidence that apoptosis triggers HSC activation and liver fibrosis and suggest that Bid inhibition may be useful as an antifibrotic NASH therapy.

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**THE LIVER EXHIBITS THERMAL VARIATIONS DEPENDING ON THE FIBROSIS DEGREE: A PROOF OF CONCEPT CONCERNING THE USE OF DIFFERENTIAL SCANNING CALORIMETRY FOR ASSESSING LIVER FIBROSIS**

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**Background and Aims:** Liver fibrosis results in a disproportionate of the hepatic composition and architecture, characterized by a progressive accumulation of fibrillar architecture at the liver parenchyma. Differential scanning calorimetry (DSC) is an experimental methodology able to determine the specific thermal
signature from any biological substance, based on the variation in heat flow and heat capacity. As these physicochemical properties are directly influenced by compositional and structural changes, we decided to study the thermal behavior of the liver during fibrosis using DSC.

**Methods:** Liver fibrosis was induced in rats by bile duct ligation or carbon tetrachloride administration. Degree of liver fibrosis was determined by histological examination using the Masson’s Trichrome stain, accompanied by hepatic expression of alpha-smooth muscle actin. The thermal analysis was performed in a differential scanning calorimeter using 20 mg of fresh liver mass.

**Results:** The liver showed a characteristic thermal signature in control animals, which progressively differed among mild (F1), moderate (F2), and advanced (F3–F4) liver fibrosis. For heat flow, the hepatic thermal signature from F3–F4 rats exhibited significant differences when compared with F1, F2, and controls. In terms of heat capacity, liver specimens provided a specific thermal signature for each stage of disease, characterized by a transition temperature onset at 95°C for controls, whereas in F1, F2, and F3–F4 animals this temperature significantly decreased to 93, 84 and 75°C, respectively.

**Conclusions:** In view of DSC is inexpensive, simple, highly reproducible, and only requires small samples of tissue, in the near future this analytic technique could be useful for hepatologists and pathologists as a complementary diagnostic tool to liver biopsy. Therefore, these results collectively emphasize the importance of taking into consideration the tissue-associated thermal properties in the study and evaluation of pathologic entities that exhibit variations in terms of composition and structure, as is the case of liver fibrosis.

**1112 Th17 CYTOKINES ENHANCE LIVER FIBROSIS VIA INCREASED SENSITIZATION OF HEPATIC STELLATE CELLS TO TGF-beta STIMULATION**

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**Background:** Activated hepatic stellate cells (HSCs) are key initiators of the fibrogenic process. Intrahepatic CD4+ T cells are major producers of hepatoprotective cytokines such as IL-10 produced by regulatory T cells (Tregs) or inflammatory and regulatory cytokines like IL-17 and IL-22 produced by Th17 cells. Th17 cells have been implicated in various conditions or liver damage but the mechanism of action of Th17 cytokines on HSC is still poorly understood.

**Aims:** To understand the role of the different Th17 cytokines (IL-17A and IL-22) in modulating HSC activation, and how the balance of Th17/Tregs ratio influences liver fibrosis progression.

**Methods:** The HSC line LX2 was stimulated with increasing doses of IL-17A or IL-22, and compared to TGF-β- and PBS-treated cells. Activation of HSCs was evaluated by examining the expression of the pro-fibrotic molecules alpha-smooth muscle actin (α-SMA), collagen type I (COL1A1) and tissue inhibitor of metalloproteinase I (Timp-I) by q-PCR. Protein expression was validated by either western blot or picro Sirius red stain. Cell surface expression of the cytokine receptors IL-10Rβ, TGF-βRII and IL-17RA was evaluated by flow cytometry. Finally, we compared the ex vivo frequency of Th17, Tc17 and Tregs in peripheral blood and intrahepatic lymphocytes from patients at different fibrosis stage.

**Results:** IL-17A and IL-22 alone did not induce LX2 activation, as no induction of α-SMA, COL1A1 and TIMP-I was observed. However, both IL-17A and IL-22 sensitized HSCs to the action of suboptimal doses of TGF-β, confirmed by strong α-SMA, collagen type I and Timp-I gene expression and protein production. IL-17A but not IL-22 upregulated TGF-βRII cell surface expression and partially inhibited TGF-βRII downmodulation upon stimulation. Preliminary results with patient samples (n=12) demonstrate that Th17/Tregs ratio is altered during liver disease and correlate with the serum level of ALT (p = 0.03).

**Conclusion:** Our results demonstrated a pro-fibrotic function for IL-17A and IL-22, as both cytokines sensitize HSC to the action of TGF-β. IL-17A acts through upregulation and stabilization of the TGF-βRII while IL-22 probably acts through an intracellular mechanism. Preliminary results suggest that dysregulation of Th17/Tregs ratio is a signature of liver inflammation and potentially liver fibrosis progression.

**1113 UNIAXIAL STRAIN FACILITATES CYTOSKELETAL REALIGNMENT AND PROMOTES A FIBROGENIC PHENOTYPE IN HEPATIC STELLATE CELLS IN VITRO**

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**Introduction:** The formation and subsequent maturation of linear scars linking adjacent portal tracts and/or central veins is a defining feature of cirrhosis. Bridging fibrosis creates a physical barrier restricting hepatocellular regeneration and tissue remodelling. The process of scar formation depends upon the action of myofibroblast populations, including hepatic stellate cells (HSCs). These highly contractile cells are responsible for the maintenance of tissue integrity following injury.

We hypothesised that linear tension produced in evolving linear scars may promote cellular realignment and matrix deposition by HSCs. Our aim was to determine the growth characteristics of HSCs in 3D-tissue culture and whether these might contribute to linear scar formation. We also aimed to determine the effect of uniaxial strain on HSC alignment and matrix deposition in 2D-tissue culture.

**Methods:** Primary murine HSCs were cultured for 1–4 days on a non-woven polyester matrix (fibre diameter 13μm) providing a 3D-framework for HSC attachment, migration and aggregation. HSCs were also cultured on collagen-I-coated deformable silicon membranes and subjected to uniaxial mechanical strain for 4–24 hours (Flexcell FX-2000 apparatus). Cyclical strain (1Hz) was applied to the membranes to give uniaxial strain of 2.5–10%. Cytoskeletal organisation was assessed by digital image analysis following fluorescent F-actin-phalloidin staining and gene expression measured with real-time PCR.

**Results:** HSCs cultured on the non-woven polyester matrix formed initial attachments with single polyester fibres. After 1–2 days, fine attachments formed between HSCs on adjacent fibres separated by distances of up to 100μm. With additional time in culture, HSCs formed linear networks of multiple cells extending for distances of up to 100μm and connected by slender cellular processes. HSCs subjected to uniaxial strain in 2D-culture demonstrated reorientation of their actin stress fibres parallel to the direction of applied stress. Increasing the magnitude of uniaxial strain applied to HSCs from 2.5 to 10% resulted in a significant upregulation of COL1A1 (1.2-fold, p < 0.05), COL3A1 (3.6-fold, p < 0.001) and TIMP1 (1.8-fold, p < 0.001) expression.

**Conclusions:** HSCs spontaneously aggregate into linear cellular networks when embedded in a 3D non-woven mesh. Uniaxial strain induces HSC cytoskeletal realignment and gene expression changes that would promote matrix accumulation and scar maturation.
1114 TRANSPLANTATION OF EpCAM+ve HUMAN HEPATIC STEM CELLS IN LIVER CIRRHOSIS AND CELLULAR IMMUNE RESPONSE
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Introduction: Liver transplantation is the only effective treatment for decompensated liver-cirrhosis. Several factors, such as non-availability of donors, operative-risks, complications associated with rejection, usage of immunosuppressive agents, and high cost of treatment, make this strategy available to only a few people. Human foetal liver derived hepatic progenitor cell transplantation (HSCT) have shown to be a good alternative to manage end-stage liver diseases. In this retrospective study, we investigated safety and efficacy of HSCT by monitoring the T-cell, NK-cell and cytokines which play major role in cellular immune response and rejection of chronic decompensated liver cirrhosis patients.

Materials and Methods: A total of 5 patients with decompensated liver cirrhosis were enrolled in the study. After giving human foetal liver-derived EpCAM positive cell transplantation, T-cell (CD3, CD4 and CD8), NK-cells (CD16) by flow cytometry and cytokine-levels (IL2, TNFα, IFNγ and IFN-γ) by ELISA were monitored four times within a month.

Result: Present study demonstrated that after HSCT patient showed marked clinical recovery and decline in the MELD score and there was no significant variation found in cell mediated response and cytokine levels between pre and post transplantation.

Conclusion: Hence this preliminary study demonstrated human foetal liver-derived EpCAM positive stem cell transplantation is safe for end stage liver cirrhosis.

1115 IDENTIFYING COREGULATED GENE CLUSTERS LINKED TO HEPATIC FIBROSIS
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Background and Aims: Liver fibrosis is a complex trait that varies considerably among individuals. The underlying networks of genetic factors have not yet been clarified. Quantitative trait loci (QTL) analysis allows the identification of genetic loci, interacting genes and gene networks associated with fibrosis progression. Here, we provide the first systematic transcriptome analysis of liver fibrosis in a ‘genetic reference panel’ of recombinant inbred (RI) lines differing in fibrosis susceptibility. The aim of our study was to identify genetic networks and regulatory mechanisms of gene expression during fibrogenesis.

Methods: We generated 96 hepatic expression profiles of fibrotic livers, using Mouse Gene 1.0 ST microarrays (Affymetrix), from 32 RI BXD lines after fibrosis induction by carbon tetrachloride (CCL4) challenge. Transcript levels were used as traits in QTL analysis and mapped to the BXD mouse genomes. Thereby, we identified loci regulating gene expression during fibrogenesis (eQTL). We defined selection criteria for candidate genes, within the hydroxyproline- and fibrosis-stage associated regions (pQTL) identified previously. These implicated the search for genes that 1) are locally regulated quantitative trait genes (cisQTL), 2) co-segregate significantly with fibrosis phenotypes and 3) show a fibrosis-specific regulation by comparing cisQTL in CCl4-treated fibrotic animals to expression data of healthy animals.

Results: Among over 1000 genes in pQTL regions, this eQTL mapping strategy identified 61 cisQTL; 35 cisQTL were differentially regulated compared to healthy animals, including known fibrosis-associated genes such as nuclear receptor LXR and tenascin C. The significant correlation of the fibrosis specific cisQTL to liver phenotypes and the presence of non-synonymous single nucleotide polymorphisms within coding regions of the cisQTL confirmed their relevance for fibrogenesis. Finally, we describe unique networks of the identified candidate genes co-regulated during fibrogenesis.

Conclusions: Integrating QTL mapping of expression and phenotype data within the BXD RI panel as a ‘genetic reference population’ allowed us to identify novel candidate genes of liver fibrogenesis. Our experimental set-up provides a basic experimental framework to dissect gene networks that drive hepatic fibrogenesis and are modulated by therapeutic interventions.

1116 THE CHEMOKINE MACROPHAGE INFLAMMATORY PROTEIN-1α (MIP-1α) IS AN IMPORTANT MEDIATOR OF LIVER FIBROSIS
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Background and Aims: Chemokines are important mediators of acute and chronic liver injury. Overall, chronic liver fibrosis is strongly linked to activation of hepatic stellate cells as well as to influx of inflammatory cells into the liver. We here investigate the role of the chemokine CCL3, also known as macrophage inflammatory protein-1α, in two experimental liver fibrosis models.

Methods: CCL3−/− mice and wild-type mice were treated with CCL1 or a MCD diet to induce liver fibrosis. Fibrosis was analyzed by Sirus red staining, hydroxyproline content and gene expression of fibrosis-associated genes. Immune cell infiltration was investigated by FACS analysis and immunohistochemical staining. The proliferation of the stellate cell line GRX was determined after stimulation with recombinant CCL3, in vitro.

Results: We could show that the protein concentration of CCL3 is increased in wild-type mice after chronic liver injury (P < 0.05). CCL3 deficient mice showed significant decreased levels of fibrosis as assessed by histological staining and intrahepatic hydroxyproline content in both fibrosis models (P < 0.05). The activation of stellate cells was decreased in the CCL3−/− mice in contrast to the wild-type counterparts. FACS analysis and immunohistochemical staining showed a significant decrease of the T-cell population in the liver (P < 0.05). Stimulation of stellate cells with recombinant CCL3 led to significantly increased proliferation when compared to vehicle treated cells (P < 0.001).

Conclusions: Our results identify the chemokine CCL3 as an important mediator of liver fibrogenesis. The inhibition of fibrosis in CCL3−/− mice in different fibrosis models is triggered by decreased activation of hepatic stellate cells and influx of recruited T-cells into the liver. Thus, therapeutic modulation of CCL3 might be a promising target for chronic liver diseases.
DELTA LIKE LIGAND4 ATTENUATES EXPERIMENTAL LIVER FIBROSIS

Results: Microarray analyses identified nine Notch signaling-related genes that were associated with fibrotic stages in HBV patients. Immunohistochemistry was performed to assess Notch ligands/receptors in liver specimens. Effects of Notch ligands in rat hepatic stellate cells (HSC) and cirrhotic fat storing cells (CFSC) were examined. Recombinant Dll4 (rDll4) was applied to mice challenged with carbon tetrachloride (CCl4).

Conclusions: Dll4 immune-microscopy analysis revealed that Dll4 and Notch1 are expressed in bile ducts. However, the roles of Notch ligands in liver fibrogenesis remain largely unknown, which were investigated in this study.

Background and Aims: Humans have five Notch ligands (Jagged1, Jagged2, Delta like ligand (Dll)1, Dll3 and Dll4) and four receptors (Notch1, 2, 3 and 4). Mutation of Jagged1 causes Aplagile syndrome, a disease characterized by defective development of intralobular bile ducts. Notch ligands in liver fibrogenesis remain largely unknown which were investigated in this study.

Methods: Genechip analysis was used to investigate Notch-associated gene expression in 121 HBV-infected patients and 7 controls. Immunohistochemistry was performed to assess Notch ligands/receptors in liver specimens. Effects of Notch ligands in rat hepatic stellate cells (HSC) and cirrhotic fat storing cells (CFSC) were examined. Recombinant Dll4 (rDll4) was applied to mice challenged with carbon tetrachloride (CCl4).

Results: Microarray analyses identified nine Notch signaling-related genes that were associated with fibrotic stages in HBV patients. Immunohistochemistry co-staining and confocal microscopy analysis revealed that Dll4 and Notch1 are expressed in activated HSCs and myofibroblasts in HBV patients. Dll4 immune-score correlated with inflammatory grades and fibrotic stages in these patients. In vitro, blocking the total Notch signaling by a g-secretase inhibitor remarkably decreased expression of collagen I, a-smooth muscle actin and connective tissue growth factor whereas rDll4 protein incubation significantly decreased TGF-b-dependent expression of collagen I in HSCs. Consistent with in vitro findings, rDll4 protein injection remarkably reduced serum ALT levels, liver inflammation and fibrosis in mice after CCl4 challenge.

Conclusions: Although the general Notch signaling promotes liver fibrosis, Dll4 exerts a complex anti-fibrotic role in experimental fibrosis models, suggesting a potential therapeutic target in liver fibrosis. Elevated Dll4 expression in fibrotic liver may act as a protective mechanism to prevent the progression of liver fibrogenesis.

EFFECT OF MESENCHYMAL STEM CELL ON HEPATIC FIBROSIS IN THIOACETAMIDE-INDUCED CIRRHOTIC RAT MODEL

Background and Aim: Cirrhosis is a long-term consequence of chronic hepatic injury with fibrosis and no effective therapy except liver transplantation is currently available for decompensated cirrhosis. However, some practical limitations in liver transplantation lead us to a need for new therapeutic paradigm in this field. Recent reports have shown that the mesenchymal stem cells (MSCs) have the plasticity to differentiate into some kinds of tissue cells and improve organ function. Hence, we investigated the effect of direct inoculation of human bone marrow derived MSCs (BM-MSCs) in thioacetamide (TAA)-induced cirrhosis in a rat model.

Methods: Adult Sprague-Dawley rats were allocated into three groups (each group, n = 15) as follows: G1, sham; G2, TAA-control; G3, TAA+BM-MSC. To induce cirrhosis, 200mg/kg TAA injection was done twice a week for 12 weeks in G2 and G3. 2.5×10⁶ cells of amplified human BM-MSCs were injected directly into the right liver lobe twice, at weeks 6 and 8 in G3. At 12 weeks, the effect of BM-MSCs on cirrhosis was analyzed histomorphologically using Laennec scores. α-Smooth muscle actin (α-SMA) expression by immunohistochemical staining, relative expression of collagen type 1, and transforming growth factor β (TGF-β) were also evaluated by real-time reverse transcriptase-polymerase chain reaction.

Results: Laennec scores were 0, 5.4±0.7 and 3.7±0.1 in G1, G2 and G3, respectively. Histologically, BM-MSCs injected group (G3) showed significant suppression of hepatic fibrosis compared with TAA-control group (G2) (P < 0.001). Expressions of α-SMA (%) were
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significantly lower in G3 than in G2 (3.08±1.26 vs. 7.00±4.12, P < 0.05). Also, the relative expression of collagen type 1 and TGF-β1 in RT-PCR were 0.64±0.24, 2.06±0.51, 1.32±0.31 and 0.62±0.28, 5.89±3.05, 2.22±1.41 in G1, G2 and G3, respectively (P < 0.005).

Conclusion: Our results showed that BM-MSCs could attenuate liver fibrosis in rats with TAA-induced cirrhosis, raising the possibility for clinical use of BM-MSCs in the treatment of cirrhosis.

1120 EFFECT OF MATRIX METALLOPROTEINASE-8 DEFICIENCY ON LIVER FIBROSIS PROGRESSION AND REVERSAL

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Background and Aims: Matrix Metalloproteinases (MMPs) are a family of enzymes involved in various processes such as modulation of inflammation, matrix remodeling and collagen turnover. MMP-8, collagenase-2, has been described as a pro- as well as antifibrotic modulator of liver fibrosis, but consistent studies in models of liver fibrosis progression and regression are lacking.

Methods: Six week old female MMP-8−/− mice and their wildtype controls (n = 10 per group, C57/BL6 background) were treated with carbon tetrachloride (CCL4) or thioacetamide (TAA) for 4 or 8 weeks according to an optimized fibrosis induction model (Popov et al, Gastroenterology 2011). For studying fibrosis regression, mice were harvested at 5 day, 2 weeks and 4 weeks after 4 weeks of toxin treatment. Parameters of liver function, fibrosis (hydroxyproline, Sirius red morphometry) and transcript levels related to fibrogenesis and fibrosis were determined at sacrifice.

Results: There was no significant difference in fibrosis and fibrosis related transcript levels between MMP-8−/− and wildtype mice, both after 4 or after 8 weeks of CCl4- or TAA-treatment, and at all time points during 4 weeks of spontaneous reversal. However, at 8 weeks both CCL4- and TAA-treated MMP-8−/− mice showed a significant (<1.5 fold) upregulation of MMP-9 compared to the wildtype controls. Moreover, TAA-treated MMP-8−/− mice demonstrated a mild but significant elevation (<1.5 fold) of transcript for proinflammatory CCR7 and CCL5. In the 4 week regression models, TAA induced more rapid fibrosis progression and recovery than CCL4. During regression MMP-8−/− KO mice in both models showed mildly to significantly attenuated levels of profibrogenic or proinflammatory transcripts such as TGFβ1, integrin β6, TIMP-1, CXCR3, CCL3, CCL5, CCR7.

Conclusion: Our studies demonstrate that constitutive deficiency in MMP-8 which is considered a central protease for the degradation of fibrillar collagen does not affect fibrosis progression or reversal significantly. This mild phenotype may be explained by MMP-8’s its proteolytic effect on a broad spectrum of cytokines and chemokines with divergent activity on fibrogenesis. Functional investigations on MMP-8 activity are currently underway.

1121 PHENOTYPE AND EXPRESSION QTL ANALYSES OF HEPATOCELLULAR DAMAGE IN VITRO AND FIBROGENESIS IN VIVO IN BDX MICE IDENTIFY EXTRACELLULAR PROTEINASE INHIBITOR (Expi) AS CANDIDATE GENE

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Background: The aim of our study was to identify risk factors and drivers of hepatic fibrosis by quantitative trait locus (QTL) mapping in a ‘genetic reference population’.

Methods: We assessed hepatocellular susceptibility to profibrogenic TGF-beta signalling in cultured primary hepatocytes in vitro as well as fibrogenesis following CCl4 injections in vivo in inbred mouse strains C57BL/6j and DBA/2j, the parental strains of the genetic reference population BXD that differs in fibrosis susceptibility (Hillebrandt et al. Nat Genet 2005), and in 21 BXD lines. Phenotype QTL (pQTL) mapping delineated the same genomic region on mouse chromosome 11 in vitro and in vivo. To identify drivers of fibrogenesis, we investigated genetic loci within this interval for their impact on hepatic gene expression during short-term liver damage by (1) ethanol or (2) CCl4 using an algorithm entitled eQTL analysis. A mouse with targeted Expi knockout was provided by the EUCOMM repository at the Sanger Centre, Cambridge, UK. Inactivation of both alleles by insertion of a Neo cassette in tested animals was verified by genomic PCR.

Results: eQTL analysis of BXD livers in response to short-term ethanol damage or CCl4 injections revealed five single nucleotide variants (SNPs) that have a regulatory impact on other genes during acute liver damage. Amino acid exchanges near two expression-associated SNPs in the QTL area indicate candidacy of Expi and Ms2 for both traits. Expression network analysis identifies Dhx40 as a common factor in response to short-term liver damage by ethanol and CCl4. Cultured hepatocytes from Expi knockout mice show enhanced susceptibility to TGF-beta induced cell death in vitro, confirming the role of this gene as implied by QTL analysis.

Conclusion: The combination of pQTL mapping in vitro and in vivo with eQTL analysis in silico has identified two novel candidate genes and a common mediator of fibrogenesis within a 6 Mb region on Chr 11. Knockout of the Expi gene renders hepatocytes more susceptible to TGF-beta induced cell death.

1122 UP-REGULATION OF THE ANGIOTENSIN-CONVERTING ENZYME 2/ANGIOTENSIN-(1–7)/Mas AXIS PROTECTS AGAINST LIVER FIBROSIS BY INHIBITING NOX4/Mad3 PATHWAY IN BILE-DUCT-LIGATION RATS

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Background and Aims: Accumulating evidence demonstrates that up-regulation of the angiotensin-converting enzyme (ACE)/angiotensin (Ang) II/Ang II type 1 receptor (AT1R) axis aggravates hepatic fibrosis. The recently discovered ACE2/Ang-(1–7)/Mas axis protects against hepatic fibrosis. However, the mechanisms by which ACE2 and Ang-(1–7) attenuate hepatic fibrosis remain unclear. We hypothesized that up-regulation of the ACE2/Ang-(1–7)/Mas axis protects against bile-duct-ligation (BDL)-induced hepatic fibrosis by inhibiting the NOX4/smad3 pathway.

Methods: In vivo, Ang-(1–7) was continuously infused into Wistar rats for four weeks that had received bile-duct-ligation. In vitro, HSC-T6 cells were pretreated with compounds that block the activity of AT1R (irbesartan), Mas (A-779) and NOX4 (DPI) before exposure to Ang II or Ang-(1–7). HSC-T6 cells were infected with lentivirus-mediated ACE2 before exposure to Ang II.

Results: Ang-(1–7) prevented BDL-induced hepatic fibrosis by inhibiting NOX4/smad3 pathway. In vitro, Ang-(1–7) inhibited Ang II-induced smad3 phosphorylation and NOX4-ROS pathway, resulting in attenuation of α-collagen I protein levels and reduced oxidative stress. These changes were reversed by administration of A779. However, treatment with Ang-(1–7) alone markedly increased the expression of NOX4. Overexpression of ACE2 decreased Ang II-stimulated phosphorylation of smad3, NOX4 and α-collagen I protein levels; these changes were reversed by administration of A779.
Conclusions: Up-regulation of the ACE2/Ang-(1–7)/Mas axis protected against BDL-induced hepatic fibrosis by inhibiting the NOX4/smads pathway.

1123 UNDERSTANDING INTEGRIN SIGNALLING DURING LIVER FIBROSIS PROVIDES INSIGHT INTO THEIR MODULATION AS A THERAPEUTIC STRATEGY TO TREAT THE DISEASE
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Background: Liver fibrosis is a leading cause of morbidity and mortality, and there is an urgent need for antifibrotic treatments. Following liver injury hepatic stellate cells (HSCs) become activated to proliferative myofibroblasts, which migrate into the liver parenchyma and secrete tissue damaging extracellular matrix (ECM) proteins, including profibrotic type 1 collagen (COL1), parenchyma and secrete tissue damaging extracellular matrix proteins, resulting in liver fibrosis. One of the receptor systems involved in ECM mediated responses relevant to fibrosis is the integrins, composed of α and β subunits. In this study we have identified that integrin β1 (ITGB1) plays a central role in HSC activation and have identified α3 (ITGAA3) and α11 (ITGAA11) as the critical β1 partners in models of liver fibrosis.

Methods: HSCs were isolated and activated by culture on plastic. LX2 cells were used as a second model. Expression was assayed by qPCR, western blotting and immunocytochemistry. Protein interaction was determined by co-immunoprecipitation (Co-IP). Datamining utilised the STRING database.

Results: Itgb1 expression was elevated in activated HSCs, closely resembling the expression of the profibrotic transcription factor Sry-box 9 (Sox9), ECM proteins Osteopontin (Opn) and Coll, and myofibroblast marker α-smooth muscle actin (α-Sma). Further analysis using an Itgb1 mouse model (Itgb1−/−; CreER), allowing Itgb1 deletion using 4-hydroxytamoxifen in cultured HSCs, resulted in cells resembling a more quiescent phenotype with reduced Sox9 and α-Sma. During activation of HSCs, Itgα2, 3, 6, 8 and 11 were increased as potential Itgb1 interacting α-subunits. Moreover, analysis of previous microarray data from Sox9 depleted HSCs, placed Itgα11 and Itgα3 in a profibrotic network involving collagen and laminin. Reassuringly, Itgα11 and Itgα3 were both decreased following abrogation of Sox9. Further analysis verified Itgα11β1 interaction and both co-localised in activated HSCs.

Conclusion: These data suggest ITGB1 is an important mediator of HSC activation with implications for increased proliferation, migration and contractility in liver fibrosis. The identification of ITGA11 and ITGA3, downstream of SOX9, are particularly interesting as both are implicated in fibrotic mechanisms along with ITGB1.

A better understanding of the role of these integrins and their signalling mechanisms during HSC activation may reveal potential new therapeutic targets for the treatment of liver fibrosis.

1124 DEFICIENT NEUTROPHIL RECRUITMENT INTO THE LIVER PARENCHYMA AFTER AN ACUTE CCl4 CHALLENGE DOES NOT AFFECT FIBROGENESIS
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Background and Aims: Neutrophils are key effectors of the innate immune response against bacterial infection. They infiltrate into the liver parenchyma and migrate to the site of injury where participate in hepatocellular necrosis by oxidative stress. Neutrophil contribution to liver injury has been reported in alcoholic hepatitis, acetaminophen overdose and post-liver transplantation. However, whether neutrophils play a direct role in fibrogenesis still remains unclear. Thus, the aim of this study was to analyse the contribution of neutrophil to fibrogenesis induce by acute CCl4 challenge in a dependent and independent TLR way.

Methods: CCl4 was acutely administrated at 1 μg/g body weight for 24, 48 and 72 hours to WT, TLR2−/−, TLR4−/−, TLR9−/− mice. Purified Ly6G antibody or IgG control (100 μg/mouse) was given to WT 12 hours prior to acute dose CCl4 ± lipoteichoic acid (LTA) (250 μg/mouse) challenge. Animals were culled at 8 and 48 hours post-CCl4. Myofibroblasts (α-SMA), neutrophils (NIMP) and macrophages (F4/80) were determined by immunohistochemistry. Hepatic inflammatory gene expression was measured by RT-PCR.

Results: Only TLR2−/− mice showed a deficient neutrophil recruitment at 24 hours after acute CCl4 injury versus WT, TLR4−/− or TLR9−/− mice, despite similar levels of liver damage. α-SMA positive area peaked at 72 hours post-CCl4 injection but no difference was observed between TLR2−/−, TLR4−/−, TLR9−/− and WT animals. TNF-α, CXCL-1 and CXCL-2 gene expression were significantly decreased in the TLR2−/− in comparison with WT, but no differences were detected in IL-6, CCL-2 and CCL-5. Ly6-G depleting antibody successfully decreased the number of neutrophils versus control IgG, however no changes were observed in fibrogenesis at 48 hours analyzed by Col1α1 and α-SMA gene expression or α-SMA positive area in liver. Supporting these findings, in a TLR independent system, S100A9−/− mice displayed reduced neutrophil recruitment after acute and chronic CCl4 challenge but showed no change in liver fibrogenesis.

Conclusions: Neutrophils do not play a key role in fibrogenesis in an acute or chronic toxic liver injury in a dependent and independent TLR2 fashion.
able to repress AKT phosphorylation after GAS6 exposure (30 min, 200 ng/ml), and overnight treatment of LX2 cells was able to dose-dependently decrease basal AKT levels and inhibit GAS6-dependent AKT activation.

**Conclusions**: Our results point to Axl/AKT axis as a relevant mechanism in HSC activation and suggest that Axl targeting may be an interesting therapeutic strategy to reduce liver fibrosis.

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**IDENTIFICATION OF p90RSK AS A NOVEL THERAPEUTIC TARGET FOR LIVER FIBROSIS**

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**Background and Aims**: There is an urgent need to identify new therapeutic targets for liver fibrosis, a condition characterized by a progressive deposition of extracellular matrix in the hepatic parenchyma. Previous proteomic studies in our laboratory identified p90RSK as a possible mediator of liver injury. There is no information on its potential role on liver fibrosis. p90RSK is an intracellular signaling kinase that regulates cell proliferation, differentiation and collagen synthesis. The aim of the current study is to investigate the role of p90RSK in liver fibrosis.

**Methods**: Hepatic p90RSK levels were analyzed by qPCR, immunohistochemistry and Western blotting in patients with severe liver fibrosis (F4) caused by alcoholic hepatitis (AH) (n=9) and chronic hepatitis C (HCV) (n=9), in fragments of normal livers (n=7) and in patients with low fibrosis caused by non-alcoholic steatohepatitis (NASH) (n=9). Furthermore, pharmacological inhibition of p90RSK by kaempferol, a natural flavonoid that inhibits this kinase, was studied in a mouse model of hepatic fibrosis by carbon tetrachloride (CCl4). Finally, p90RSK in vitro phosphorylation and effects in hepatic stellate cell (HSC)'s activation were studied in primary human HSC.

**Results**: p90RSK gene and protein expression and phosphorylation were found up-regulated in livers of patients with a high degree of liver fibrosis compared to normal livers and patients with low-mild fibrosis (p<0.01). Tissue distribution of activated p90RSK revealed a nuclear translocation of this kinase in hepatocytes. Furthermore, kaempferol administration caused a reduction of collagen deposition and expression of pro-fibrogenic genes (Collagen type I, TIMP-1, TGF-b and MMP-2) and pro-inflammatory cytokines (TNF-a and MCP-1) in CCl4-treated mice (p<0.05). In addition, these mice had lower increase of serum transaminases (p<0.05). Finally, the in vitro studies revealed that pro-fibrogenic mediators (PDGF, LPS and TNF-a) induced a transient phosphorylation of p90RSK. Importantly, kaempferol reduced the basal expression of pro-fibrogenic genes (Collagen type I, TIMP-1 and TGF-b) in activated HSC (p<0.05).

**Conclusions**: p90RSK is functionally activated in patients with advanced liver fibrosis and drives experimental hepatic fibrosis as well as the fibrogenic properties of HSC. These results suggest that p90RSK could be a new therapeutic target for liver fibrosis.

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**LOW DOSE STEADY STATE H2O2 INDUCES FIBROLYTIC MMP-3 IN VITRO AND IN VIVO**

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**Introduction**: Inflammation associated reactive oxygen species (ROS) such as H2O2 are considered a key event during development of fibrosis/cirrhosis. This contrasts with other diseases such as rheumatoid arthritis where inflammation and ROS are causing fibrolysis and cartilage destruction. We here study the effect of sustained low H2O2 levels on fibroblasts, hepatic stellate cells and in a mouse model.

**Methods**: Human NIH 3T3 fibroblasts and LX-2 hepatic stellate cells were incubated for 6 or 24h with 0.5-6μM H2O2 under different oxygen tensions using the recently developed GOX/CAT system (enzymatic generation of H2O2 by glucose oxidase and catalase). RNA was isolated and molecules involved in fibrogenesis and fibrolysis were analyzed by qRT-PCR. Additional Western blotting was performed for MMP3.

**Results**: In contrast to MMP13, TIMP1 and collagen 1α1, MMP3 was significantly upregulated (x3.5) after 6 hours in both cell types in the presence of non toxic steady state H2O2. The MMP-3 induction could be completely blocked by co-incubation with the antioxidant N-acetylcysteine (2 mM). Notably, MMP-3 was even more drastically upregulated (20x) if the experiment was performed in a slightly lower oxygen environment (10% instead of 21% oxygen) that mimic more closely an inflammatory and hepatic environment. We finally confirm these findings in a recently established mouse model of hepatic oxidative stress by injecting mice with glucose oxidase i.v. (0.2U/g body weight) which home to the liver and continuously exposes liver cells to toxic levels of H2O2. Analysis of MMP-3 in the liver of these mice showed significant upregulation of MMP-3.

**Summary**: We here demonstrate in vitro and in vivo that non-toxic concentrations of H2O2 comparable to those released by inflammatory cells drastically induced MMP-3, a major fibrolytic player in tissue remodeling.

1128

**THERAPEUTIC EFFECT OF BONE MARROW-DERIVED CD45+ CELLS IN CONTROL OF LIVER FIBROSIS IN MOUSE MODEL**

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**Background and Aims**: CLD is a serious global health problem, it involves with excessive collagen matrix deposition (collagen scar) leading to inhibition of normal liver regeneration and function. End-stage CLD is treated with LDLT or cadaver liver transplantation, though in most of the cases patients die due to unavailability of donor liver. Furthermore, recipients often suffer from infection and graft reactions. There is a need for an alternate treatment protocol, which is affordable and less complicated. In this investigation, we have examined the role of BM-derived cells in the regression of liver fibrosis and tissue regeneration.

**Methods**: Experimental liver fibrosis model was established in mouse by repeated injection of CCl4 for 10 weeks, and its progression was monitored by adopting Metavir scoring system and determining percentage collagen equivalent area. Mice were intraspleenically transplanted with eGFP-expressing CD45+ cells on 3rd day of last CCl4 dose (Metavir stage III). The regression of fibrosis was followed for 4 months. The engrafted donor cell types were assessed by immunohistochemistry (IHC). The activation and
deactivation of HSCs in the absence or presence of donor cells were examined in vivo and by co-culture experiments.

**Results:** Mice received donor cells showed significant regression of fibrosis. The extent of steatosis was also low in the experimental groups of mice. Mice showed decreasing trend of alpha-smooth muscle actin expression in HSCs and collagen I synthesis, confirming decline of HSCs activation and fibrosis. The engrafted cells persisted in the liver till the end of this investigation. A major fraction of them expressed albumin, some of them expressed von Willebrand factor and a few expressed F4/80 antigen. In vitro experiments confirmed that CD45+ cells can suppress proliferation and activation of HSCs.

**Conclusions:** Hematopoietic cell therapy improves clinically relevant parameters in experimental mouse model of chronic liver injury. Donor cells appear to extend paracrine effects for suppressing HSCs activity in turn assist regression of scar tissue. Engrafted cells also involve in the regeneration of liver. These results suggest a clinical potential of this therapy to treat chronic liver injury.

Acknowledgement: This work was funded by Department of Biotechnology, India under CMM programme.

**1129**

**SERPIN-B3 AS A PRO-FIBROGENIC MEDIATOR CONTRIBUTING TO CHRONIC LIVER DISEASE PROGRESSION**

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**Background and Aims:** SERPINB3 (S-B3) is a cystein protease inhibitor known to be over-expressed in a high percentage of cirrhotic patients carrying hepatocellular carcinoma. In addition, previous studies have suggested the existence of a significant correlation between TGF-β1 and S-B3 in liver biopsies from patients with chronic hepatitis and cirrhosis, with both proteins correlating also to the extent of liver fibrosis. In the present study we have investigated the putative pro-fibrogenic role of S-B3.

**Methods:** Morphological, molecular and cell biology techniques have been employed to evaluate S-B3 expression, fibrosis-related parameters or responses in the following conditions: i) in the liver of patients with HCV-related chronic liver disease; ii) in transgenic mice overexpressing S-B3 in the liver and in related wild type mice, both submitted to chronic CCl4 administration; iii) in primary cultures of human activated hepatic stellate cells (HSC/MFs), which were also exposed to recombinant S-B3.

**Results:** S-B3 expression has been detected in 45% of chronic HCV patients analysed, with an expression level that was found to increase progressively from F1–F2 to F4 patients (METAVIR score). In S-B3 positive specimens, this serpin was mainly expressed in hepatocytes close to fibrotic septa and some α-smooth muscle-actin (αSMA) positive cells. S-B3 transgenic mice exposed chronically to CCl4 (12 weeks) were characterized by a significantly higher increase of extracellular matrix deposition, αSMA-positive myofibroblasts as well as of collagen type 1 transcripts, compared to wild type mice. Experiments on human HSC/MFs led to the following major results: (i) untreated HSC/MFs, which express low but detectable levels of S-B3, up-regulated S-B3 expression and release in response to both hypoxia and TGF-β1; (ii) exposure of HSC/MFs to recombinant S-B3 protein resulted in a significant increase in TGF-β1 expression and other fibrosis related parameters. In addition, S-B3-induced oriented migration of HSC/MFs through a redox-mechanism involving activation of c-Jun-NH2-terminal kinases (JNK) isoforms 1 and 2.

**Conclusions:** S-B3, up-regulated in conditions of chronic liver injury, can act as a paracrine pro-fibrogenic mediator and contribute to chronic liver disease progression.

**1130**

**HIGH DOSE ORAL THIOACETAMIDE INDUCES IRREVERSIBLE HEPATIC FIBROSIS IN MICE**

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**Introduction:** Myofibroblast apoptosis, accumulation of specific subsets of pro-resolution macrophage and matrix degradation by matrix metalloproteinase (MMP) enzymes are key mechanisms in liver fibrosis regression following the cessation of injury. However, in certain circumstances, hepatic fibrosis does not resolve entirely. The exact factors that govern whether fibrosis is reversible or irreversible are not fully known.

**Aim:** To develop and characterise an irreversible model of murine hepatic fibrosis and utilise this to identify factors determining irreversibility.

**Methods:** Liver fibrosis was induced in C57BL/6 mice by continuous administration of oral thioacetamide (TAA) (300 mg/l or 600 mg/l) for up to 52 weeks followed by conversion to normal drinking water for reversibility studies (control animals received normal water throughout). Comparisons were made to mice injured with intraperitoneal CCl4, a well characterised model of reversible liver fibrosis. Livers were analysed by immunohistochemistry, qPCR and flow cytometry of hepatic non-parenchymal cells.

**Results:** Oral TAA 600 mg/l, but not 300 mg/l, induced progressive liver fibrosis, with scarring detectable by 8 weeks and established cirrhosis by 52 weeks. Following either 8 or 52 weeks of TAA treatment, conversion to water for up to 8 additional weeks resulted in no detectable fibrosis resolution. Gene expression of Collagen 1 and 3 in addition to key MMPs, rapidly returned to baseline following TAA cessation, suggesting a failure of matrix degradation as a reason for the persistent scar. Analysis of hepatic macrophages, a key source of MMPs, following TAA cessation demonstrated a lack of the dynamic changes in subsets seen in the reversible CCl4 model. Specifically, no increase in the recently defined restorative MMP-expressing CD11b+Ly-6Ch+ hepatic macrophage subset was observed. Furthermore, TAA-induced liver fibrosis was not associated with a significant increase in α-SMA expression but did show a robust increase in desmin. This is in stark contrast to the strong α-SMA upregulation following CCl4 injury and suggests an alternative myofibroblast population in the TAA model.

**Conclusions:** Oral TAA induces irreversible liver fibrosis in mice. This irreversibility is likely to be due to a lack of accumulation MMP-expressing restorative macrophages and activation of an α-SMA+ desmin+ myofibroblast population, resulting in a more resistant scar.

**1131**

**THE MYOSTATIN SYSTEM IS EXPRESSED IN THE LIVER AND ITS ACTIVATION MEDIATES PROFIBROGENIC ACTIONS IN HEPATIC STELLATE CELLS (HSC) VIA c-jun N-TERMINAL KINASE (JNK)**

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**Background and Aims:** Myostatin, a member of the transforming growth factor-β superfamily, is widely expressed in several organs and in the muscle. The biological functions of myostatin are mediated by different receptors, including activin receptor IIB (ActRIIB). Myostatin has been implicated in the biology of cachexia
during cancer and cirrhosis, but it also participates in the pathogenesis of metabolic syndrome. Recently, myostatin levels have been associated with fibrosis during NAFLD. However, no information is available on the possible direct role of myostatin in the biology of HSC. Aim of the study was to evaluate the effect of myostatin on biological actions and intracellular signaling in human HSC.

Methods: We employed both an immortalized human HSC line (LX-2) and primary HSC isolated from human livers. Cell proliferation was evaluated by MTT. Cell migration was assessed with modified Boyden chambers. Gene expression and secretion of cytokines or ECM proteins were measured by qRT-PCR and ELISA, respectively. Intracellular signaling pathways were evaluated using phosphorylation-specific antibodies.

Results: Transcripts for ActRIIB were expressed by HSC. Exposure to myostatin (50 ng/ml) induced a significant increase in cell migration in both LX-2 and primary HSC, and time-dependently reduced cell proliferation. In addition, myostatin increased mRNA expression of TGF-beta and procollagen type I. We tested the ability of myostatin to activate intracellular signaling pathways in HSC. Myostatin rapidly and markedly induced phosphorylation of Smad3 and JNK. In contrast, p38MAPK and ERK1/2 were only modestly activated. Pretreatment of both LX-2 or primary HSC with the selective JNK inhibitor, SP600125 (20 μM), caused a marked inhibition of myostatin-induced cell migration. Transcripts for myostatin and ActRIIB were measurable in normal liver. Induction of steatohepatitis by administration of a methionine and choline-deficient diet significantly increased the expression of both molecules.

Conclusions: HSC express ActRIIB and respond to myostatin with increased chemotaxis, reduced proliferation, and increased expression of profibrogenic genes. At least some of these require JNK activation. Expression of myostatin and its receptor is present in the liver and up-regulated following chronic liver injury, indicating a possible role for this molecule in liver fibrosis.

The first two Authors contributed equally to the present study.

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EFFECT OF ADENOSINE 2B RECEPTOR ANTAGONIST MRS1754 ON FIBROSIS PROGRESSION AND REGRESSION IN MICE
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Background and Aims: There are four known subtypes of adenosine-receptors by selective agonists and antagonists has potential for the treatment of various cardiovascular inflammatory and neurological diseases. Recent studies demonstrated a role for the adenosine-receptors in liver diseases, including NASH, hepatic inflammation and fibrosis. However, it is unclear how far the A2BR-receptor modulates fibrogenesis. We investigated antifibrotic potential of the selective A2B-receptor antagonist MRS1754 in liver fibrosis progression and regression.

Methods: Mdr2−/− mice were injected daily over 4 weeks either with vehicle (0.2% DMSO in PBS, n = 10), low-dose MRS1754 (0.5 mg/kg, n = 10) or high-dose MRS1754 (2 mg/kg, n = 10) intraperitoneally. In the other model 18 mice were treated according to the CCl4 fibrosis model in a 2 weeks frame. Some mice were administered low-dose (0.5 mg/kg, n = 6) or high-dose (2 mg/kg, n = 6) MRS1754 in the second week additionally. In the fibrosis regression group after 6 weeks of fibrosis induction with carbon tetrachloride (CCl4) or thioacetamide (TAA), mice were injected daily with the two doses of MRS1754 for 4 weeks (each n = 10). Inflammation and fibrosis were quantified by histology, collagen determination, Sirius red morphometry, serum biochemistries and qPCR for fibrosis and fibrolysis related transcripts.

Results: In the CCl4 progression cohort low-dose A2B receptor blockade had no effect on collagen deposition, while high-dose treatment lowered hepatic-collagen by 18%, with a significantly reduced fibrotic area after Sirius red staining. Mdr2−/− mice treated with high dose A2B receptor antagonist displayed a 32% and 37% reduction of relative and total hepatic-collagen respectively, with downregulation of TGFβ1, αSMA, TIMP-1, MMP-2, 9 and MMP-13 transcripts. Mice in the CCl4-induced fibrosis regression cohort showed significantly reduced hepatic-collagen accumulation, and mice in the TAA-induced fibrosis regression cohort showed a significant but mitigated decrease of collagen deposition in a dose dependent manner. In the, regression model after CCl4 levels of TGFβ1, αSMA mRNA were downregulated, and those of MMP-2, 3 and TIMP-1 significantly elevated.

Conclusion: In the models of fibrosis progression and regression, specific A2BR antagonism alleviated liver fibrosis characterisation of the exact antifibrotic mechanisms of cell specific A2B antagonism are currently underway.

1133

THE NCU-G1gt/gt MOUSE AS A LIVER FIBROSIS MODEL ORGANISM: A STUDY OF THE DEVELOPMENT OF FIBROSIS AND RESPONSE TO ANTI-FIBROTIC TREATMENT
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Background and Aims: NCU-G1 is a novel protein. First described as a regulator of transcription[1], and later as a lysosomal integral membrane protein [2]. We created a novel mouse model with no expression of NCU-G1 (NCU-G1−/− mouse). These animals spontaneously develop hepatic fibrosis early in life, without any other detectable phenotypes, and could become a new model for this condition [3]. Hepatic fibrogenesis is a response to continued insults to the liver. Increased formation of extra cellular matrix (ECM) (mainly collagen type I and III) results from an imbalance between synthesis and degradation of ECM-proteins. Different cell types and enzymes are involved in this process, including the ECM-degrading matrix metalloproteinase’s (MMPs), and their inhibitors, tissue inhibitor of metalloproteinase (TIMPs). Alterations in their expression indicates an on-going fibrogenesis [4]. If left untreated, fibrosis will develop and lead to permanent damages to the liver. Several compounds are used to reverse this process. One such is sodium hydrosulfide (NaHS), which has been shown to attenuate fibrosis in rodent models [5, 6]. This study investigates the aplicability of NCU-G1−/− mice as a model organism for hepatic fibrosis, with a focus on the progression of fibrosis and response to anti-fibrotic treatment by NaHS.

Methods: NaHS (0.6 mg/g body weight) was administered intraperitoneally every other day for 30 days. Changes in transcription of fibrotic regulators were analyzed using real-time qRT-PCR. Protein levels of MMP-2 and MMP9 were assessed by zymography, and collagen levels by hydroxyproline assay.

Results: Gene expression analyses indicate changes in expression of MMPs and TIMPs, indicating fibrogenesis. Hydroxyproline levels increase with age in NCU-G1−/− liver, confirming this finding. A positive response to treatment with NaHS was indicated by a drop in hydroxyproline levels.

Conclusion: The hepatic fibrosis in NCU-G1−/− mouse increases with age, but the condition was improved upon NaHS treatment. Our mouse model may be used in experimental trials of novel anti-fibrotic compounds.
POSTERS

1134 FATTY ACID AMIDE HYDROLASE IS THE HEPATIC GATEKEEPER AGAINST ENDOCANNABINOID-INDUCED CELL DEATH AND HOLDS ANTI-FIBROTIC PROPERTIES

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Background and Aims: The hepatic endocannabinoid (EC) system becomes activated and plays a crucial role in fibrosis. We have previously shown, that the levels of multiple ECs are upregulated during fibrogenesis and ECs selectively induce cell death in activated HSCs, but not in hepatocytes. However, the role of different putative EC degradation enzymes in cell death susceptibility and hepatic fibrosis are still unclear. We analysed the cellular expression of fatty-acid-amide hydrolase (FAAH), monoacyl-glycerol lipase (MGL), catechol-O-methyl transferase (COMT), alpha-beta-hydrolase domain (ABHD)-6 and -12 and their functions in EC-induced cell death.

Methods: Primary hepatocytes and hepatic stellate cells (HSCs) were isolated from healthy mouse liver by collagenase perfusion. Liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) were isolated by collagenase perfusion and MACS. Cell death induced by the ECs anandamide, 2-AG, NADA and virodhamine was evaluated by LDH assay and AnnexinV/PI. Expression of the degradation enzymes was determined by qRT-PCR and western blot. Liver injury and fibrosis was induced by CCl4 in wildtype and FAAH−/− mice (n=4 each) for 14 days.

Results: The major anandamide-degrading enzyme FAAH was expressed in hepatocytes (>100x vs. KCs), LSECs (>14x), KCs, but not in HSCs. This expression pattern correlated not only with the cellular resistance against anandamide-, but also 2-AG-, NADA- or virodhamine-induced cell death. Pharmacological FAAH inhibition rendered these celltypes susceptible toward all tested ECs. Moreover, all celltypes isolated from FAAH−/− livers were also highly sensitive for EC-induced cell death. Conversely, FAAH overexpression rendered HSCs resistant against EC-induced death. The major 2-AG-degrading enzyme MGL was expressed in LSECs (>140x vs. hepatocytes), HSCs (>9x), KCs (>12x) and hepatocytes. However, MGL inhibition did not sensitize any celltype toward cell death induced by any EC. The putative NADA-degrading enzyme COMT and the alternative 2-AG-degrading enzymes ABHD-6 and -12 were not expressed in any celltype. FAAH−/− mice showed increased liver injury and fibrogenesis after CCl4, underlining the importance of this enzyme for the resistance against EC-induced cellular damage in vivo.

Conclusions: FAAH holds a key role as the major protecting enzyme in the liver not only against anandamide – but other EC-induced cell death and bears antifibrotic properties.

1135 Rev-erb alpha: NOVEL REGULATOR OF HEPATIC STELLATE CELL ACTIVATION

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Background and Aims: Activation of hepatic stellate cells (HSCs) is a major contributor to hepatic fibrogenesis, a process characterized by accumulation of scar matrix, hepatic cell death and disruption of normal liver architecture. Increasing evidence suggests metabolic reprogramming plays an essential role in regulation of HSC activation. Rev-erbs, members of the nuclear receptor superfamily, are key regulators of lipid and glucose metabolism; therefore, we hypothesize that Rev-erbs control the process of HSC activation.

Methods: Primary rat HSCs were isolated via standard pronase-collagenase digestion and subsequent density gradient centrifugation. LX-2 cells were kindly provided by Dr. Scott Friedman (Mount Sinai School of Medicine). Cells were treated with adipogenic mixture (MDI), 5 ng/ml of recombinant transforming growth factor-beta (TGFβ), or plated on Matrigel™ (growth condition which promotes HSC quiescence) for various time points. Gene expression was determined by RealTime PCR and Western blot. Immunocytochemistry followed by confocal microscopy was used to determine sub-cellular localization. Rev-erbα and Rev-erbβ expression vectors were produced by standard molecular cloning. LX-2 cells were transfected with expression vectors or controls using Fugene® HD.

Results: Using an in vitro model (primary rat cells) of HSC activation, we detected upregulation of two Rev-erb alpha (Rev-erba) isoforms (Rev-erba1 and Rev-erba2) at the protein level, with immunocytochemistry demonstrating significant cytoplasmic accumulation. When stimulated with known adipogenic (MDI) and fibrogenic (TGFβ) compounds, expression patterns of RE isoforms were analogous. Human HSCs (LX-2) manipulated to ectopically express recombinant Rev-erba1 and Rev-erba2 mirrored the results found in primary cells. Compared to control vector, forced expression of Rev-erba1 and Rev-erba2 potentiated TGFβ-dependent activation of plasminogen activator inhibitor-1 and type I collagen. Overexpression of Rev-erba1 conferred previously non-responsive LX-2 cells sensitive to Rev-erb agonist SR6452, while overexpression of Rev-erba2 did not, suggesting functional differences between the two isoforms. Moreover, when cultured in Matrigel™, cells expressing a truncation mutant of Rev-erba (known to localize to the cytoplasm) showed significantly higher smooth muscle alpha actin expression, supporting a fibrogenic functionality of cytoplasmic Rev-erba.

Conclusion: Taken together, we conclude upregulation and cytoplasmic accumulation of Rev-erb alpha are integral to both the myofibroblast phenotype and the fibrogenic response during HSC activation.

1136 REGRESSION OF FIBROSIS AND REVERSAL OF CIRRHOSIS IN THIOACETAMIDE-INDUCED LIVER FIBROSIS FOLLOWING TREATMENT WITH GALECTIN INHIBITORS

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Introduction and Objective: Galectin-3 (gal-3) protein is critical to the development of liver fibrosis because gal-3 null mice have attenuated fibrosis after liver injury. Therefore, we examined the ability of novel complex carbohydrate gal-3 inhibitors to treat toxin-induced fibrosis and cirrhosis.

Methods: Fibrosis was induced in rats by intraperitoneal injections with thioacetamide (TAA); some groups were treated
with GR-MD-02 (galactoarabino-rhamnogalaturonan) or GM-CT-01 (galactomannan), which bind gal-3>gal-1.

**Results:** In initial experiments, 4 weeks of treatment with GR-MD-02 (60 mg/kg 2/wk) following completion of 8 weeks of TAA (150 mg/kg 2X/wk) significantly reduced collagen content by almost 50% based on Sirius red staining (6.5±0.8% n=7 vs. 11.4±0.3% n=7, p<0.05). Rats were then exposed to more intense and longer TAA treatment (150 mg/kg 3X/wk for 11 wks), which included either GR-MD-02 or GM-CT-01 during weeks 8 through 11. TAA rats treated with vehicle developed extensive fibrosis (26±4.5% collagen; n=9) and pathological stage 6 Ishak fibrosis (ie., cirrhosis). Treatment with either GR-MD-02 (90 mg/kg ip) or GM-CT-01 (180 mg/kg ip) given once weekly during wks 8–11 led to marked reduction in fibrosis (9.5±2.5% (p<0.001) and 15±5.6% (p<0.001), respectively). Based on blinded pathological scoring, all vehicle-treated animals had cirrhosis (stage 6) whereas fibrosis stage was significantly reduced (median stage=4.5), with evidence of resolved or resolving cirrhosis in the treated animals. Portal pressure in TAA treated rats given vehicle (20.3±2.4 cm H2O) was significantly elevated compared to normal rats (10.5±2.4 cm H2O, p<0.001), which was significantly reduced by GR-MD-02 or GM-CT-01 (17.1±2.4 cm H2O (p<0.05) and 18.5±3.7 cm H2O (ns), respectively). Expression of collagen I mRNA and protein, and alpha-smooth muscle actin protein were significantly reduced in GR-MD-02 and GM-CT-01 treated rats compared to vehicle-treated control rats. While AST and ALT were not reduced by treatment with GR-MD-02, treatment with GM-CT-01 resulted in a normalization of AST/ALT levels.

**Conclusions:** Treatment with two galectin inhibitors with different chemical compositions, but having common structural elements, significantly reduced fibrosis and reversed cirrhosis in a toxic model of liver fibrosis, even while continuing TAA, which were associated with reduced portal hypertension.

### 1137

**A ROLE FOR ALDH ACTIVITY DURING HEPATIC STELLATE CELL ACTIVATION**


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**Background:** Hepatic fibrosis is the major complication of virtually all types of chronic liver damage. Hepatic stellate cells (HSCs) remain the main contributors of the increased liver scar tissue formation. HSCs are the liver pericytes that store 80–90% of total liver retinoid in their lipid droplets. It is generally accepted that when HSCs activate retinyl ester levels progressively decrease. Retinyl esters can be degraded by aldehyde dehydrogenase (ALDH) enzymes into retinoic acid. Throughout a healthy liver, the HSC population displays heterogeneity based on their ALDH activity and size, expression of markers (collagens, desmin, αSMA), location, and in their capacity for retinoid storage. It is not yet entirely clear why HSCs lose retinol during activation, which enzymes are involved in this process and what role the retinol metabolites precisely play in HSCs upon liver injury.

**Aim:** Determine whether ALDH enzymes are involved in the HSC activation process by regulating the degradation of retinyl esters.

**Methods:** We isolated HSCs either from wild type or CCl4-injured livers and is upregulated in end-stage human liver diseases. aLMFs have higher levels of Fn14 mRNA than HSCs. bα-SMA and Vimentin. These findings are confirmed in primary HSCs with various cytokines including TGF-β.

**Results:** Relative levels of Fn14 and α-SMA in liver myofibroblasts (αLMFs) were assessed for Fn14 and α-SMA expression by qPCR and immunofluorescence. Expression of collagen I mRNA and protein, and alpha-smooth muscle actin protein were significantly reduced in GR-MD-02 and GM-CT-01 treated rats compared to vehicle-treated control rats. While AST and ALT were not reduced by treatment with GR-MD-02, treatment with GM-CT-01 resulted in a normalization of AST/ALT levels.

**Conclusions:** Treatment with two galectin inhibitors with different chemical compositions, but having common structural elements, significantly reduced fibrosis and reversed cirrhosis in a toxic model of liver fibrosis, even while continuing TAA, which were associated with reduced portal hypertension.

### 1138

**THE FN14/TWEAK RECEPTOR-LIGAND SYSTEM REGULATES HEPATIC STELLATE CELL FUNCTION IN LIVER FIBROSIS**

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**Background:** Fibrosis represents a common pathway of most chronic liver diseases. Hepatic stellate cells (HSCs) are the key contributors to liver fibrosis. Tumour necrosis factor-like weak inducer of apoptosis (TWEAK) and its cognate receptor fibroblast growth factor-inducible molecule 14 (Fn14) have been implicated in liver fibrosis. In other organ systems TWEAK is involved in various cellular processes including proliferation, apoptosis, and inflammation. In murine models Fn14 is expressed at low levels in normal livers but is upregulated during injury and carcinogenesis. Fn14 knock-out mice have been shown to have less liver fibrosis. To date it is unknown whether human HSCs express Fn14 and how such expression is regulated.

**Aims:** To assess the expression of Fn14 and TWEAK in normal and diseased human livers and to investigate the regulation of Fn14 on primary HSCs in vitro.

**Methods:** Tissue was obtained using explanted specimens from the liver transplant programme based at the Queen Elizabeth Hospital, Birmingham, UK with ethical approval and patient consent. Whole human liver samples, isolated primary HSCs and tissue was obtained using explanted specimens from the liver transplant programme based at the Queen Elizabeth Hospital, Birmingham, UK with ethical approval and patient consent. Whole human liver samples, isolated primary HSCs and healthy liver is heterogeneous based on their ALDH activity and inhibition of this activity hampers activation of HSCs in vitro and in vivo.}

**Conclusions:** Our results suggest that the HSC population in a healthy liver is heterogeneous based on their ALDH activity and inhibition of this activity hampers activation of HSCs in vitro and in vivo.
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DOES HEPATITIS B AND HEPATITIS C CO-INFECTION INCREASE THE RISK OF DRUG INDUCED HEPATITIS DURING ANTI TUBERCULOUS CHEMOTHERAPY?

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Aims and Objectives: We evaluated whether HBV +ve and HCV +ve patients are at high risk for developing drug induced hepatitis than control subjects during treatment for tuberculosis with standard short course regimens.

Study design: Observational cohort study.

Material and Methods: All newly diagnosed active tuberculosis patients were included in the study population and they were further screened for hepatitis B surface antigen and HCV antibodies. All patients were divided into three groups. One having no co-infection with hepatitis B and Hepatitis C and was taken as control group, second group was co-infected with hepatitis B and third was co-infected with hepatitis C. Short course anti tuberculous regimen was started and patients were followed for six months.

Results: One hundred and twenty eight tuberculous patients were divided into three groups. 92 in control groups without any co-infection with hepatitis B and C, 10 were HBV +ve and 26 were HCV +ve. During follow up 24 developed drug induced hepatitis, 8 (38.33%, n=24) in control group, 2 (8.33%, n=24) in hepatitis B group and 14 (58.33%, n=24) in hepatitis C group.

Conclusion: These findings suggest that treatment for tuberculosis in HCV seropositive patients is a risk factor for the development of hepatitis exacerbation and HBV seropositive patients shows no any increased risk of hepatitis exacerbation.

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HEPATITIS C VIRUS AND LIPID DROPLETS: ROLE OF ADIPOSE DIFFERENTIATION-RELATED PROTEIN IN LIPID DROPLET MORPHOLOGY AND VIRAL LIFE CYCLE

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Background and Aims: Hepatitis C virus (HCV) is a positive-strand RNA virus of the Flaviviridae family, whose life cycle is tightly associated with lipid metabolism. HCV assembly and maturation start at the surface of lipid droplets, while viral egress depends on very-low density lipoprotein secretion. In order to better understand the relationship between HCV and lipid metabolism, we analyzed the impact of lipid droplets on HCV life cycle with a particular focus on Adipose Differentiation-Related Protein (ADRP), a lipid droplet-associated protein.

Methods: We transduced human hepatoma cells (Huh-7) with a lentiviral vector expressing ADRP and monitored the impact of ADRP overexpression on

i. lipid droplet morphology and
ii. HCV life cycle and viral particle production in the setting of infection with a cell cultured-derived HCV (full length Jc1 construct).

To assess the effect of ADRP on HCV entry, HCV receptors (i.e. CD81, Low-Density Lipoprotein Receptor, Scavenger receptor class B member 1, Claudin, Occludin, Niemann-Pick disease type C1) expression were assessed by quantitative RT-PCR.

Results: ADRP mRNA expression level was increased by 2-fold during the course of Jc1 infection. The lentiviral-mediated overexpression of ADRP induced the appearance of large lipid droplets, and this modification in lipid droplet morphology was accompanied by an increase of main lipid droplet components (1.5- and 3.5-fold increase of triglycerides and cholesterol esters respectively). HCV secretion of infectious particles – but not HCV replication – was significantly increased by ADRP overexpression (extracellular HCV RNA level and infectivity were respectively increased by 2-fold and 5-fold). Interestingly, ADRP overexpression likewise enhanced occludin mRNA level by approximately 3-fold.

Conclusion: These findings suggest that ADRP is a critical factor for HCV life cycle.

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IDENTIFICATION OF LONG NON-CODING RNAs INDUCED BY INTERFERON

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Long non-coding RNAs (lncRNAs) play important roles in cell proliferation and differentiation. However, few studies have analyzed their role in cell homeostasis or in cell response to extracellular factors. Interferon (IFN) a in a potent cellular stimuli against viral infection and has a crucial role in the treatment of HCV chronic infection. Many protein coding genes have been described to mediate IFN response, however the relevance of non-coding RNAs in the response to IFN is unknown. To determine whether lncRNAs could mediate IFN functionality, we have first analyzed the transcriptome of Huh7.5 controls or IFN-treated cells by Sure Print microarray and RNASeq. Analysis of the microarray showed altered expression with high statistical significance of 845 probes, 90% of which were up-regulated and represent the well-characterized transcription activation pattern of IFN. Similar results were obtained after analysis of RNASeq. We have analyzed the genes annotated as lncRNAs in the array. In contrast to coding genes, 40% of the probes were down-regulated, suggesting an unexpected role of the IFN response in transcription inhibition. We have validated 12 of the 18 lncRNAs up-regulated annotated in Ensembl. The results show different kinetics of activation ranging from 2 to more than 100 fold. Most of these IncRNAs respond to IFN in all cell-lines tested and accumulate preferentially in the nucleus. However, some of them are mostly in the cytoplasm or move to the cytoplasm after IFN treatment. Most respond to IFN a, b and l, but interestingly one seems to be more sensitive to IFN l, a type III IFN. Thus, differences in IncRNA expression could result in the different strength and kinetics of response observed for type I or type III IFNs. Finally we also determined whether some of the IncRNAs upregulated by IFN are also induced after HCV infection. The results show that eight out of eighteen IncRNAs were also upregulated in cells infected with HCV. Further studies will be required to understand the role of these IncRNAs both in IFN response and HCV infection.
RIBAVIRIN IS A TLR7 AGONIST AND ITS ANTIVIRAL EFFECT ON HCV IS AT LEAST PARTLY RELATED TO THE INDUCTION OF IRF7 EXPRESSION

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Background: In chronic hepatitis C treatment, ribavirin significantly enhances the clinical effect of IFN. With DAA, ribavirin also improves the rates of SVR in both IFN-containing and IFN-free regimens. The main effect of ribavirin is to prevent virological breakthroughs and relapses. Although multiple mechanisms of action have been suggested for ribavirin, the main antiviral mechanism has not yet been clearly elucidated.

Objective: To understand the molecular mechanism of ribavirin antiviral action in HCV infection.

Methods and Results: Gene expression analysis of uninfected Huh7 treated with ribavirin for 24 hours showed that the expression of genes implicated in IFN responses, including TLR7, IRF7 and IRF9, was induced by ribavirin administration. Using the subgenomic replicon Con-1 (SGR) in Huh7.5 cells, ribavirin exerted dose-dependent antiviral activity against HCV. To investigate how ribavirin affects gene transcription in the context of viral infection, we first investigated whether the promoter of IRF7 gene expression is modulated by ribavirin. Hepatoma cells expressing the SGR and one or both functional interferon-sensitive response elements from different reporter plasmids were treated with different doses of ribavirin, alone and in combination with IFN. Ribavirin activated the IRF7 promoter via IRF-E in a dose-dependent manner. In contrast to IFN, ribavirin had no effect on ISRE. In addition, shRNA-mediated suppression of IRF7 resulted in a significant reduction of HCV replication inhibition. Although IRF7 may be of particular importance because of its role in amplifying the IFN signaling cascade, IFN production is initiated by the recognition of TLR, one of the two best-known pattern-recognition receptors. Ribavirin had an effect on TLR7 mRNA expression and, using cells expressing human TLR and an inducible reporter gene, ribavirin selectively activated TLR7 in a dose-dependent manner. In addition, co-incubation of imiquimod or loxoribin with ribavirin significantly increased activity of both compounds on TLR-7 expressing HEK cells. TLR7 stimulation by ribavirin induced cytokine secretion, including type I interferon in hepatoma cells.

Conclusion: Ribavirin is a selective TLR-7 agonist, leading to increased type I interferon production through promoting the activation of IRF-7, a key factor because of its direct antiviral effect and its role in amplifying the IFN signaling cascade.

INHIBITION OF THE INSULIN-MEDIATED AS160 ACTIVATION IS AN IMPORTANT EVENT LEADING TO INCREASED HCV EGRESS

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Background and Aims: Infection by the hepatitis C virus (HCV) induces insulin resistance. We recently found that insulin activation of AS160, the main PKB target involved in Glut4 translocation, is specifically inhibited in uninfected cells (myocytes, adipocytes and liver cells) cocultured with HCV-infected cells, leading to impaired glucose uptake and substantially contributing to insulin resistance. Nevertheless, this finding does not bring any clue about the benefits for HCV to induce such disorders in cells. Therefore, we decided to challenge the potential role of AS160 in HCV life cycle. In addition, as activation/inactivation cycle of AS160 is regulated by insulin, we also tested the ability of insulin to impact HCV life cycle.

Methods: The effect of silencing or overexpression of AS160, as well as of a physiological concentration of insulin (1 nM) on HCV secretion, was assessed in Huh-7 cells expressing the genomic-length Jc1 construct. HCV replication was assessed using the subgenomic replicon pFK_i389LucNS3–3′JFH1.

Results: Insulin treatment for 6 or 16 hours leads to a 50% inhibition of HCV secretion, while unafflicting the absolute number of HCV genomes secreted. While the silencing of AS160 increased the number of infectious particles secreted (measured as TCID50/ml) by more than 1 log in a dose dependent manner, it induced only a four-fold increase of viral particle number (measured as secreted Jc1 HCV RNA) and of HCV replication (measured as luciferase activity after transfection with the subgenomic replicon). In a mirror experiment, overexpression of AS160 significantly reduced HCV virion secretion.

Conclusion: These results suggest that, in HCV infection, inhibition of insulin-mediated AS160 activation may lead to an increased infectious viral particle production, suggesting that HCV may benefit from the insulin resistant state.

ROLE OF EMT SIGNALING IN THE EARLY STEPS OF HCV INFECTION

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Background and Aims: Hepatitis C virus (HCV) is an important blood-borne pathogen, which can cause severe liver diseases including fibrosis, cirrhosis and hepatocellular carcinoma. The hepatocyte is its primary cellular target, but the C virus is also able to infect B lymphocytes and dendritic cells. HCV entry into target cells occurs through a complex mechanism, although still debated and not fully understood, involving several host cell surface molecules and structural HCV proteins. It’s well known that viral proteins can alter the typical epithelial architecture of hepatic cells inducing epithelial-to-mesenchymal transition (EMT) promoting increased motility and invasiveness. The HCV-related EMT involves the modulation of several pathways such those governed by Sonic hedgehog (SHH), recently associated with HCV replication, and Tumor Growth Factor (TGF) beta signaling. The aim of the study is to identify the possible role of EMT pathways during the early phases of HCV infection.

Materials and Methods: To perform our investigation we used J6/JFH1 HCV strain to infect Huh 7.5, human hepatocarcinoma cell line. At different time point after the infection we have analyzed the EMT signaling through Western blot analysis, immunofluorescence staining and RT-qPCR. The same experiments were performed after EMT chemical inhibition.

Results: Our results highlight a marked up-regulation of EMT signaling effectors during the early steps of infection. In particular SHH and its effectors, Gli1 and Gli2, were significantly overexpressed at 4 and 8 hours after infection. Similar results were obtained analyzing TGF beta and its effectors, such as Snail and Twist. To analyze their role in HCV infection, we have evaluated the presence of HCV (5′UTR) in the early steps of infection after a treatment with a chemical inhibitor of EMT. Interestingly chemical EMT inhibition causes a strong reduction of HCV presence into the hepatocytes.

Conclusions: Our study confirms the involvement of SHH and TGF beta pathways during the early steps of HCV infection. Using an
EMT chemical inhibitor we have obtained a significant reduction of the presence of HCV inside the hepatocytes. In light of our results, affecting EMT pathways it’s possible to counteract the early steps of infection suggesting novel and effective anti-HCV therapies.

1145
PRE-TREATMENT PREDICTION OF TELAPREVIR RESPONSE USING A NOVEL CAPTURE-FUSION ASSAY FOR HCV REPLICATION
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Background: Telaprevir enhances treatment response in genotype (G)1 HCV, but cure rates remain low amongst patients with prior treatment null response. G3 HCV is insensitive to telaprevir but a subset of patients (~30%) may respond. We have developed a novel capture-fusion assay to study patient-derived HCV. Here we examined whether this assay can identify pre-treatment telaprevir sensitivity in patients with G1 and G3 HCV.

Methods: Pre-treatment serum samples (supplied blinded to virological response) were obtained from a published trial of eight G1 patients treated with telaprevir monotherapy. Pre-treatment and post-breakthrough samples were obtained from three G1 patients (previous ‘null responders’) who failed retreatment with telaprevir, post-breakthrough samples were obtained from three G1 patients who treated with telaprevir monotherapy. Pre-treatment and post-transplantation measures are needed to prevent graft infection. One potential strategy to prevent such infection is the administration of potent broadly neutralizing antibodies (bnAbs).

Here, we tested whether three previously published [Giang et al. PNAS (2012) 109(16):6205–10.] antibodies (AR3A, AR3B and AR4A), either administered as passive immunotherapy or as vector-mediated gene transfer, could prevent HCV infection and/or interfere with established infection. In NOD-Rag1−/−IL2rγ−− (NRG) mice a single intramuscular injection with a recombinant adeno-associated vector (rAAV) lead to stable serum expression of human antibodies in the range of 10–1000 ug/ml for more than 100 days.

In genetically humanized mice, rAAV expression of one or all three bnAbs was able to prevent HCV entry. To test the effects of bnAbs on established HCV infection, we inoculated HCV genotype 1a infected human liver chimeric FAH−/−NOD-Rag1−/−IL2rγ−− (FNRG) mice with AR3A, AR3B and AR4A-expressing rAAVs. In a separate experiment viremic FNRG mice were given the three rAAVs in combination with high doses of the three antibodies. Whereas ongoing viremia remained stable for 3 months after administration of the three rAAVs, the combination of passive immunotherapy and rAAVs lead to a decrease in viremia over 5 weeks, close to the limit of detection. The virus from mice that received only rAAVs associated vector (rAAV) led to stable serum expression of human antibodies in the range of 10–1000 ug/ml for more than 100 days.

To determine whether escape variants are present in these sera we are currently testing the ability of the three antibodies to sterilize serum from mice infected with cell culture HCV.
Our results suggest that successful translation of this approach to humans could hold promise as one strategy to protect the liver graft from becoming infected with HCV.

**1147 EFFECT OF GENETIC POLYMORPHISMS IN THE CD81 GENE ON HEPATITIS C VIRUS CELL ENTRY AND NEUTRALIZATION WITH ANTI-CD81 ANTIBODIES**

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**Background:** The tetraspanin CD81 is an essential host factor for the HCV cell entry. Moreover it has been shown that it contributes to HCV host range. Antibodies directed against CD81 are being developed as a therapeutic approach for HCV infection. In genome sequencing projects different single nucleotide polymorphisms (SNP) have been described in the CD81 gene. Here we investigated whether seven coding non-synonymous SNPs that exist with low frequency in human populations have an influence on the cellular permissiveness to HCV infection or the inhibitory activity of different CD81 antibodies.

**Methods:** Using a cell line with low endogenous levels of CD81, we first generated cell lines expressing different SNP variants of human CD81 by lentiviral gene transfer. Expression of endogenous and exogenous CD81 was monitored by FACS. Permissiveness to HCV infection was assessed using state of the art in vitro assays probing all steps of the viral replication cycle, i.e. cell entry, RNA replication and assembly and release of infectious particles.

**Results:** In direct infection experiments using cell culture grown HCV, cells expressing any of the seven CD81 variants can be infected, however one SNP located in exon 7 appears to be associated with lower permissiveness for the virus. Whether this variant also affects other replication cycle steps, most notably viral replication, is currently under investigation. The anti-CD81 antibodies JS81, 1.3.3.22 and 5A6 recognize the CD81 variants equally and are able to prevent HCV cell entry with similar efficiency compared to the presence of wildtype CD81.

**Conclusion:** One out of seven coding non-synonymous SNP’s in the CD81 gene appears to be associated with a less efficient entry of HCV into its target cells. The variants do not impede the ability of CD81 antibodies to block infection.

**1148 EFFECT OF THE IL28B SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ON THE FIRST PHASE HEPATITIS C VIRUS KINETICS IN PATIENTS TREATED WITH PEG-INFERENCE AND RIBAVIRIN (PR)**

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**Background:** The rs12979860 SNP upstream to the IL28B gene is a strong predictor of sustained virological response in chronic hepatitis C patients treated with PR. The actual mechanism(s) through which this SNP influences treatment is unclear. The on-treatment kinetics of serum HCV-RNA follows a bi-phasic decline, where the first phase, encompassing the initial 24–72 hours, is a measure of interferon sensitivity. We assessed whether IL28B polymorphism affected the first phase viral decline.

**Methods:** Patients with liver biopsy-proven, HCV-RNA+, chronic active hepatitis C undergone antiviral treatment with PR between 2007 and 2011 were studied. IL28B status was analysed by means of a commercial real-time PCR assay (Roche diagnostics). HCV-RNA quantitation was performed by Cobas Taqmian 5 minutes before and 48 to 72 hours after treatment start.

**Results:** 118 patients were studied (median age 56 yrs, males 58%, cirrhosis 24%). 69 patients (59%) harboured HCV genotype 1, 37 (31%) genotype 2, 12 (10%) genotype 3. IL28B genotype distribution was: CC 37 (31.5%); CT 63 (53.5%); TT 18 (15%) patients. The prevalence of viral genotypes did not differ across IL28B genotype groups. TT carriers had higher median fibrosis score (3 vs 2 in CC or CT) but similar necroinflammation. Baseline viremia (median, log_{10} IU/ml) was 6.22 in CC, 6.06 in CT and 5.98 in TT (p<0.02). The first phase viral decline was significantly higher in CC than either CT or TT patients (2.48 vs 1.56 and 1.21 log_{10} IU/ml, respectively; p<0.0001). It was lowest in genotype 1-CTT (0.47) and highest in genotype 2-3-CC (2.73 log_{10} IU/ml) subjects. Rates of RVR and SVR were as follows: CC, 70% and 84%; CT, 36% and 46%; TT, 44% and 50%. Univariate predictors of first phase HCV-RNA decline were: HCV genotype, IL28B grading, ALTs and GGT. In a linear regression model including these variables, HCV genotype and IL28B were independently related to the first phase HCV-RNA kinetics.

**Conclusions:** The first phase kinetics of HCV-RNA decline on PR treatment is an accurate measure of interferon sensitivity and is strictly related to both IL28B polymorphism and viral genotype. Its measurement might prove useful to correctly tailor antiviral treatment.

**1149 REDUCTION OF mir-122 EXPRESSION IN IL28B CT/TT CHRONIC HEPATITIS C PATIENTS WHO FAILED TO PEGYLATED-INFERENCE AND RIBAVIRIN TREATMENT**

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**Background and Aims:** Mir-122 is highly expressed, in the liver, where it represents 70% of the total miRNAs. Mir-122 binding within hepatitis C virus (HCV) genome stimulates its replication, in vitro. Reduction of mir-122 expression has been associated with non-response (NR) in patients with chronic hepatitis C. Genome wide association studies identified a strong association between IL28B polymorphisms and sustained virological response (SVR), in patients with CHC. The aim of the study was to investigate, in vivo, the association of mir-122 expression and IL28B polymorphism with SVR.

**Methods:** Pre-treatment liver biopsies and serums from 110 patients with CHC were included. Fifty six patients achieved a SVR, and 54 failed to respond to the treatment. 36 were NRs and 18 were responder-relapsers (RR). 56 were complete early virological responders (cEVR) and 36 were primary non-responders (pNR). Hepatic mir-122 expression was assessed by RT-q-PCR. IL28B rs12979860 polymorphism was analyzed by direct sequencing.

**Results:** IL28B CC genotype was associated with an increase of mir-122 expression, in the total population (p=0.008) and in NRs (p=0.001), at baseline. For IL28B CT/TT genotypes, the response was associated to an increase of mir122 (p=0.027) whereas for CC genotype, the response was not associated to mir-122 levels (p=0.90). Mir-122 expression was reduced in pNR compared to
cEVR (p = 0.003). We found no relationship with the expression of mir-122 for total cholesterol, triglycerides and HCV viral load. Mir-122 expression showed a 50% reduction in HCV infected patients and is not modified at the different stages of fibrosis.

Conclusions: Patients IL28B CT/TT who failed to PEG-IFN plus ribavirin presented a reduction of mir-122 expression. Whereas, in vitro mir-122 stimulates HCV replication, there was no correlation between viral load and hepatic mir-122 expression. The stage of the disease was not associated with a modification of mir-122 expression. Altogether, these results suggest that the level of expression of mir-122 needs to be maintained to regulate its different activity.

1150 HEPATITIS C VIRUS LIVER TRANSPLANTATION ESCAPE VARIANT IS CHARACTERIZED BY BOTH ENHANCED TRIGLYCERIDE-RICH LIPOPROTEIN ASSOCIATION AND SENSITIVITY TO apoE ANTIbODIES

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A unique feature of hepatitis C virus (HCV) is that virions circulate in plasma associated with host triglyceride-rich lipoproteins (TRL), though the functional impact of this association remains undefined. Virus/lipoprotein complex formation may facilitate HCV escape from host neutralizing antibodies by concealing epitopes, although the mechanisms are unknown. We previously demonstrated that escape from antibody-mediated neutralization is a key factor for viral persistence in acute liver graft infection and chronic infection (Faï-Kremer et al. J Exp Med 2010; Fofana et al. Gastroenterology 2012). Aiming to address the role of HCV-TRL associations for viral evasion, we investigated the role of lipoprotein association using an HCVcc chimera expressing the structural proteins of a well characterized post-transplantation escape variant (VL). Fractionation of this virus by iodixanol density gradients and assaying by limiting dilution assay revealed two distinct subpopulations with more than half of infectious virions in densities below 1.05 g/mL. This density profile, which is unique compared to both Jc1 and JFH1, was further investigated for differential sensitivity to neutralizing antibodies from patient serum. Remarkably, the low-density subpopulation was capable of escape, while the high-density subpopulation was highly sensitive to neutralization. Introduction of residue exchange F447L, a mutation identified from a highly neutralized pre-transplant serum. Remarkably, the low-density subpopulation was capable of neutralizing the VL variant than the F447L variant, suggesting a functional role for apoE in the observed phenotype. These findings demonstrate that HCV-TRL associations contribute to viral evasion in acute liver graft infection and identify a potential genetic element within HCV envelope glycoprotein E2 that contributes to lipoprotein association. Taken together, these findings are highly relevant for the development of strategies to prevent liver graft infection and prophylactic B cell vaccines.

*DJF was supported by the EASL Dame Sheila Sherlock Postdoctoral Fellowship.
claudin-1 monoclonal antibodies (mAbs) inhibit HCV infection of primary human hepatocytes (PHHs). However, the physiological relevance of claudin-6 and claudin-9 in HCV entry and infection of primary liver cells remains unknown.

**Methods:** Using genetic immunization, we produced claudin-6 and -9 specific mAbs in order to explore the role of these claudins as functional HCV receptors in PHHs. The effects of antibodies were analyzed in a non-HCV-permissive 293T cells engineered to express exogenously claudin-1, -6 or -9. Huh7.5.1 cells and PHHs.

**Results:** Expression of exogenous human claudin-6 or claudin-9 in 293T cells conferred HCV pseudoparticle (HCVpp) entry that was inhibited by our new mAbs, demonstrating their functional activity. In contrast to claudin-1 specific mAbs, neither claudin-6 nor claudin-9 specific mAbs bound PHHs or inhibited HCVpp entry of different genotypes into Huh7.5.1 cells or PHHs. These data are consistent with the limited or absent detection of claudin-6 or claudin-9 mRNA in normal or inflamed human liver tissue.

**Conclusions:** Claudin-6 and 9 can serve as HCV entry factor in HCV permissive transformed cell lines (over)expressing these molecules. In contrast, cell surface expression of claudin-6 and 9 in PHH is low or absent and HCV entry into PHH is dependent on claudin-1. Functional studies suggest that claudin-1 is the key entry factor in PHH. These findings are relevant for the understanding of claudins as HCV entry factors and development of claudin-specific entry inhibitors for prevention of liver graft infection and antiviral resistance.

**1153 CYCLOPHILIN INHIBITOR ALISPORIVIR (ALV) COMBINATIONS WITH DIRECT ACTING ANTIVIRALS REVEAL STRONG SYNERGISTIC ANTI-HCV EFFECTS**

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**Background and Aims:** Two classes of HCV antivirals exist: direct-acting antivirals (DAAs) and host-targeting antivirals (HTAs). DAAs target viral proteins including the protease NS3, the polymerase NS5B and NS5A, while HTAs target various host proteins critical for HCV replication. Both classes of antivirals dramatically decrease HCV viremia in infected patients. In this study, we investigated whether specific DAAs exhibit synergistic, additive or antagonistic effects with the HTA ALV, which targets cyclophilin A, a host factor vital for HCV replication.

**Methods:** We established a Huh 7.5.1 cell line expressing the HCV Con1 replicon encoding an NS5A-YFP fusion protein. This cell line was used as part of a novel, reproducible, sensitive and quantitative FAC-based assay to conduct combination drug studies of ALV with the protease inhibitor boceprevir, polymerase inhibitors mericitabine (R7128) and sofosbuvir (PSI-7977) and NS5A inhibitors daclatasvir (BMS-790052) and EDP-239.

**Results:** We found that ALV-boceprevir combination has an additive effect in inhibiting HCV replication. In contrast, the combinations of ALV with NS5B polymerase inhibitors (mericitabine or sofosbuvir) or with NS5A inhibitors (daclatasvir or EDP-239) exhibit remarkable antiviral synergistic effects. Combining ALV with the NS5A inhibitors inhibit HCV replication more efficiently than combining ALV with the polymerase inhibitors. Importantly, we obtained similar results with genotypes 2a and 3. ALV was shown to block the contact between cyclophilin A and the domain II of NS5A, and NS5A inhibitors were shown to target the domain I of NS5A. Thus, our present data strongly suggest this is a molecular basis for the use of two classes of inhibitors, which act on two distinct domains of NS5A. Similar studies with other genotypes are ongoing to determine whether the ALV-NS5A inhibitor highest synergistic effect is pan-genotypic.

**Conclusions:** We showed for the first time that the combination of cyclophilin inhibitors with specific classes of DAAs provides strong synergistic effects against genotypes 1b, 2a and 3. Interestingly, the combination of the cyclophilin inhibitor ALV and NS5A inhibitors provides the highest synergistic antiviral effect and represents an attractive strategy to block effectively HCV replication.

**1154 ROLE OF PROTEIN TYROSINE-PHOSPHATASE 1B (PTP1B) ACTIVITY IN HCV-INDUCED HEPATOCELLULAR CARCINOMA: AN IN VITRO ANALYSIS**

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**Background and Aims:** Several studies indicated that PTP1B, an insulin pathway inhibitor, is involved in different types of cancer. Studies of spontaneous mammary carcinomas in mice have demonstrated a potentially critical role for PTP1B in metastatic progression. Hepatitis C infection is one of the main risk factors for hepatocellular carcinoma (HCC) development. A putative role for PTP1B in HCC has been recently pointed out. We aim to analyze PTP1B abundance and enzyme activity using an in vitro model of HCV infection and further HCC progression.

**Methods:** Huh7.5 (IL28B rs12979860-CT genotype) and Huh7 (CC) cells were grown and infected with the JFH1 replicon (1 particle/cell). Metformina (2mM) were added to the cells. Total RNA and protein extraction were performed 24 or 48 hours after the media was changed. The expression of the different genes was quantified using the qRT-PCR Quantace (Bioline) kit and analysis of specific proteins by Western-Blot. Tyrosine phosphate activity was quantified by Tyrosine phosphate assay system (Promega Biotech Ibérica, SL Branch Office, Madrid, Spain).

**Results:** PTP1B protein abundance was increased after 48 hours post-infection in Huh7.5 cells, although was decreased in the early step of infection (24 hours). PTP1N gene expression was not increased in Huh7.5 (IL28B CT genotype), but did in Huh7 (CC genotype) (9.1±1.2 fold). PTP1B gene expression was down-regulated in Huh7 infected and treated with metformin (4.7±0.7 fold). Total tyrosine phosphate activity was found strongly inhibited in both cells lines (Huh7 and Huh7.5 cells) both in JFH1 infected and JFH-1treated with metformin (1.5±0.1). Metformin (2mM) had cytostatic effect on Huh7.5 cells.

**Conclusions:** PTP1B is a critical protein involved in several types of cancer including HCC. Tyrosine phosphate activity was modified by HCV infection. This effect was observed in gene expression (in an IL28B polymorphism-dependent manner), protein expression and enzyme activity. These evidences suggest that the beneficial effect of metformin could be linked to the down-regulation of PTP1B gene expression and modulation of protein activity.

**1155 PRIMARY HUMAN HEPATOCYTES AND CLINICAL STRAINS OF HEPATITIS C VIRUS: A HIGHLY RELEVANT MODEL FOR ANTIVIRAL DRUG DEVELOPMENT**


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**Background and Aims:** So far, drug development against hepatitis C virus (HCV) was hampered by the lack of physiologically relevant in vitro models. Adult primary human hepatocytes (PHH) are
sensitive to HCV-clinical strains infection (HCVser), support the complete replication cycle, and remain the cell-culture system that most closely mimics the in vivo situation. Nevertheless, the model is limited, mainly because few sera give measurable level of intracellular HCV RNA. These considerations prompted us to study the relationship between host cell phenotype in culture, characteristics of viral isolates and serum infectivity. Our aim was then to establish a well-defined sera collection allowing studies of HCV infection in its natural host cell and evaluation of anti-HCV compounds.

Methods: From kinetic experiments, we defined the optimal conditions to infect PHH. A standard test allowed classifying ~100 sera in 3 groups, based on their infectivity toward PHH. Sera were then carefully characterized (clinical profile of patient, viral load, genotype, and cytokine/growth factor content).

Results: PHH support HCVser replication for at least two weeks with high level of RNA copies per cell and de novo virus production. We found that 12% of the sera tested are highly infectious (HI; more than 5x10^4 HCV RNA copies/μg of total RNA), 68% are poorly infectious (PI; less than 1.3x10^4 copies/μg) and 20% are intermediate (Inter). Infectivity toward PHH cannot be predicted from clinical characteristics of the serum donors, HCV viral load or genotype. The HI sera have a cytokine profile that clearly distinguishes them from other groups, with low levels of the majority of the 52 analytes tested, including cytokines involved in the regulation of immune responses and inflammatory reactions. Finally, the activity of antiviral compounds was evaluated against different clinical isolates.

Conclusion: We defined optimal conditions to successfully infect PHH with HCVser and showed that this highly relevant model can be useful for understanding the mechanism of HCV infection, for selection of new HCV clones able to replicate in hepatoma cell lines and to determine the potency of new antiviral drugs toward various HCV strains.

1156 THE RIBOSOMAL PROTEIN RACK1 IS A SPECIFIC HOST FACTOR REQUIRED FOR IRES-MEDIATED TRANSLATION OF HEPATITIS C VIRUS
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Background: Treatment of chronic viral infection is challenged by variability of viral targets and development of resistance. Viruses depend on host factors for their life cycle, which are attractive alternative antiviral targets, provided that they are not mandatory for normal cell functions. Using a functional proteomic screen, we recently identified Receptor for Activated C Kinase 1 (RACK1) as a specific host factor required for replication of internal ribosome entry site (IRES)-containing viruses such as Drosophila C virus (DCV).

Methods: Using state-of-the-art cell culture models for HCV infection, replication and translation, we investigated the functional impact of RACK1 as a host factor for HCV infection.

Results: Silencing of RACK1 expression in Huh 7.5.1 cells resulted in a marked, specific and significant decrease in HCV Jc1 infection and infectious virion production. A similar effect was obtained when RACK1 expression was silenced in HCV replicating cells, demonstrating a crucial role of this host factor in HCV replication. In contrast, infection of non IRES-translated viruses such as adenovirus or vesicular stomatitis virus remained unchanged in RACK1 silenced cells. In order to discriminate between the translation and the replication steps of the HCV life cycle, we established stable cell lines expressing either an IRES<sub>HCVRNA</sub>-luciferase reporter or a classical capped luciferase reporter, respectively. Silencing of RACK1 markedly and exclusively decreased IRES<sub>HCVRNA</sub>-dependent translation, but not classical cap-mediated translation, demonstrating that RACK1 is specifically required for IRES-mediated translation of HCV.

Conclusions: Collectively, our results demonstrate that RACK1, a component of the ribosome, is a specific host factor for IRES-dependent HCV translation. Our data conceptually advance the understanding of viral translation and reveal a novel host target for the development of antivirals addressing resistance.

1157 EARLY HCV RNA DYNAMICS AND FACTORS ASSOCIATED WITH HIGH EARLY HCV RNA LEVEL DURING ACUTE HCV INFECTION
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Background and Aims: Viral dynamics during acute HCV infection can provide insights into immunopathogenesis. We aimed to assess early HCV RNA dynamics, including factors associated with high early HCV RNA level and impact on spontaneous clearance.

Methods: Data were drawn from an international collaboration of nine prospective cohorts evaluating HCV infection risk and outcomes among people who inject drugs (Inc3 Study). Individuals with incident HCV were identified (seroconversion within two years or symptomatic infection with seroconversion illness) and HCV RNA dynamics during acute infection evaluated. Factors associated with high early HCV RNA level (i.e. ≥6 log IU/mL, one month post-infection) were assessed and impact on spontaneous clearance evaluated using logistic regression analyses.

Results: Overall, 669 participants with incident HCV were included (35% females, mean age 29 years, 49% rs12979860 IL28B CC genotype). Peak median HCV RNA levels occurred one month following infection in those with spontaneous clearance (6.0 log IU/mL, IQR=4.4–7.2) and persistence infection (5.4 log IU/mL, IQR=4.5–6.4; P=0.091), followed by declines between months one and three of 4.2 and 0.8 log IU/mL, respectively (Figure 1A). In multivariate logistic regression among those with HCV RNA levels one month following infection (n=189), IL28B CC genotype (vs. TT/CT, adjusted odds ratio (AOR)=3.55; 95% CI=1.67–7.56; P=0.001) was associated with high early HCV RNA level. Age, sex and HCV genotype were not associated in adjusted analyses, but a significant interaction between IL28B genotype and sex was observed (P=0.043). Among females, IL28B CC genotype was strongly associated with high early HCV RNA level (AOR=6.68; 95% CI=1.73–25.73; P=0.006), but among males, only marginally associated (AOR=2.53; 95% CI=1.00–6.40; P=0.050). After adjusting for age, sex, IL28B genotype and HCV genotype, high early HCV RNA
level was not associated with subsequent spontaneous clearance (AOR = 1.33; 95% CI=0.48–3.69; \( P = 0.583 \)).

**Conclusion:** HCV RNA level decline was observed between one and months following infection, with continued decline among those with spontaneous clearance. IL28B genotype influenced early HCV dynamics, particularly among women, suggesting gender-specific HCV innate immune responses.

**Figure:** Monthly medians of HCVRNA levels in acute HCV.

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**HEPATITIS C VIRUS CORE PROTEIN BASIC AMINO ACID REGION 1 IS RESPONSIBLE FOR THE IMPAIRMENT OF IRF-3 ACTIVATION**

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**Aim:** Hepatitis C virus (HCV) causes persistent disease in infected individuals. The innate immune system is activated immediately upon infection as the first line of host defense against invading pathogens, with type I interferon (IFN) signaling being the crucial step in the antiviral response. The IFN system is a prime target of HCV for persistent infections. IRF-3, a key transcriptional factor in the type I interferon system, is frequently impaired by HCV, in order to establish persistent infection. However, the exact mechanism by which the virus establishes persistent infection has not been fully understood yet. The present study aimed to investigate the effects of various HCV proteins on IRF-3 activation, and elucidate the underlying mechanisms.

**Methods:** To achieve this, full-length HCV and HCV subgenomic constructs corresponding to structural and each of the nonstructural proteins were transiently transfected into HepG2 cells. IFN-\( \beta \)-induction, plaque formation, and IRF-3 dimerization were elicited by Newcastle disease virus (NDV) infection. The expressions of IRF-3 homodimer and its monomer, Ser386-phosphorylated IRF-3, and HCV core protein were detected by immunofluorescence and western blotting. IFN-\( \beta \) mRNA expression was quantified by real-time PCR (RT-PCR), and IRF-3 activity was measured by the levels of IRF-3 dimerization and phosphorylation, induced by NDV infection or polyriboinosinic: polyribocytidylic acid [poly(I:C)]. Switching of the expression of the complete HCV genome as well as the core proteins, E1, E2, and NS2, suppressed IFN-\( \beta \) mRNA levels and IRF-3 dimerization, induced by NDV infection.

**Results:** Our study revealed a crucial region of the HCV core protein, basic amino acid region 1 (BR1), to inhibit IRF-3 dimerization as well as its phosphorylation induced by NDV infection and poly (I:C), thus interfering with IRF-3 activation. Therefore, our study suggests that rescue of the IRF-3 pathway impairment may be an effective treatment for HCV infection.

**Conclusion:** A crucial region of the HCV core protein interferes with IRF-3 activation and thereby inhibits the IFN signaling cascades. Future studies involving DDX3 modification by the HCV core protein may be interesting to explore the cell growth-dysregulation mechanisms.

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**SEQUENCING THE HEPATITIS C VIRUS: A SYSTEMATIC REVIEW**

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**Background and Aims:** Viral population sequencing has long been important in understanding HCV classification, epidemiology, evolution, transmission clustering, treatment response and natural history. The length and diversity of the HCV genome has resulted in analysis of particular regions of the virus, however there has been limited standardisation of protocols. This systematic review was undertaken to map the location and frequency of population sequencing on the HCV genome in peer reviewed publications, with the aim to produce a database of population sequencing primers and amplicons to inform future research.

**Methods:** Medline and Scopus databases were searched for English language publications based on keyword/MeSH terms related to sequence analysis (16 terms) or HCV (3 terms), plus “primer” as a general search term. Exclusion criteria included non-HCV research, review articles, duplicate records, and incomplete description of HCV sequencing methods. The PCR primer locations of accepted publications were noted, and purpose of sequencing was determined.

**Results:** A total of 435 studies were accepted from the 2042 identified, with 608 HCV population sequencing amplicons identified and mapped on the HCV genome. As seen in Figure 1 there is great diversity in the positioning of HCV population sequencing amplicons. The most commonly sequenced region was the Hypervariable Region-I, often utilised for studies of evolution and clustering/transmission analysis. Studies related to genotyping/classification and epidemiology favoured a defined segment of the NS5B region, likely as a result of the consensus guidelines...
for HCV genotyping released in 1994, in addition to the highly conserved 5′-UTR and Core regions. Whereas treatment response/resistance was assessed mainly in the Interferon sensitivity determining region (ISDR) region of NS5A.

**Conclusions:** While sequence analysis of HCV is generally constricted to certain regions of the HCV genome there is little consistency in the positioning of population sequencing primers, with the exception of a few highly referenced manuscripts. This study demonstrates the heterogeneity of HCV sequencing in the field, and provides a comprehensive database of previously published primer sets that may be utilised in future sequencing studies.

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**1160**

HCV GENOTYPE 1 PROTEASE INHIBITOR RESISTANCE MUTATIONS AT NS3 PROTEASE AMINO ACIDS 155 AND 156 CONFER RESISTANCE TO GENOTYPE 2A, 3A, 5A, AND 6A VIRUSES

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**Background and Aims:** Hepatitis C virus (HCV) NS3 protease (NS3P) mutations R155K, R155T, A156S, and A156T mediate resistance to protease inhibitors (PIs) for genotype 1. We studied the effect of these mutations on HCV recombinants with genotype 2a, 3a, 5a, and 6a specific NS3P.

**Methods:** Following HCV RNA transfection of Huh7.5 cells, viral fitness was evaluated by comparison of viral spread monitored by immunostaining and of supernatant infectivity titers. Following viral passage to naïve Huh7.5 cells, NS3P was directly sequenced. Concentration-response profiles were determined for linear PIs by immunostaining and of supernatant infectivity titers. Following HCVRNA transfection of Huh7.5 cells, viral spread in culture. MK-5172 showed exceptional potency against viral passage naïve Huh7.5 cells, NS3P was directly sequenced.

**Results:** Regarding fitness, 2a, 5a, and 6a R155K and A156S mutants were comparable to original recombinants and introduced mutations were maintained in passaged viruses. For 3a, only the R155K mutant spread in culture and maintained the mutation. R155T was only permissive in 2a and 6a, acquiring another known resistance mutation, D168A. A156T reverted in all mutants showing spread in culture. MK-5172 showed exceptional potency against all original recombinants: for 2a, 5a, and 6a, EC50 was 0.5–1.5 log₁₀ lower, and for 3a, EC50 was 1.5–2.5 log₁₀ lower compared to previously tested macrocyclic inhibitors. For linear inhibitors, EC50 values of mutants were 1.5–18-fold greater than for respective original viruses; a similar degree of resistance was found for a newly developed 1a virus with R155K or A156S. Results were more heterogeneous for macrocyclic compounds: for Vaniprevir, mutants showed 2.5–65-fold resistance; for Simeprevir, mutants showed 2–50-fold resistance, except A156S mutants, showing 5–13-fold greater sensitivity. For MK-5172, 2a, 5a, and 6a mutants showed 1.5–3.5-fold resistance, while 3aR155K showed 2.5-fold greater sensitivity. All determined EC50 differences proved highly significant (P<0.0001). Further, when promoting viral escape by treatment with subtherapeutic doses of linear inhibitors, original 2a, 3a, 5a, and 6a recombinants acquired mutations at NS3P position 155 and/or 156.

**Conclusions:** For developed HCV protease-mutants, great fitness differences were found. Overall, resistance mutations identified in genotype 1 infected patients seem to be of importance for resistance of genotype 2a, 3a, 5a, and 6a. Resistance levels depended on HCV genotype, the specific mutation, and the specific PI.

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**1161**

INDUCTION OF HEME OXYGENASE-1 IN HUMAN HEPATOCYTES PROVIDES HEPATOPROTECTIVE EFFECTS AND SUPPRESSES HCV REPLICATION IN CHIMERIC uPA/SCID MICE

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**Background:** Hepatitis C Virus (HCV) infection represents one of the leading causes of chronic hepatitis worldwide. Enhancement of host anti-oxidant enzymes, such as heme oxygenase-1 (HO-1), may attenuate liver disease by reducing hepatocellular injury. Besides displaying hepatoprotective effects, the induction of HO-1, which can be triggered by cobalt-protoporphyrin-IX (CoPP) administration, was shown to reduce HCV replication in vitro (Lehmann et al, Hepatol. 2010).

**Aim** of this study was to investigate the antiviral and hepatoprotective effects of HO-1 in vivo using HCV-infected humanized mice. The antiviral effect of HO-1 was evaluated also in combination with interferon alpha administration.

**Methods:** Patient-derived HCV-positive serum (genotype 1) was used to establish HCV infection in uPA/SCID mice displaying high levels of human chimerism. HO-1 was induced by intraperitoneal injection of CoPP (5 mg/kg; twice/week), while human peg-interferon-alpha (peg-IFNα) (2.5 ng/g; twice/week) was given either alone or in combination with CoPP. Viremia changes and intrahepatic expression levels of human genes were measured by qRT-PCR.

**Results:** Two weeks of CoPP administration to humanized mice stably infected with HCV (median 1.4E6 HCV-RNA copies/ml) induced significant increase of human HO-1 RNA levels (10-fold induction), and considerable suppression of HCV replication (median 2log viremia reduction). Furthermore, HO-1 induction attenuated the HCV-driven enhancement of some human interferon-stimulated genes (ISGs), such as ISG-15 and Mx1, as well as the expression of human-specific inflammatory cytokines, like TGFβ and IFNγ, thus confirming the protective function of HO-1 in human hepatocytes in vivo. Notably, 2 weeks of treatment with CoPP and peg-IFNα given in combination induced an even stronger suppression of HCV viremia (3log reduction), whereas HCV replication was reduced by 1log in mice receiving the same dosage of peg-IFNα as monotherapy.

**Conclusions:** Enhancement of the antioxidant enzyme HO-1 in human hepatocytes not only mitigated the proinflammatory cytokine milieu in HCV-infected livers, but also provoked significant
suppression of viral replication. The synergistic antiviral effects determined by treating humanized mice with CoPP and peg-IFNα hinted at a potential role for HO-1 induction in antiviral therapy, while providing therapeutic protection of the hepatocytes from HCV-mediated hepatocellular injury.

1162 TURMERIC CURCUMIN INHIBITS ENTRY OF ALL HEPATITIS C VIRUS GENOTYPES INTO HUMAN LIVER CELLS
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Background and Aims: Hepatitis C virus (HCV) infection causes severe liver disease and affects more than 160 million individuals worldwide. Individuals undergoing liver organ transplantation face universal reinfection of the graft. Therefore, affordable antiviral strategies targeting the early stages of infection are urgently needed to prevent the recurrence of HCV after liver transplantation. The aim of the study was to determine the antiviral activity of turmeric curcumin, a natural compound with antiviral and anti-cancer activity, against HCV.

Methods: The antiviral activity of curcumin and its derivates was evaluated using HCV pseudogenotypes (HCVpp) and cell-culture derived HCV (HCVcc) in hepatoma cell lines and primary human hepatocytes. Its mechanism of action was disected by R18-labelled virions and a membrane fluidity assay.

Results: Curcumin treatment had no effect on HCV RNA replication or viral assembly and release. However, co-treatment with curcumin potently inhibited entry of all major HCV genotypes. Similar antiviral activity was also exerted by other curcumin derivates but not by tetrahydrocurcumin suggesting the importance of α,β-unsaturated ketone groups for the antiviral activity. Expression levels of known HCV receptors were unaltered while curcumin inhibited cell-to-cell transmission and was effective in combination with other antiviral agents.

Conclusion: Turmeric curcumin inhibits HCV entry independent of the genotype and in primary human hepatocytes by affecting the membrane fluidity, and hence impairing the virus binding and fusion ability of HCV.

1163 EDA-STREPTavidIN FUSION PROTEIN CONJUGATED TO BIOTI NyLATED HCV-NS3 PROTEIN INDUCES STRONG T CELL IMMUNE RESPONSES AGAINST NS3
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Background and Aims: Recombinant proteins are generally poor immunogens and do not induce strong T cell immune responses if they are not combined with appropriate adjuvants, or modified to improve their capture by dendritic cells (DCs). In previous works we have shown that fusion of an antigen to the extra domain A from fibronectin (EDA) targets the antigen to dendritic cells via TLR4 and improves its immunogenicity. In this work we have prepared a fusion protein between EDA and streptavidin (EDAvidin) to allow its interaction with biotinylated antigens. We have tested the immunogenicity of biotinylated NS3 combined with EDAvidin to induce strong anti-NS3 cellular immune responses as a potential vaccination strategy against HCV infection.

Methods: Recombinant EDAvidin, EDA-NS3 fusion protein and NS3 were produced in E. coli and purified by affinity chromatography. NS3 was biotinylated using Sulfo-NHS-biotin reagent. The capacity of EDAvidin to bind to biotinylated proteins was tested by surface Plasmon resonance, by western blot and by ELISA. The efficacy of antigen capture by DC was tested by flow cytometry using biotinylated GFP. Immunogenicity of the recombinant proteins was tested in HHD transgenic mice (expressing human HLA-A2) by ELISPOT and by in vivo killing assays.

Results: Recombinant EDAvidin tetramerizes and binds very efficiently to biotinylated proteins. EDAvidin retains the proinflammatory properties of EDA, activating TLR4 signaling pathway. When combined with biotinylated GFP, EDAvidin favours GFP capture by dendritic cells. Immunization of mice with EDAvidin combined with biotinylated NS3 induce a strong anti-NS3 cellular immune responses similar to that induced by the fusion protein EDA-NS3.

Conclusions: EDAvidin can bind biotinylated proteins and improve their immunogenicity in vivo. EDAvidin combined with biotinylated NS3 can be considered as a vaccination strategy against hepatitis C virus infection.

1164 INDUCTION OF A PREDIABETIC STATE IN TRANSGENIC MICE EXPRESSING THE HCV PROTEINS
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HCV infection is an independent risk factor of type-2 diabetes (T2D) in humans. We tried to understand the mechanisms of HCV-related T2D using the FL-N/35 transgenic mouse model expressing the full HCV-ORF. FL-N/35 mice displayed similar baseline glycemia, but significantly higher insulinemia and HOMA-IR than control littermates (WT). Glucose tolerance tests (IPGTT) resulted in significantly higher glycemia levels in transgenic mice, demonstrating that HCV protein expression is associated with glucose intolerance, like in HCV-infected patients. We then assessed whether this pre-diabetic state was due to a defect in β-cell function and/or to insulin resistance (IR).
During IPGTT, the early peak of insulin secretion was absent in FL-N/35 mice, whilst the pancreatic β-cell mass and total pancreatic insulin pools were identical to WT, suggesting altered insulin secretion in HCV mice. It has been established that plasmatic IL6 influences glucose-stimulated insulin release. Indeed, we observed lower levels of plasmatic IL6 in FL-N/35 mice compared to WT. To confirm this, we either injected IL6 or stimulated its production using CCl4 injections and performed IPGTT. We found that boosting plasmatic IL6 in FL-N/35 mice corrected their glucose intolerance and restored the insulin secretion. Thus, low plasmatic levels of IL6 might encompass, at least in part, for the pancreatic β-cell secretion defect. However, transgenic hepatocytes did not show reduced capacity to produce IL6 in vivo and in vitro, which suggests that other hepatic cells might be involved.

To assess whether HCV transgenic mice are insulin resistant, we performed hyperinsulinaemic euglycaemic clamps. We observed a significantly lower glucose infusion rate and a higher endogenous glucose production, and a concomitant drastic diminution of soleus striated muscle glucose uptake. These results demonstrate that FL-N/35 mice are insulin resistant and that HCV-related IR is both of muscular and hepatic origins.

In conclusion, like patients with chronic hepatitis C, mice expressing the full HCV ORF are intolerant to glucose. This pre-diabetic state is therefore due to a direct role of HCV protein expression in the liver. The HCV-related pre-diabetic state appears to be the combined result of IL6-dependent insulin secretion impairment and IR of muscular and hepatic origins.

1165
HCV INFECTION REPROGRAMS THE HEPATIC GLUCOSE AND GLUTAMINE METABOLISM
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Background and Aims: Hepatitis C virus (HCV) is the only virus that is known to perturb hepatic glucose and lipid metabolism with important patho-physiological consequences. Chronic carriers often develop steatosis, insulin resistance and type 2 diabetes, which resolve with successful antiviral treatment. Therefore it is thought that HCV interferes directly with the lipogenic and glycolytic pathways and requires these changes for its replication. However, the exact circumstances of this metabolic reprogramming still remain vague and require further analysis. Here we investigate some fundamental changes in glucose and glutamine metabolism linked to HCV infection.

Methods: RNA derived from Huh7.5 cells infected or not with JFH1 and from biopsies of chronic HCV patients were used for RT-qPCR analysis with primers targeting metabolic genes. Nutrient deprivation and biochemical and NMR-based metabolic flux analysis were performed with JFH1 infected Huh7.5 cell culture extracts.

Results: We show that HCV modulates the transcript levels of some key regulators of glucose metabolism (HIF-1α, PKM2, G6PD) in the hepatocyte-derived cell-line Huh7.5 as well as liver biopsies of patients with chronic hepatitis C, which hinted at changes to glycolytic fluxes. In addition, we found enzymes and factors regulating glutamine metabolism (MYC, SLC1A5, SLC7A5, GLS) to be upregulated by HCV. Indeed, cell proliferation rates of HCV infected and uninfected cells in conditioned growth media showed that infected cells become dependent on glutamine and lose their glucose dependence. NMR-based metabolomic assays further corroborated these findings. We then showed that silencing of MYC, an oncogene and metabolic transcription factor known to induce glutamine addiction, as well as silencing of GLS, considerably reduced HCV infection.

Conclusions: Altogether, these data suggest that HCV reprograms the hepatocyte metabolism and establishes glucose dependence. This HCV-induced metabolic reprogramming is similar to that commonly found in many types of tumor cells. Because these changes seem to be required for viral replication, we are currently investigating their roles in the various steps of the viral life cycle, and their impact on the pathological features associated with chronic hepatitis C.

1166
THE HEPATITIS C VIRUS (HCV) F PUTATIVE-PROTEIN: A NOVEL MODULATING FACTOR OF THE PREDICTIVE VALUE TO ANTIVIRAL TREATMENT RESPONSE OF THE IL28B REGION
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Introduction: Previous works have suggested that core polymorphisms can be prognostic marker of interferon sensitivity. However, there are not data about the role of the F coding region of HCV, an predictive overlapping open reading frame (+1ORF) with that of the core protein. The aim of this study was to analyze the role of F-putative and core sequences as predictive markers of antiviral response alone or in combination with IL-28 genotypes.

Patients and Methods: We included basal sera from 28 Caucasian patients with chronic HCV genotype 1b treated with pegIFN alfa-2a + ribavirin. HCV-RNA was quantified with the Abbott kit 2000. The coding region of the viral core (127 amino acids) and protein F (first 122 amino acids) was analyzed by bulk-PCR/sequencing. IL28B polymorphisms (rs12979860) were determined by standard TaqMan methodology.

Results: The distribution of the IL28B genotypes was: 10 (36%) CC, 14 (50%) CT and 4 (14%) TT. The SVR rate was 70% (7/10) in CC; 57% (8/14) in CT patients, and 0% in TT patients. Core protein was significant more conserved than putative F protein (88% vs 57% of amino acid homology; p < 0.001). The most significant amino acid differences between responder and non responder patients in both CC and CT patients were observed in the putative F coding region (Table 1). So, only one position of the core and 9 positions of the F protein showed opposite changes between responders and non responders patients into the CC genotype. two positions, one in each ORF, discriminates responder and non responders into the CT patients, although only the change in the protein F was significant (p = 0.05). A segment of 3 amino acids of the F protein (67–69) which allow the simultaneous discrimination of CC responders (aa 67 and 68) and CT responders (aa: 69).

Conclusions: Data obtained suggest that the putative F-protein sequence of HCV can be a good predictor marker of interferon sensitivity, and that can be used to identify potentially responder patients harboring IL28B CT genotype.

1167
A POSSIBLE NOVEL ROLE OF KRÜPPEL-LIKE TRANSCRIPTION FACTOR 15 DURING HCV INFECTION IN PBMCs FROM HCV POSITIVE PATIENTS
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Background and Aims: HCV infection leads to a wide spectrum of liver diseases ranging from mild chronic hepatitis to end-stage cirrhosis and hepatocellular carcinoma. Although HCV is considered
essentially hepatotropic, recent studies suggest that it can also infect peripheral blood mononuclear cells (PBMC). HCV infection has been clinically associated with serum lipid abnormalities. The aim of this study is to analyze the possible roles of genes involved in HCV-mediated lipid metabolism in PBMC from HCV infected patients.

**Materials and Methods:** Using The Human Adipogenesis RT² Profiler™ PCR Array, we have analyzed the expression profile of 84 genes in chronic HCV PBMCs patients normalized with PBMCs from healthy donors. To compared the results obtained from HCV+ PBMCs with the expression in the hepatocytes, we have used a JFH1/HCV in vitro system after 48h post infection normalized with uninfected Huh 7.5.1 cell line.

**Results:** Both in vivo and in vitro models HCV was able to up-regulate the same genes (14% in both of them) involved in lipid metabolism such as SREBP1c, FASN, FABP4, ACACB and in signal transduction (29% and 27% respectively) resulted modulated both in HCV PBMCs and J6/JFH1-infection system. In both models the Krüppel-like transcription factor 15 (KLF15). KLF15 results significantly upregulated in HCV+ PBMCs as well as the J6/JFH1 in vitro infection. In addition, in PBMCs, KLF15 upregulation is statistically and positively correlated to the Body Weight, the BMI and the ALT levels. Furthermore, in PBMCs from HCV+ donors, KLF15 expression correlates with the HCV Viral Load and the antibody titer (anti-NS3 anti-NS4 and anti-capsid), suggesting a possible intriguing role of this factor in HCV infection.

**Conclusions:** Our results confirm the ability of HCV to affect lipid metabolism; we have highlighted a similar modulation both in HCV patients lymphoid cells and in J6/JFH1 HCV infection model. In particular, the virus-related KLF15 enhanced expression, in both in vivo and in vitro models, suggests this protein as new possible marker of HCV infection and as a potential therapeutic target useful to counteract HCV infection.

**1169**

**BL-8030: A NOVEL, POTENT, SELECTIVE, ORALLY AVAILABLE INHIBITOR OF HEPATITIS C VIRUS NS3/4A PROTEASE**

P. Halfon1, J. Courcambeck1, T. Whitaker2, P.M. Tharnish2, T.R. McBrayer2, S.J. Coats2, J.FH1/HCV PBMCs with the expression in the hepatocytes, we have used a JFH1/HCV in vitro system after 48h post infection normalized with uninfected Huh 7.5.1 cell line. To compared the results obtained from HCV+ PBMCs with the expression in the hepatocytes, we have used a JFH1/HCV in vitro system after 48h post infection normalized with uninfected Huh 7.5.1 cell line.

**Results:** Both in vivo and in vitro models HCV was able to up-regulate the same genes (14% in both of them) involved in lipid metabolism such as SREBP1c, FASN, FABP4, ACACB and in signal transduction (29% and 27% respectively) resulted modulated both in HCV PBMCs and J6/JFH1-infection system. In both models the Krüppel-like transcription factor 15 (KLF15). KLF15 results significantly upregulated in HCV+ PBMCs as well as the J6/JFH1 in vitro infection. In addition, in PBMCs, KLF15 upregulation is statistically and positively correlated to the Body Weight, the BMI and the ALT levels. Furthermore, in PBMCs from HCV+ donors, KLF15 expression correlates with the HCV Viral Load and the antibody titer (anti-NS3 anti-NS4 and anti-capsid), suggesting a possible intriguing role of this factor in HCV infection.

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**1168**

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P. Halfon1, J. Courcambeck1, T. Whitaker2, P.M. Tharnish2, T.R. McBrayer2, S.J. Coats2, J.

**Background and Aims:** Hepatitis C Virus (HCV) NS3/4A protease inhibitors (PI) are likely to be a key component of future combination therapies for patients with chronic HCV infections. These agents demonstrate dramatic antiviral effects, but tend to be genotype 1 specific and associated with unpleasant adverse effects. Furthermore, PIs have demonstrated a high risk for development of resistant virus. Based on structure-based drug design and NS3 molecular resistance mechanisms, a set of peptidomimetic NS3/4A protease inhibitors were synthesized. Here, we report the pharmacodynamic and pharmacokinetic profile of BL-8030 (formerly GNS-227), a low-nanomolar inhibitor of HCV.

**Methods:** The antiviral activity and selectivity of BL-8030 was assessed versus:
1. a Huh-7 cell based HCV replicon assay;
2. wild type and PI mutant enzymes and
3. a panel of human proteases to demonstrate specificity.

**Results:** BL-8030 demonstrated excellent potency, in the low nanomolar range, in HCV replicon genotypes 1a, 1b and 2a. Its pan-genotypic activity was tested on a panel of NS3 enzymes using a biochemical fluorometric protease assay. BL-8030 demonstrated potent activity against genotypes 1a, 1b, 2a and 4. BL-8030 was also potent against the main, clinically relevant resistance mutations. BL-8030 showed several thousand-fold selectivity relative to a panel of human proteases. PK studies in rats indicated good oral bioavailability after single 10 and 100 mg/kg dosing (17.4 and 33.7%). Following oral administration, BL-8030 was distributed in the liver with a liver to plasma ratio ranging from 28.4 to 45.4 (24hr and 12hr post dosing). Importantly, BL-8030 levels in the liver 24 hours post dosing were above its EC50, suggesting the potential for once a day dosing in the clinic.

**Conclusions:** BL-8030 is a novel, highly potent and specific HCV NS3/4A protease inhibitor as demonstrated by cellular and biochemical assays and has the potential to prevent and treat emerging resistance variants. BL-8030 has a favorable pharmacological and safety profile with good oral bioavailability, liver distribution and the potential for once daily dosing.

**1169**

**GENERATION OF PERMISSIVE BCLC5 CELL LINES FOR THE STUDY OF HCV REPLICATION: ROLE OF miR122 IN REPLICATION ENHANCEMENT**

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Robust hepatitis C virus (HCV) cell-culture propagation is restricted almost exclusively to the human hepatoma cell line Huh7 and its selected subclones, which are highly permissive for HCV replication; e.g., Huh-7.5 cells. Recent studies suggest that host factors such as innate immunity, gene polymorphisms, and/or miR122 are associated with the permissiveness of cells for HCV infection. We have recently characterized the BCLC5 cell line, which is derived from human hepatocellular carcinoma, and which can support subgenomic (SGR) HCV RNA replication for prolonged periods of time. In order to obtain highly permissive cell lines for HCV replication, clonal BCLC5 cells-(designated BCLC5-C8 cells) harboring a selectable neomycin (neo)-SGR were cured of HCV RNA with IFN-alpha treatment (designated BCLC5-C8i cells). In vitro transcribed neo-SGR RNA was then electroporated into the cured BCLC5-C8i cells and transduction efficiencies were determined 21 days post-G418 selection by counting the resulting colonies. Colony formation efficiency increased in BCLC5-C8i cells compared to their parental BCLC5 or Huh-7.5 cells (6- and 3-fold, respectively). Similarly, short-term replication efficiency was increased in BCLC5-C8i cells as deduced by the reporter activity of a SGR-carrying Glasgow luciferase (GLuc-SGR). Since miR122 has been shown to facilitate HCV replication, we assessed miR122 expression in the BCLC5 cell line and its derivatives by real time qRT-PCR. Neither BCLC5, BCLC5-C8, nor BCLC5-C8i cells expressed endogenous miR122. Therefore, we examined whether ectopic miR122 expression could enhance HCV replication in BCLC5-C8i cells. We then selected one clone that expressed miR122 at comparable levels to Huh-7.5 cells and analyzed HCV replication in these cells. BCLC-5 C8i/miR122 cells exhibited a 3- to 4-fold increase in GLuc-SGR replication as deduced by reporter secretion and intracellular HCV RNA determination. Similar results were obtained upon electroporation of full-length HCV RNA. In conclusion, we have established two new cell lines, BCLC5-C8i and BCLC5-C8i/miR122, which are permissive for HCV replication. Our results reveal that miR122 is not essential for HCV replication;
rather, it acts as an enhancer only in those cells with increased HCV replication tolerance.

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THE CYCLOPHILIN INHIBITOR SCY-635 RESTORES THE INNATE RECOGNITION OF HCV BY PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) FROM HCV POSITIVE SUBJECTS

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Background and Aim: Viral immune evasion strategies lead to persistent HCV infections by interfering with the innate response to the virus and blocking the initiation of an anti-viral T cell response. In a Phase 1b clinical study in adults with chronic HCV infections, treatment with SCY-635 resulted in the induction of endogenous IFNs. More recently, a Phase 2a study demonstrated that short-term treatment with SCY-635 enhanced the antiviral effect of Peg-IFN/RBV in difficult to treat, HCV genotype-1 infected subjects in a manner consistent with having ameliorated viral immune escape mechanisms and restored innate immune responses to the virus. The purpose of this study was to evaluate the ability of SCY-635 to modulate the innate immune recognition of HCV by PBMC isolated from subjects chronically infected with HCV.

Method: PBMC were isolated from heparinized blood obtained from HCV-positive subjects and HCV-negative healthy control subjects. Cells were incubated in serum free medium in the presence or absence of SCY-635 or direct acting antivirals (DAAs). Stimulation with the toll-like receptor 3 agonist poly I:C was included as a positive control. Interferon and cytokine levels were determined by ELISA in supernatants taken 18h after treatment.

Results: SCY-635 treatment induced the production of IFN-α, IFN-β, IFN-γ and IFN-λ, by PBMC from chronically infected HCV subjects in a dose dependent manner. In addition, SCY-635 treated PBMC secreted IL-6 and TNF-α. PBMC from healthy HCV-negative study subjects did not produce IFNs or cytokines in response to SCY-635 treatment. Treatment with poly I:C resulted in IFN-λ secretion and there was an additive effect on secretion of IFN-α by addition of SCY-635 in HCV+ PBMC. Preliminary data suggest that DAAs did not induce IFN in HCV+ PBMC. Experiments to confirm these observations are ongoing. Follow up experiments to identify the cells source(s) of SCY-635-induced IFNs and inflammatory cytokines are also underway.

Conclusions: Release of type I, II and III IFNs by SCY-635-treated PBMC from HCV positive subjects indicates that SCY-635 restores the innate recognition of HCV-associated molecular patterns. The data support the hypothesis that SCY-635 exerts its anti-HCV activity via modulation of the innate immune system.

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EFFECTS OF A NEW LIPOSOME-ENCAPSULATED FORMULATION OF SILYBIN ON HEPATITIS C VIRUS INFECTION

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Introduction: Silybin, the active component of Silybum Marianum, has been described to prevent HCV entry and to have a direct inhibitory activity on viral polymerase NS5B, with a variable efficacy depending on its pharmaceutical formulation and pharmacokinetic properties. The goal of our study was to evaluate whether increasing the concentration of the molecule at its target site resulted in higher efficacy. This hypothesis was tested by encapsulating silybin into liposomes (SILL) and comparing the pharmacological activity of this new silybin formulation with the compound dissolved in DMSO (SILD).

Methods: Experiments were performed on human-derived permissive hepatoma cell lines: Huh7.5/Con1/FL-Neo, stably transfected with a plasmid coding for HCV genotype 1b, Huh7.5 transiently transfected with JC1-GFP chimeric plasmid (Huh7.5/pJC1-GFP), coding for HCV genotype 2a, and Huh7.5 infected for 72h with supernatants (MOI 0.1) derived from Huh7.5 cells electroporated with pC1 plasmid. qRT-PCR, western blotting and immunofluorescence were used to assess silybin antiviral activity.

Results: Treatment of Huh7.5/Con1/FL-Neo and of Huh7.5/pJC1-GFP cells with 75 μM of SILD for 72h did not affect HCV RNA and protein content. No difference in HCV replication levels were also observed when replication-competent cells were treated with SILL. When tested on the infectious system, SILD pretreatment was able to prevent HCV infection (HCV RNA 37.52%±15.6 vs ctrl p < 0.0001 and protein content 41.5±6.36 vs ctrl p < 0.01) and, more surprisingly, both SILL and the empty liposomes (EL) abolished almost completely viral protein expression, causing a strong reduction of HCV RNA content, with SILL being 4 fold more potent than EL (EL 0.4%±0.13 p < 10^-28 SILL 0.094%±0.015 p < 10^-28 vs ctrl). Finally liposom-based formulations reduced viral particles release of 150 fold vs ctrl.

Conclusions: Our data demonstrated that silybin is able to prevent HCV entry, but showed no effect on HCV replication in genotype 1b and 2a cell systems. Liposome-encapsulation of silybin enhanced the prevention of infection (4 hundred fold vs SILD and 1000 fold vs ctrl), combining the antiviral activity of the compound with the reduction of virus entry and the inhibition of viral particles release mediated by the liposomes themselves.

Funded by Fondazione Cassa di Risparmio di Puglia

1172
FUNCTIONAL ANALYSIS OF THE DIACYLGLYCEROL ACYLTRANSFERASE ACTIVITY-1 (DGAT1) IN THE REPPLICATION OF HEPATITIS C VIRUS: EFFECT OF QUERCETIN

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Background and Aims: Hepatitis C virus requires lipid droplets for its assembly and interaction with DGAT1 and Core proteins. Quercetin is a flavonoid that inhibits viral replication through two different mechanisms (a) targeting NS3 and NS5 synthesis, (b) avoiding lipoviroparticle formation through DGAT-1 activity blockade. We aim to analyze DGAT1 role in viral replication, as well as the impact of IL28B polymorphism in Huh7.5 cells (genotype CT) vs Huh7 (genotype CC) and viral genotype on DGAT activity.

Methods: We selected serum of patient with high viral load, genotype 1b (7.1logIU/ml) and genotype 3a (6.6 logIU/ml). Huh7.5 (CT) and Huh7 (CC) cells were incubated in DMEM medium supplemented with 10% FBS, antibiotics, L-glutamine and nonessential amino acids. At 24 hours later, infective particles of JFH-1 (it was used how control) and serum of patients were added to the cells. After 4 days, cells were collected and total RNA and proteins were extracted. Gene expression was determined by qRT-PCR and DGAT total activity was calculated by fluorescence assay. (Synergy HT, BioTek, Bedfordshire, UK). Quercetin was added to cell cultures (50μM) for 72 hours to evaluate its effect on DGAT activity.

Results: DGAT1 gene expression levels (expressed as fold induction) in Huh7.5 cells (IL28B: CT) infected with different HCV genotypes were: JFH1: 1.5±0.3; G1: 1.5±0.4; G3: 1.6±0.2; p < 0.05; for Huh7 cells (CC): JFH1: 2.35±0.12; G1: 1.5±0.04; G3: 2.7±0.02; p<0.05. When Huh7.5 cells were treated with quercetin (50μM), DGAT-1
gene expression levels were decreased (30–45%), independently of viral genotype. Total DGAT activity was found increased in Huh7.5 cells infected by JFH1 (1.6 ± 0.2), genotype 1b (2.1 ± 0.2) and genotype 3a (1.9 ± 0.1). DGAT activity in Huh7 cells was increased upon HCV infection (JFH1: 1.6 ± 0.03, G1a: 1.54 ± 0.04 and G3a: 1.9 ± 0.04). Quercetin had an inhibitory effect on DGAT activity (1.5 ± 0.1 fold) when cells were infected by genotype 3a and 1a. Quercetin inhibited viral replication in a dose-dependent manner; quercetin 25uM: 31.23%; an inhibitory effect on DGAT activity (1.5 ± 0.1).

**Conclusions:** HCV infection increased DGAT-1 gene expression and DGAT activity, improving viral replication. Quercetin decreased DGAT activity and reduced viral replication. DGAT1 protein can arise as a new target for hepatitis C therapy.

### 1174 A NOVEL METHOD FOR NON-INVASIVE DIAGNOSIS OF HEPATITIS C VIRUS USING ELECTROMAGNETIC SIGNAL DETECTION: A MULTICENTER INTERNATIONAL STUDY

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**Background:** Chronic hepatitis C virus (HCV) infection is a worldwide major health problem. RT-PCR is the standard technique for detection of HCV viremia. A simple, rapid, non-invasive electromagnetic sensor (C-FAST device) was patented; in which the resonant electromagnetic signal of HCV-RNA nucleotides (molecule signature) is recorded as a consensus frequency and replayed for its identification.

**Aim:** The study compared the diagnostic utility and reliability of C-FAST device in detection of HCV viremia with the standard HCV-PCR.

**Subjects and Methods:** The first phase was done as pilot in Egypt on 79 participants, the second phase was done both nationally and internationally in five centers: one center from Egypt, two centers from Pakistan and two centers from India (800, 92 and 113 subjects respectively). The third phase was done nationally as multicenter study on (1600) participants for ensuring its representativeness. Consent signing subjects were subjected to both C-FAST device and the reference Gold Standard “PCR” to assess the device validity under the same described procedures and methodology throughout the three phases that ensured independent, blind comparison with PCR. The device was also assessed nationally and internationally by two different observers independently on the same participants to test its reliability.

**Results:** When compared to PCR technique, C-FAST device during all phases revealed sensitivity ranges from 95% to 100%, specificity ranges 95.5% to 100%, PPV ranges 89.5% to 100%, NPV ranges from 95% to 100% and positive likelihood ratios ranges from 21.8% to 38.5%. The results of Kappa (95.5% to 99.9%) indicate that the two observers have the same percent agreement. They classify everyone exactly the same way on using the C fast device.

**Conclusion:** This study confirmed the efficiency of C-FAST device in recording and replaying the HCV electromagnetic signal. It is practical evidence that nucleotides emit electromagnetic signals that can be used for its identification. As compared to PCR, C-FAST is an accurate, valid and non-invasive device.

### 1175 HEPATITIS C VIRUS PROPAGATION IN HUMAN CD4+ AND CD8+ T LYMPHOCYTES

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**Introduction and Aim:** Accumulated molecular and clinical evidence indicate that immune cells can support replication of hepatitis C virus (HCV). To investigate the ability of patient-derived, wild-type HCV to infect CD4+ and CD8+ T lymphocytes and to assess properties of the virions produced, we employed a previously established in vitro HCV replication system in which normal human T cells served as targets.
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Methods: Plasma of a patient chronically infected with HCV genotype 1, carrying viral load of 1.2x10⁷ copies/ml that was prescreened for its infectivity towards total T cells, served as inoculum to infect normal human CD4+ and CD8+ T cell subsets (>97% pure by flow cytometry). The cells were pre-stimulated with phytohemagglutinin (PHA; 5 μg/ml), exposed to HCV and cultured under alternating stimulation with PHA and/or interleukin-2 (IL-2) for 14 days post-infection, as reported (JGV 2006; 87:3577; Hepatology 2008;49:1431; JVI 2012;86:3723). HCV RNA positive (genomic) and negative (replicative) strands were detected by strand-specific RT-PCR followed by nucleic acid hybridization (RT-PCR/NH). Intracellular HCV NS5a and core proteins were identified by confocal microscopy. Released HCV RNA-reactive particles were examined by sucrose and iodixanol gradient ultracentrifugations. Clonal sequencing of the HCV 5’-UTR region served to compare the HCV virions harboured by inoculum and in in vitro infected cells.

Results: HCV RNA positive and replicative strands, as well as NS5a and core proteins were detected in both CD4+ and CD8+ T cells after infection. Up to 1.2% cells were found NS5a protein positive. HCV RNA-reactive particles displaying distinct sedimentation velocity and buoyant density occurred in inoculums from CD4+ and CD8+ T cells exposed to this inoculum. Clonal sequencing revealed different HCV variants in infected cells compared to plasma used as inoculum.

Conclusions: Patient-derived, wild-type HCV can infect and establish productive replication in normal human both CD4+ and CD8+ T cells as evidenced by detection of HCV RNA replicative strand and intracellular expression of NS5a and core proteins. De novo HCV infection of the T cell subsets was confirmed by identification of unique variants in infected cells and HCV RNA-reactive particles with distinct physical properties in cell culture supernatants.

T FOLLICULAR HELPER CELLS: DO THEY PLAY A ROLE IN CHRONIC HCV?


Patients with chronic HCV infection are characterized by a weak CD8+ T cell response likely due to diminished CD4+ T cell help. Conflicting data on the role of CD4+ T follicular helper (TFH) cells and B cells in HCV infection still exist and although liver follicles are considered as responders and non-responders before treatment. This however is an invasive biomarker, making it difficult to apply to patient management.

Using ex vivo stimulation, we measured the induced phosphorylation of intracellular-Stat1 in peripheral blood mononuclear cells (PBMC) of HCV-infected patients before and during treatment and correlated the results to the virological response.

Patients and Methods: Our study is a prospective study. Twenty-nine chronic HCV-infected patients, genotype 1 (G1) or 4 (G4), treated with pegylated-IFN-alpha/ribavirin/protease inhibitor have been recruited thus far. PBMC from patients are harvested before initiation (D0) and after four weeks of treatment. Phosphorylation of intracellular-Stat1 is measured ex vivo by flow cytometry in different subsets of PBMC, before and after stimulation with IFN-alpha. Patients are classified as responders and non-responders based on the viral load at W12.

Results: Results for the first 18 patients (15 reached W12 of treatment and 3 W4) were detailed. 15 patients had HCV-G1 and 3 had HCV-G4. The median viral load before treatment was 5.83 log IU/ml. Eight patients received boceprevir and 10 telaprevir. At W12, 11 patients were responders (undetectable viral load) and 4 were non-responders. Three patients were considered as responders at W4 (viral load <1000 IU/ml). At D0, the level of P-Stat1 was higher in PBMC and in T-lymphocytes of responders than non-responders: at baseline (P=0.02 and P=0.04) and after ex vivo stimulation of cells with IFN-alpha (P=0.006 and P=0.006). Among T-lymphocytes, naïve CD8+T cells expressed the highest level of P-Stat1. This “insensitivity” to endogenous and exogenous IFN-alpha in T-lymphocytes of non-responders was not explained by intracellular-Stat1 or IFNAR1/2 receptor levels. Moreover, stimulation with IFN-gamma (Jak1/Stat1-cross-pathway) showed a similar level of P-Stat1 induction, suggesting that the differences are due to differential Jak1-Stat1 activation.

Conclusions: Before initiation of treatment, sensitivity of PBMC to IFN-alpha, as measured by P-Stat1 level, acts as a non-invasive biomarker of early virological response to anti-HCV triple-therapy.
1178  
A NANOBODY RECOGNIZING A NOVEL EPITOPE IN HEPATITIS C VIRUS GLYCOPROTEIN E2 BROADLY NEUTRALIZES VIRUS ENTRY AND INHIBITS CELL–CELL TRANSMISSION  

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Introduction: Treatment of hepatitis C virus (HCV) infection with the current generation of direct-acting antiviral therapies (DAAs) is limited by the emergence of resistance mutations. As such, novel targets for clinical intervention need to be identified. An attractive approach is immunotherapy targeting the HCV entry cascade. However, the efficacy of antibody therapy is limited by the ability of HCV to infect cells by neutralization resistant cell-to-cell transmission.  

Objectives: To address these limitations to antibody therapy we chose an alternative approach to identifying entry inhibitors, isolating nanobodies from alpacas. In addition to conventional IgG, alpacas naturally produce homodimeric heavy chain IgG molecules (HcAbs) devoid of light chains. The epitope-binding variable region domains are called nanobodies. When expressed as a single domain, nanobodies have the advantage of being small, stable, non-immunogenic, and able to recognize epitopes inaccessible to antibodies.  

Design: We exploited these unique features by immunizing alpacas with a recombinant, purified form of the HCV E2 envelope glycoprotein. Affinity enrichment of a recombinant phage library of resulting nanobody sequences was performed, and the nanobody clones from the immunized animal assessed for their neutralizing potency. A combination of mapping techniques revealed the fine specificity of this neutralizing nanobody.  

Results: We isolated four discrete nanobodies that bound to E2. The most prevalent clone (D03) potently inhibited entry of a genetically diverse array of HCV patient isolates and was able to inhibit antibody-resistant cell-to-cell transmission of cell cultured HCV. We determined that D03 recognized a novel, highly conserved epitope on the surface of E2, overlapping the CD81 binding site. Determination of the crystal structure of this nanobody revealed an immunoglobulin domain with an unusually long CDR3 loop stabilized by a disulfide bond between the CDR2 and CDR3 loops.  

Conclusions: This is the first description of an nanobody inhibiting HCV entry and cell–cell transmission of HCV. These in vitro investigations define nanobodies as a new class of entry inhibitors that have the potential to be used in combination with existing therapeutics.

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ACCELERATED LIVER DISEASE IN AGGRESSIVE HEPATITIS C VIRUS GENE RECURRENT POST-LIVER TRANSPLANTATION MAY BE DUE TO ENHANCED APOPTOSIS MEDIATED BY BOTH VIRUS AND IMMUNOSUPPRESSANTS  

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Introduction: In post-liver transplant (OLT) hepatitis C (HCV) recurrence, the combination of immunosuppressants and HCV is postulated to increase hepatocyte apoptosis and liver fibrosis. We investigated (1) hepatocyte apoptosis in liver biopsies of HCV-infected patients pre- and post-OLT, (2) the effects of HCV and immunosuppressants on cell death in primary mouse hepatocytes (PMoH) and human hematoma cells (HuH7), (3) reversal of these effects using inhibitors of apoptosis and necroptosis.  

Methods: Hepatocyte apoptosis was assessed in pre- and post-OLT liver biopsies via immunohistochemistry for M30 CytoDEATH and cleaved PARP (cPARP). PMoH harvested from C57BL/6 mice and HuH7 cells were infected with adenoviral constructs encoding HCV structural (rAdHCV-CoreE1E2) or non-structural (rAdHCV-NS3–5B) genes, in the presence or absence of physiologically relevant concentrations of cyclosporine and/or mycophenolate mofetil (MMF). Pan-caspase inhibitor Q-VD-Oph and RIP-kinase inhibitor necrostatin-1 were used to inhibit apoptosis and necroptosis respectively. Cell viability and apoptosis were evaluated using crystal violet assays and Western immunoblots probed for cleaved caspase 3 (cCasp3) and cPARP.  

Results: Pre-OLT, patients with HCV cirrhosis had a 2.7–3.5- and 7.8–fold increase in M30 CytoDEATH and cPARP compared to non-HCV cirrhotics and non-cirrhotics respectively. Patients with post-OLT HCV recurrence had a 4–6-fold increase in M30 CytoDEATH and cPARP compared to HCV-negative post-OLT patients. In PMoH and HuH7, cyclosporine alone had minimal effect on cell death. MMF improved cell viability and reduced apoptosis, although the combination of cyclosporine and MMF reduced cell viability by 2.1-fold and increased cCasp3 and cPARP by 2.5–2.8-fold. rAd-HCV infection reduced cell viability by 1.6-fold and increased cCasp3 and cPARP by 2.4–3.2-fold. The addition of cyclosporine and MMF to HCV infection, particularly in combination, further reduced cell viability and increased cCasp3 and cPARP. Both Q-VD-Oph and necrostatin-1 significantly improved cell viability but only Q-VD-Oph reversed hepatocyte apoptosis.  

Conclusions: Hepatocyte apoptosis was significantly increased in livers of HCV-infected patients pre- and post-OLT compared to HCV-negative patients. Immunosuppressants increased HCV-mediated hepatocyte cell death, but these effects were reversed by the addition of a pan-caspase inhibitor. These results provide an insight into the mechanisms responsible for accelerated liver fibrosis and possible novel therapeutic targets in post-transplant HCV.

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ANTI-HCV DYNAMICS FOLLOWING ACUTE HCV INFECTION IN HIV-INFECTED MEN WHO HAVE SEX WITH MEN  

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Introduction: Loss of hepatitis C virus (HCV) antibodies, indicated by lower anti-HCV reactivity and ultimately seroreversion, has been reported in both acute and chronic HCV infection, but all in the absence of HIV-infection. We describe anti-HCV dynamics in HIV-infected men who have sex with men (MSM) during and following acute HCV infection.  

Methods: Anti-HCV dynamics were studied in 48 HIV-infected MSM with acute HCV infection with <6 months between the last negative and the first HCV RNA positive test. Presence of HCV RNA was determined using the TMA VERSANT® HCV RNA Qualitative Assay (Siemens). Anti-HCV reactivity was measured annually using the AxSYM HCV v3.0 Assay (Abbott) during a median follow-up of 3.3 years (IQR 0.7–5.0 years). Kaplan Meier methods were used
to calculate time to development and subsequent loss of HCV antibodies.

**Results:** Among 48 MSM, 54 HCV (re-)infections were documented. Most frequent genotypes were 1a (71%) and 4d (20%). Median age was 42 years (IQR 36–47). Median time between the last negative and the first positive HCV RNA test was 114 days (IQR 80–137). The first positive HCV RNA sample had detectable HCV antibodies in 12/48 subjects; median time to HCV seroconversion was 60 days (IQR 0–112). HCV was spontaneously cleared in 3 subjects and 23 subjects were treated during the acute phase of infection, resulting in a sustained virologic response (SVR) in 15/23 (65%). Interestingly, all 23 were treated during the acute phase of infection, resulting in a sustained virologic response (SVR) in 15/23 (65%).

Seroreversion was common when compared to reports on mono-infected patients. Upon reinfection, antibody response seems comparable to the response after initial infection. This may indicate that monitoring anti-HCV reactivity could be a cost-effective approach for diagnosis of reinfection. The implementation of Prometheus index represents a useful and inexpensive non-invasive tool for the prediction of sustained virological response in patients coinfected with HCV-HIV.

**Conclusions:** Having elected the convenient cut-off points, this study concludes that Prometheus index represents a useful and inexpensive non-invasive tool for the prediction of sustained virological response in patients coinfected with HCV-HIV.

**Background:** The rate of liver fibrosis progression and the development of hepatocellular carcinoma in hepatitis C virus and human immunodeficiency virus (HCV-HIV) coinfected patients are faster than HCV monoinfected individuals. The treatment of HCV with pegylated interferon plus ribavirin is controversial, especially HCV-HIV coinfected individuals whom show suboptimal response. Recently, Prometheus index (PI) has been proposed as a predictive index of sustained virological response (SVR) in patients coinfected with HCV and HIV whom received this treatment. Therefore, it could be clinically relevant to evaluate the diagnostic efficacy of PI as a screening of patients affected of HCV-HIV in our population. The implementation of Prometheus index represents a useful and inexpensive non-invasive tool for the prediction of sustained virological response in patients coinfected with HCV-HIV.

**Introduction and Objectives:** IL28B CC polymorphism is associated with a better virological response in patients with chronic hepatitis B (HBV) or C (HCV). However, the effects of this polymorphism on immune functions of the liver, the site of virus production and the mechanism of SVR remained still known. The aim of this work was to study and to compare the degranulation activity of liver immune cells in patients with histological proven chronic hepatitis B or C. The implementation of Prometheus index represents a useful and inexpensive non-invasive tool for the prediction of sustained virological response in patients coinfected with HCV-HIV.

**Conclusions:** Among HIV-infected MSM, a relatively fast decline of anti-HCV reactivity was associated with viral clearance. Seroreversion was common when compared to reports on mono-infected patients. Upon reinfection, antibody response seems comparable to the response after initial infection. This may indicate that monitoring anti-HCV reactivity could be a cost-effective approach for diagnosis of reinfection.

**Methods:** From a total population of 103 HCV infected patients, a group of 32 HCV-HIV coinfected individuals were included in this prospective study. These patients had completed a course of pegylated interferon plus ribavirin therapy. PI was calculated using the variables liver stiffness (in kPa), HCV genotype (1 and 4 versus 2 and 3), HCV RNA level (in log IU/mL) and the rs12979860 polymorphism of IL28B (CT or TT versus CC). The liver stiffness was measured using FibroScan. The diagnostic accuracy of PI was evaluated by ROC curve analysis. The cut-off points used from ROC curves were: 0.25, 0.50 and 0.75. Statistical analysis was performed using SPSS 17.0.

**Results:** The mean age was 37.4 (SD: 6.9) and 65.6% of the population were men (n=21). The area under the ROC curve (AUC) for predictive index of SVR was 0.847 [95% confidence interval (CI): 0.696–0.998] which was statistically significant (p=0.001). The AUC and predictive values for the cut-off points 0.25, 0.50 and 0.75 were: AUC=0.567 (p>0.05), positive predictive value (PPV)=56.7%, negative predictive value (NPV)=100%; AUC=0.700 (p=0.05), PPV=65.4%, NPV=100% and AUC=0.782 (p=0.002), PPV=81.3%, NPV=75%, respectively. We improved the predictive SVR establishing a cut-off point of 0.80 or PI=80% [AUC=0.816 (p=0.002), PPV=86.7%, NPV=76.5%, OR=18.5 (95% CI: 3.2–165.9)].

**Conclusions:** Having elected the convenient cut-off points, this study concludes that Prometheus index represents a useful and inexpensive non-invasive tool for the prediction of sustained virological response in patients coinfected with HCV-HIV.
was significantly higher in HCV patients ($p=0.012$ and $p=0.003$) respectively than in HBV patients.

**Conclusion:** These results suggested that intra-hepatic T lymphocytes activity is higher in patients with chronic hepatitis C and IL28B CC than in HBV patients. In HBV patients, IL28B polymorphism don't play a role in immune response.

**1183 ANTIVIRAL SAFETY AND PK STUDIES OF A NOVEL HIGHLY POTENT HCV INHIBITOR ZN2007 EXCELLENT FOR CLINICAL TRIALS**

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**Background:** Hepatitis C virus (HCV) is a single-stranded positive RNA virus and becoming a major health problem. It is confirmed that NS3 protein in HCV possesses serine protease activity and is essential for viral replication and infectivity.

**Results:** This presentation discloses development of a novel antiviral compound highly potent as one of the best HCV NS3 protease inhibitor. It is found that a novel HCV inhibitor ZN2007 is not only highly potent (EC$_{50}$ for Ia, Ib, Ia and Iva, respectively) but also shows excellent safety and PK in all tested rats, dogs and monkeys. There is no any serious side effect determined in combination of ZN2007 with different potential targets such as hERG, Cytochrome P450, Chymotrypsin, Chymase, Cathepsins, etc, respectively. ZN2007 shows negative for the Ames, chromosome aberration, bacterial reverse mutation and rat bone marrow micronucleus assay. Furthermore, its metabolic stability in liver and plasma is very good and much better than other two referred micronucleus assay. Furthermore, its metabolic stability in liver and plasma is very good and much better than other two referred.

**Conclusions:** ZN2007 has finished all of preclinical studies, and its antiviral compound is determined as one of the best HCV NS3 protease inhibitors. It is found that a novel HCV inhibitor ZN2007 is not only highly potent (EC$_{50}$ for Ia, Ib, Ia and Iva, respectively) but also shows excellent safety and PK in all tested rats, dogs and monkeys. There is no any serious side effect determined in combination of ZN2007 with different potential targets such as hERG, Cytochrome P450, Chymotrypsin, Chymase, Cathepsins, etc, respectively. ZN2007 shows negative for the Ames, chromosome aberration, bacterial reverse mutation and rat bone marrow micronucleus assay. Furthermore, its metabolic stability in liver and plasma is very good and much better than other two referred micromucleus assay. Furthermore, its metabolic stability in liver and plasma is very good and much better than other two referred.

**80d. VIRAL HEPATITIS C: CLINICAL (NEW COMPOUNDS, RESISTANCE)**

**1184 FRAGMENT BASED DRUG DISCOVERY OF NEW CYCLOPHILIN INHIBITORS UNRELATED TO CYCLOSPORINE A THAT POTENTLY INHIBIT HCV REPLICATION**

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Cyclophilin A, a host cell peptidyl prolyl cis-trans isomerase, is required for efficient HCV replication. Cyclophilin inhibitors appear as very promising candidates for the treatment of HCV infection. However, cyclophilin inhibitors in development are all derived from cyclosporine A, raising a number of issues regarding their synthesis and tolerability, emphasizing the need for new families of cyclophilin inhibitors unrelated to cyclosporine A.

**Methods and Results:** We used Fragment-Based Drug Design (FBDD), an original approach based on X-ray crystallography and nuclear magnetic resonance spectroscopy (NMR), to rationally design a new family of potent inhibitors of cyclophilins. Our hit compound exhibited IC50s of 0.1, 0.3, and 0.1 micromolar against cyclophilin A, B and D, respectively. This compound was found to have sub-micromolar anti-HCV activity in vitro (EC50: 0.2 micromolar in the genotype 1b subgenomic replicon). This antiviral activity was not influenced by the HCV genotype and the compound had a high barrier to the emergence of resistance. Early ADMX experiments showed promising "drug-like" properties, with high solubility and stability, high absorption potential, low shifts in biological potential in presence of human plasma and a moderate predicted clearance. The compound was not a substrate for CYP1A2, CYP2B6, CYP3A4 and a moderate substrate for CYP2C19 and CYP2D6. It moderately inhibited CYP2C8 but potently inhibited CYP3A4, with no activity on the human aryl hydrocarbon receptor (AhR) at concentrations up to 50µM. This new original chemical structure will avoid class issues associated with cyclosporine analogues. Our hit cyclophilin inhibitor represents a very promising candidate for subsequent optimization and clinical development in anti-HCV therapy.
POSTERS

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ITPA GENE VARIANTS PREDICT HEMOLYTIC RIBAVIRIN INDUCED ANAEMIA IN PATIENTS TREATED WITH THE INTERFERON-FREE REGIMEN OF FALDAPREVI, BI207127 AND RIBAVIRIN IN SOUND-C2

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Background and Aims: Single nucleotide polymorphisms (SNPs) in the inosine triphosphatase (ITPA) gene have been found to be associated with haemolytic anaemia and ribavirin dose reductions in HCV patients treated with pegylated interferon and ribavirin. However, anaemia is aggravated by the myelosuppressive effects of pegylated interferon and the effect of ITPA polymorphisms on interferon-free treatments is unknown. We examined the effect of two ITPA SNPs on events associated with anaemia in patients treated with faldaprevir, BI 207127 and ribavirin in the SOUND-C2 study in which up to 85% of patients achieved SVR but >10% of patients experienced anaemia and 6% had ribavirin dose reductions.

Methods: In this open-label Phase 2b study, 362 treatment-naive patients with genotype 1 HCV were randomised. Two SNPs within the ITPA gene region, rs1127354 and rs6051702, were genotyped by melting curve analysis in 314 patients. ITPA genotypes were defined as favourable (rs1127354 AA or AC and rs6051702 CC or CA) or unfavourable (rs1127354 CC and rs6051702 AA) in terms of their association with haemolytic anaemia. Anaemia (haemoglobin <10 g/dL), ribavirin dose reduction and erythropoietin use were assessed for patients with favourable and unfavourable SNPs.

Results: Twenty-nine ligands out of forty were identified to bind to five cavities within the E2 binding site on CD81. Four of these molecules effectively block E2 binding to CD81-LEL and have been used as drug leads to design a series of compounds. Four other ligands of low to moderate affinity were used to synthesize the first two bidentate HCV SHALs targeting the E2 binding site on CD81 by solid phase synthesis.

Conclusion: Two SHALs have been synthesized and will be further tested for their inhibitory effect on HCV entry.

Table 1 (abstract 1186).

<table>
<thead>
<tr>
<th>ITPA SNP and genotype</th>
<th>rs1127354 AA or AC</th>
<th>rs1127354 CC</th>
<th>rs6051702 CC or CA</th>
<th>rs6051702 AA</th>
<th>Unfavourable genotype at both positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin &lt;10g/dL, n (%)</td>
<td>0 (0)</td>
<td>32 (12)</td>
<td>7 (6)</td>
<td>25 (12)</td>
<td>25 (13)</td>
</tr>
<tr>
<td>Ribavirin dose reduction for anaemia*, n (%)</td>
<td>4 (9)</td>
<td>14 (5)</td>
<td>6 (5)</td>
<td>12 (6)</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Erythropoietin use, n (%)</td>
<td>0 (0)</td>
<td>9 (3)</td>
<td>2 (2)</td>
<td>7 (3)</td>
<td>7 (3)</td>
</tr>
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</table>

*Anaemia as an adverse event as defined by investigators (not a laboratory event).
Background: The effect of viral kinetic (VK), pharmacokinetic (PK) and pharmacodynamic (PD) modeling in predicting response to direct acting antiviral (DAA) Interferon-free therapy has not been fully elucidated. The objective of this study was to evaluate the ability of a detailed PK–PD modeling in predicting treatment response to an interferon-free anti-HCV DAA therapy.

Methods: Sixty HCV GT-1 subjects were treated with the potent NS5B inhibitor GS-7977 and either full dose or low dose ribavirin (RBV) for 24 weeks. A substudy of 25 patients underwent an intense early VK/PK study with frequent blood draws for HCV viral load monitoring and drug levels of GS-7977 and its metabolite GS-331007. A mathematical model of viral kinetics accounting of the pharmacokinetics was used to estimate efficacy and viral kinetic curves. Overall, viral clearance rate (c), infected cell loss (delta) and mean and maximum efficacy (epsilon) were estimated.

Results: All 25 patients in this substudy showed a rapid early virologic decline and maintained viral suppression through the end of treatment. Six patients relapsed after treatment completion while 18 patients maintained viral suppression through SVR8 (range: 8–24 wks. post treatment completion). One patient discontinued treatment at week 3 voluntarily and was lost to follow up. Median values of mean and maximum estimated efficacy was 99.87% and 99.94% in the patients who relapsed compared to 99.94% and 99.97% in patients without relapse (p = 0.0058 and p = 0.047), respectively. Median infected cell loss rate delta was 0.27/day and 0.19/day in patients with and without relapse respectively (p = 0.14). Median estimated viral clearance rate was comparable in both groups (4.2 and 4.0/day, p > 0.20). There were no significant associations observed in these parameters between low and full dose ribavirin, genotype 1a and 1b, CC and non-CC IL28B genotype, early or advanced fibrosis, gender, BMI.

Conclusions: GS-7977 in combination with RBV resulted in high efficacy rates. A detailed PK–PD analysis showed significant differences in efficacy between relapsers and patients achieving a sustained virologic response. However, estimates of infected cell loss and viral clearance rate (c) were comparable to that observed in previous studies with interferon and ribavirin treatment.
were tracked via 454 sequencing over a 10 year period in two patients who failed to achieve viral clearance after SOC combination therapy supplemented with BI 201335. Frequencies of characterised resistance mutations pre- and post-therapy were also assessed. Treatment failure protease genes were engineered into sub-genomic replicons (SGRs) for phenotyping.

**Methods:** 454 sequencing was performed on amplicons generated from seven pre-treatment samples, in addition to one post-treatment sample, for both patients. Nested PCR, followed by barcoded adaptor ligation and bidirectional sequencing ensured complete protease gene coverage at high-depth. Genomic cDNA input for each 454 amplicon was ascertained via qPCR to avoid making erroneous claims about viral population diversity. Pre- and post-therapy protease genes were engineered into SGRs for cross-resistance testing.

**Results:** 454 sequence re-sampling due to low genomic copy number input was identified by qPCR. Protease amino acid population consensuses remained stable for over a decade in both patients, with a large excess of synonymous mutations observed. Post BI 201335 therapy, a quantitative reduction in sites exhibiting polymorphism occurred. In one patient, enrichment of Q80L mutations (7667/8104 reads) was observed. This mutation was present in pre-therapy samples, but below conventional sequencing detection levels (2135/5152 reads). Engineering the Q80L mutation into SGRs, as well as pre- and post-treatment proteases, indicate Q80L does not confer cross-resistance to Boceprevir or Telaprevir.

**Conclusions:** Quantification of viral genomic input prior to 454 sequencing is essential to avoid resampling. 454 sequencing reveals a quantitative reduction in the total amount of sites exhibiting polymorphism post BI 201335 therapy, indicating a population bottleneck occurred. Enrichment of Q80L mutations occurred post-treatment. Interestingly, previous studies indicate Q80L does not confer resistance to BI 201335 and our phenotyping suggest Q80L does not confer cross-resistance to Boceprevir or Telaprevir. Future therapeutic options are thus not compromised. Additionally these data suggest Q80L is not selected for during therapy, and is selectively neutral, but becomes fixed in the population due to the post-therapy bottleneck.

**S190 STUDY OF ABT-267 2-DAY MONOTHERAPY FOLLOWED BY 12-WEEK COMBINATION THERAPY IN TREATMENT-NAÏVE PATIENTS WITH CHRONIC HCV GENOTYPE 1 INFECTION**


**Background:** ABT-267 is an HCV NSSA inhibitor currently being studied in combination with ABT-450/r (HCV protease inhibitor dosed with ritonavir, identified as a lead compound by Abbott and Enanta) and ABT-333 (non-nucleoside HCV NS5B inhibitor) +/− ribavirin (RBV) for treatment of patients with chronic HCV infection. This study assessed safety and efficacy of two doses of ABT-267 monotherapy followed by 12 weeks of combination therapy supplemented with the pegIFN-free combination regimen of ABT-267+ABT-450/r+ABT-333+RBV achieved SVR12.

**Methods:** Antiviral activity was studied in 3-day HCV replicon assays using stable or transient replicons encoding the NSSA gene from different genotypes. Site-directed mutagenesis was used to introduce major polymorphisms or resistance mutations. Colony reduction assays were performed in stable GT1–4 and GT6 replicon cells to assess resistance barriers.

**Results:** GS-5816 has potent and consistent antiviral activity against representative GT1–6 HCV strains (EC50=7–9 pM; Table-1) with low cytotoxicity (CC50>44 mM). Relative to nM potencies for the first-generation NSSA inhibitors, GS-5816 has potent activity against consensus GT2a and GT2b replicons encoding the dominant NSSA residue M31 (EC50>17 and 10 pM respectively). Furthermore, GS-5816 has consistent potency (EC50 values varying <3-fold) against >50 replicons representing >90% of NSSA polymorphism in GT1–4. In colony reduction assays and in vitro resistance selections
GS-5816 has a significantly higher resistance barrier than the first-generation NS5A inhibitors; and GS-5816 has potent activity against major clinical resistance mutations (e.g. EC_{50} = 130 and 121 pM against GT1a L31M and Y93C respectively, and 210 pM against GT3a A30K). GS-5816 is fully-active against mutants resistant to other classes of HCV inhibitors (e.g. Protease inhibitor resistant mutants R155K or D168Q in NS3, and nucleotide NS5B inhibitor resistant mutant S282T in NS5B), and shows additivity to moderate synergy when combined with other anti-HCV agents including sofosbuvir.

**Conclusions:** GS-5816 is a second-generation HCV NS5A inhibitor with potent and selective antiviral activity, broad genotypic and polymorphic coverage, and a favorable resistance profile for HCV genotypes 1 to 4 and 6. This preclinical activity profile of GS-5816 together with its preclinical pharmacokinetics supports its development as part of a regimen to treat chronic HCV infection worldwide.

### Table 1. Broad Genotypic Activity of GS-5816

<table>
<thead>
<tr>
<th>Genotype</th>
<th>EC_{50} (pM)</th>
<th>GT1a</th>
<th>GT1b</th>
<th>GT2a</th>
<th>GT2b</th>
<th>GT3a</th>
<th>GT4a</th>
<th>GT5a</th>
<th>GT6a</th>
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<td>GT1b</td>
<td>H77</td>
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<td>15</td>
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<td>17</td>
<td>10</td>
<td>13</td>
<td>9</td>
<td>59</td>
</tr>
<tr>
<td>Con-1</td>
<td>MD2b</td>
<td>SS2</td>
<td>ED43</td>
<td>SA13</td>
<td>HK6a</td>
<td></td>
<td></td>
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<td>Day 24</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Day 31</td>
<td></td>
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**1192 VAST MAJORITY OF DETECTED NSSA RESISTANT VARIANTS ARE NOT AMPLIFIED IN HCV PATIENTS DURING 3-DAY MONOTHERAPY WITH THE OPTIMIZED NSSA INHIBITOR PPI-668**

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**Background:** HCV NS5A inhibitor PPI-668 was assessed in a 3-Day Phase 1b monotherapy trial in HCV gt-1 patients using QD oral doses of 40, 80, 160 and 240 mg, with 8/10 patients receiving PPI-668 per cohort. Mean maximal viral load reductions of 3.54–3.75 log_{10} IU/mL were observed with doses ≥280 mg QD. A fifth cohort of gt-2/3 patients (160 mg QD) was also evaluated.

**Methods:** Comprehensive monitoring of HCV resistant variants was performed during dosing and post-dosing periods. HCV RNA was extracted from patient plasma samples, RT-PCR amplified and the resulting DNA subjected to population sequencing. Clonal sequencing was performed to determine if detected substitutions were genetically linked. PPI-668 susceptibility was assessed in transient transfection assays using HCV replicons encoding specific substitutions or population NS5A gene inserts from clinical samples.

**Results:** Known NS5A primary resistance substitutions at residues 28, 30, 31 and 93 were detected early during PPI-668 monotherapy. Among the 40 enrolled gt-1 patients, eight (one placebo) had detectable PPI-668 resistant substitutions at baseline. One patient (gt-1b 240 mg) was a non-responder and fully resistant at baseline, with 100% of his circulating virus encoding genetically linked R30Q+L31I+Y93H substitutions. The other six PPI-668 treated patients with baseline resistance substitutions responded well (RNA reductions of 2.82 to 3.95 log_{10} IU/mL). Resistance substitutions became detectable in all but one PPI-668 treated patient by 24–48 hr, as WT virus was rapidly eliminated. Importantly, these observed substitutions (≤30% of patient population) were not further amplified with continued monotherapy, suggesting that PPI-668 concentrations were sufficient to suppress these single-substitution HCV variants. Susceptibility (EC_{50}) of replicons derived from PPI-668 treated patient samples were generally at or below C_{mn} levels, confirming the advantageous PK profile of PPI-668 and its ability to cover single-substitution resistant variants. No significant differences were observed in the overall resistance patterns across the four PPI-668 treated gt-1 cohorts.

**Conclusions:** NS5A resistance variants frequently pre-exist among HCV patients, emphasizing the need for combination therapy and optimized NS5A inhibitors such as PPI-668 that achieve plasma/liver levels high enough to suppress single substitution HCV variants. Further studies of PPI-668 in combination with other DAAs are warranted.

**1193 RESISTANCE ANALYSES USING DEEP AND POPULATION SEQUENCING AFTER 3 DAY MONOTHERAPY WITH GS-9669, A NOVEL NON-NUCLEOSIDE NSSB INHIBITOR IN GENOTYPE 1 HCV PATIENTS**

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**Background:** GS-9669, a novel NS5B non-nucleoside inhibitor (NNI, site II), displayed potent antiviral activity in HCV genotype (GT) 1 subjects during a multiple ascending dose clinical trial at 50, 500 mg QD and 50 mg, 100 mg, 500 mg BID for 3 days. This study characterizes the virologic resistance observed in this phase I trial.

**Methods:** The full-length NS5B gene was amplified and population sequenced for all patients (GT1a, n=50; GT1b, n=20) at baseline (BL), Day 4 (or earlier timepoint if viral load was <1000 IU/mL) and Day 17. Illumina deep sequencing analysis was performed for GT1a (n=7) and GT1b (n=7) patients dosed with 500 mg QD at multiple timepoints through Day 17 (n=90). NS5B from 46 patients was cloned into an NS5B shuttle vector and phenotypic analyses were performed.

**Results:** Population sequencing showed that from the on-treatment (Day 1 or 2) or end of treatment (Day 4 or 5) samples, resistance-associated mutations (RAMs) at NS5B positions A486, R422 and L419 were detected in the majority of patients who received 500 mg QD and ≥50 mg BID, but only in 1/8 patients receiving 50 mg QD. Substitutions at position M423 were observed only in GT1a patients receiving low doses (50 and 100 mg BID). Deep sequencing detected RAMs as early as 24 hr post dosing in 5/8 patients receiving 500 mg QD. At end of treatment, 100% of tested samples had at least 4 RAMs by deep sequencing compared to 75% by population sequencing. On Day 17, mutations were still detected in 92% of patients (compare to 44% by population sequencing). New mutations in GT1b isolates (M423T, V494A/I I482N/T) were observed by deep sequencing but not by population sequencing. Phenotypic analysis demonstrated that viral isolates with multiple RAMs had reduced susceptibility to GS-9669 and VX-222, but wild-type susceptibility to other classes of HCV inhibitors including sofosbuvir (NI), GS-9451 (PI), GS-5885 (NS5A) and ribavirin.

**Conclusions:** Similar to other NNIs, RAMs were detected shortly after suppression of the wild-type virus. The lack of cross-resistance between GS-9669 resistant-mutants and sofosbuvir, GS-5885, GS-9451 and ribavirin, makes GS-9669 a candidate for use in combination with these inhibitors.

**POSTERS**
NS5A INHIBITOR WITH POTENTIAL FOR ONCE-DAILY DOSING

GS-5816, A NOVEL SECOND GENERATION BROAD-GENOTYPIC

provide efficient strategies to prevent liver graft infection.

or sofosbuvir by taking advantage of synergy and marked efficacy

efficiently inhibited HCV infection of DAA-resistant viruses.

IC50 of the compounds up to 100-fold. Furthermore, entry inhibitors

persistent HCV infection over a broad range of concentrations with

sofosbuvir resulted in a marked and synergistic inhibition of

Results:

in combination with DAAs, including polymerase (sofosbuvir) and

antiviral efficacy and toxicity of entry inhibitors (receptor-specific

for prevention and treatment of HCV infection, we investigated the

Methods:

Background and Aims: Although the clinical development of
direct-acting antivirals (DAAs) improves the virological
response of standard-of-care of chronic hepatitis C virus (HCV)
infected, adverse effects and resistance remain major challenges.
Furthermore, strategies for prevention of liver graft infection are absent and options for difficult-to-treat patients remain unsatisfactory. Thus, there is an unmet medical need for novel antiviral approaches with improved efficacy and safety. Viral entry is required for initiation and maintenance of infection and a promising target for antiviral therapy. Indeed, we have shown that compounds targeting HCV cell entry factors efficiently inhibit infection of all major HCV genotypes and resistant strains (Fofana et al. Gastroenterology 2010, Lupberger et al. Nat. Med. 2011, Zahid et al. Hepatology 2012).

Methods: To explore the potential of entry inhibitors as antivirals for prevention and treatment of HCV infection, we investigated the antiviral efficacy and toxicity of entry inhibitors (receptor-specific monoclonal antibodies, kinase inhibitors erlotinib and dasatinib) in combination with DAAs, including polymerase (sofosbuvir) and NSSA (daclatasvir) inhibitors, in cell culture models. Synergy was assessed by two independent methods comprising the combination index and the method of Prichard and Shipman.

Results: Combinations of entry inhibitors with daclatasvir or sofosbuvir resulted in a marked and synergistic inhibition of persistent HCV infection over a broad range of concentrations with undetectable toxicity. Combinations of receptor-specific monoclonal antibodies or erlotinib with daclatasvir or sofosbuvir decreased the IC50 of the compounds up to 100-fold. Furthermore, entry inhibitors efficiently inhibited HCV infection of DAA-resistant viruses.

Conclusion: Our results provide the rationale for the development of antiviral strategies combining entry inhibitors with daclatasvir or sofosbuvir by taking advantage of synergy and marked efficacy against resistant viruses. The uncovered combinations open novel perspectives for IFN-α-free regimens in multiresistant patients and provide efficient strategies to prevent liver graft infection.

1195

HEALTHY VOLUNTEER FIRST-IN-HUMAN EVALUATION OF GS-5816, A NOVEL SECOND GENERATION BROAD-GENOTYPIC NSSA INHIBITOR WITH POTENTIAL FOR ONCE-DAILY DOSING

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Background: GS-5816, a novel second generation NSSA broad-genotypic inhibitor, is in development for the treatment of chronic HCV infection. GS-5816 demonstrates 7–59 picomolar antiviral potency against GT1–6 replicons and is not cross-resistant with mutants resistant to other classes of HCV inhibitors. In nonclinical studies, GS-5816 exhibits modest bioavailability (25–30%), high metabolic stability, low systemic clearance and low potential for metabolic drug–drug interactions. This Phase 1 first-in-human study evaluated the pharmacokinetics (PK), safety and tolerability of GS-5816 in healthy volunteers.

Methods: This was a randomized, double-blind, placebo-controlled study with 4 staggered dose-escalation and 2 food effect (FE: low calorie and high-calorie/high fat) cohorts. Within each dose-escalation cohort, unique healthy subjects were randomized to receive single (SD) and multiple (MD) daily doses (7 days) of GS-5816 (N = 12) or matching placebo (N = 3) of 5 mg, 50 mg, 150 mg or 450 mg in the fasted state, with a 5-day washout between SD and MD. In FE cohorts, subjects (N = 12/cohort) received SD GS-5816 100 mg in the fasted and fed states with a 5-day washout in-between. GS-5816 PK parameters were estimated and summarized by dose; dose proportionality of GS-5816 was examined across evaluated doses. FE was assessed. Safety was examined throughout the study.

Results: All enrolled subjects completed the study. GS-5816 was well-tolerated at all doses; no subjects discontinued therapy early, and no clinically significant laboratory or ECGs abnormalities were observed. GS-5816 was absorbed quickly following single and multiple fasted oral doses with the maximum plasma concentrations (Cmax) occurring between 1.50 and 3.25 hours (median Tmax). GS-5816 exhibited nonlinear PK from 5 mg to 50 mg and from 100 mg to 450 mg. Median Tmax ranged from 13–17 hours, supporting QD dosing. The mean plasma concentrations 24 hours post-dose were >70-fold above the protein-adjusted EC50 for all genotypes at all doses ≥50 mg. FE cohorts are under evaluation.

Conclusion: GS-5816 was well tolerated as a single or multiple daily doses in healthy subjects. The in-vitro antiviral potency, safety and PK profile of GS-5816 supports further evaluation of its antiviral activity in HCV-infected subjects.
second phase of viral decline. Combining the drugs did not led to a substantial increase in viral decline kinetics.

1197 MK-5172 IN COMBINATION WITH PEG-INTERFERON/RIBAVIRIN DEMONSTRATES ROBUST EFFICACY AND A LOW RATE OF RESISTANCE-ASSOCIATED VIROLOGIC FAILURE IN TREATMENT NAIVE GENOTYPE 1 CHRONIC HCV-INFECTED PATIENTS

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Background: MK-5172 is a potent inhibitor of HCV NS3/4A protease which maintains potency across a broad array of resistant variants. In a Phase 2a study, treatment-naive HCV G1-infected patients received MK-5172 100-, 200-, 400-, or 800-mg QD + PR for 12 weeks followed by an additional 12 weeks (RVR achieved) or 36 weeks (no RVR) of PR. Virus isolated from patients who did not achieve SVR was further analyzed for resistance.

Methods: Samples from patients who have not achieved SVR were selected for resistance analysis. Samples (viral load >1000 IU/mL) were analyzed by population-based sequencing of NS3/4A, with select samples further analyzed by clonal or deep sequencing.

Results: Among 266 patients assigned to MK-5172+PR, 82 to 96% achieved SVR. At baseline, 33 had variants associated with reduced susceptibility to other protease inhibitors. Six patients experienced protocol-defined virologic failure. None had such baseline resistance variants. One of the 6 “failures” was actually a de novo HCV gt3a infection (no baseline detection of HCV gt3a by deep sequencing). Four cases occurred among patients who had low/undetectable levels of MK-5172 prior to failure. Three had sufficient viral titers at failure for analysis; population sequencing showed that two had no polymorphisms at failure, while the third had no polymorphisms within protease. Clonal analysis is ongoing and full dataset will be presented; interim analysis for two of these patients shows predominantly wild-type sequences with a minority of sequences encoding variants within protease. In the final case a patient achieved undetectable HCV RNA while on MK-5172+PR and for an additional 16 weeks of PR before experiencing failure.

Conclusions: MK-5172+PR demonstrated robust antiviral activity. The high response rate was achieved despite the presence in ~10% of patients of baseline resistance to other protease inhibitors in development. The present work demonstrates a low rate of resistance-associated virologic failure among patients who received MK-5172+PR. The low MK-5172 concentration in four patients at the time of breakthrough suggests the cause of viral breakthrough was the result in part of non-adherence.
1200
FINDINGS FROM CLINICAL VIROLOGY STUDIES ON SOVAPREVIR, A PHASE 2 HCV NS3 PROTEASE INHIBITOR, INDICATE A HIGH PHARMACOLOGICAL BARRIER TO VIRAL RESISTANCE

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Background: Sovaprevir (ACH-1625) is a potent HCV NS3 protease inhibitor in phase 2 that will be investigated in interferon-free regimens. In phase 1b 5-day monotherapy studies, sovaprevir at 400 mg QD demonstrated robust antiviral activity in both GT-1a and 1b hepatitis C patients. In phase 2a studies, the combination of sovaprevir at doses as low as 200 mg QD with PEG-IFN and RBV (P/R) in GT-1 hepatitis C patients led to significantly improved virologic responses as compared to P/R alone. Here we summarize viral genotypic and phenotypic analyses of patient plasma samples collected from these studies.

Methods: PCR products from plasma samples were analyzed by population or clonal sequencing. A transient replicon assay was used for phenotypic analyses of replicons carrying NS3 protease from patients or with specific mutations of interest.

Results: The mean EC50 values of sovaprevir against chimeric replicons carrying NS3 protease from GT-1a or GT-1b clinical isolates differed by less than 3-fold. Consistent with this observation, comparable HCV RNA reductions were observed in GT-1a and 1b hepatitis C patients administered with the same monotherapy dose. Viral mutations associated with in vitro sovaprevir resistance were detected during monotherapy at frequencies correlated with HCV RNA reduction, indicating enrichment of pre-existing resistant variants. Nevertheless, HCV RNA declined continuously during 5-day treatment even in patients with high frequencies of resistant variants 24 hours after the first dose, indicating the clinical efficacy of sovaprevir against these variants. During the 12-week dosing period with sovaprevir plus P/R, no subjects experienced viral breakthrough, including one subject with an R155K variant at baseline.

Conclusions: During sovaprevir monotherapy, resistant variants were readily uncovered yet viral rebound was not observed. Rather, continuous HCV RNA decline was observed even in those patients in whom resistant variants were detected at high frequencies 24 hrs after the first dose. Combined with the observation that no viral breakthrough occurred during the 12-week dosing period with sovaprevir plus P/R, these results indicate that sovaprevir is able to impose a high pharmacological barrier to viral resistance in vivo, thereby warranting clinical investigation in interferon-free regimens.
30 days. Patients were followed for 60 days for safety parameters and serum levels of liver enzymes, virus, cytokines, and regulatory T cells.

**Results:** Oral anti-CD3 immunotherapy was safe and well tolerated; no treatment-related adverse events were noted. The following improvements were noted relative to pretreatment levels in statistical analyses of data for treatment groups: HCV viral load – Day 30 [−10.9X10^5 group B (p < 0.10), and −1.7X10^5 group A], AST – Day 30 [−9.7 u/L group D (p < 0.10), −2.8 u/L group A], ALT – Days 14 [−11.7 u/L group D (p < 0.04), 0.6 u/L group A] and 21 [−8.8 u/L group D (p < 0.08), +0.8 u/L group A]. These effects were associated with promotion of regulatory T cells (CD4^+CD25^+) – Day 30 [+0.31 group C (p < 0.02), −0.02 group A]. The positive effects were somewhat more apparent in subjects with initially elevated liver enzyme levels.

**Conclusions:** Oral anti-CD3 MAb immunotherapy for non-responder HCV patients was safe and well tolerated. Trends and statistically significant improvements were observed as reductions in viral load and liver enzyme levels along with an increase in regulatory T-cell levels. These data support a role for the immune system in the pathogenesis of HCV infection, and suggest that this immunotherapy is worthy of evaluation in combination with HCV anti-viral drugs.

**1203 SAFETY PROFILE OF DACLATASVIR IN COMBINATION WITH PEGINTERFERON alfa AND RIBAVIRIN IN 1100 PATIENTS WITH CHRONIC HCV INFECTION TREATED IN PHASE 2 STUDIES**

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**Background:** Daclatasvir, a selective HCV NSSA replication complex inhibitor in phase 3 development for treatment of chronic HCV infection, has demonstrated pangenotypic activity and high SVR rates in combination with peginterferon/ribavirin (alfa/RBV) or other direct-acting antivirals. This analysis includes data from six phase 2 studies of daclatasvir+alfa/RBV in treatment-naive and experienced patients of varying ethnicities.

**Methods:** All available safety data through February 2012 from patients receiving daclatasvir in combination with alfa/RBV were aggregated into 3 groups by regimen and compared with placebo/alfa/RBV.

**Results:** Analysis included 1100 patients: 64% male, mean age 50.5 years, 78.7% Caucasian, 8.5% black, 10.4% Asian, 52.5% HCV genotype (GT) 1a, 30.5% GT1b, 6.5% GT2, 7.3% GT3, 2.8% GT4. Overall, frequencies of AEs, lab abnormalities and discontinuations due to AEs were similar comparing patients receiving alfa/RBV with/without daclatasvir. There was no clear evidence of daclatasvir-specific safety signals and no discernible effect of daclatasvir dose or treatment duration.

**Table 1.**

<table>
<thead>
<tr>
<th>Event, %</th>
<th>DCV 60 mg + alfa/RBV (N=285)</th>
<th>DCV 60 mg + alfa/RBV Duration not 24 wks (N=220)</th>
<th>DCV other dose + alfa/RBV Any duration (N=421)</th>
<th>Alfa/RBV Any duration (N=174)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment-related SAEs</td>
<td>2.8</td>
<td>2.3</td>
<td>3.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Discontinuations due to AEs</td>
<td>6.3</td>
<td>5.9</td>
<td>4.0</td>
<td>8.6</td>
</tr>
<tr>
<td>Selected grade 3–4 lab abnormalities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>5.6</td>
<td>4.1</td>
<td>5.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3.5</td>
<td>2.3</td>
<td>2.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>23.2</td>
<td>23.7</td>
<td>27.9</td>
<td>31.0</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>16.9</td>
<td>12.8</td>
<td>15.1</td>
<td>15.2</td>
</tr>
</tbody>
</table>

**Conclusions:** Once-daily daclatasvir plus alfa/RBV was generally well tolerated, with a safety profile comparable to the known profile of alfa/RBV. Addition of daclatasvir to alfa/RBV did not increase the frequency of serious AE, discontinuations due to AE or grade 3–4 lab abnormalities. These results support ongoing phase 3 clinical development.

**1204 THE PEPSI PROJECT: HCV RESISTANCE SCREENING AND PREDICTION OF PI-CONTAINING THERAPY OUTCOME**

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**Background and Aims:** Response to triple HCV treatment of peg-interferon-alpha (IFN), ribavirin (RBV) and protease inhibitor (PI) depends on different host and viral factors. This prospective, non-interventional study collects patients’ and viral data to develop and continuously improve the geno2pheno[HCV] tool for prediction of PI-containing-therapy outcome.

**Methods:** Blood and/or sera samples from the University Hospitals of Cologne, Düsseldorf, and Essen-Duisburg, Medical School Hannover and diverse private practices are being analysed. The IL-28B polymorphism is analysed by sequencing patient’s DNA. Viral genotyping+subtyping is performed by analysis of the NS5B sequence with the same tool. Based on the NS3-pro tease sequence with the same tool. Clinical data are provided by the treating centres, and encrypted and stored at the Institute of Virology in Cologne. Encrypted data are used at the MPI Saarbrücken for the development and improving of the geno2pheno[HCV] tool.

**Results:** The first version of the geno2pheno[HCV] tool has been developed and is freely available online. It allows viral genotyping+subtyping and determination of PI resistance. Until November 2012, blood and/or sera samples of 107 HCV patients on dual (IFN+RBV) or triple therapy have been analysed, at least at baseline. The IL-28B polymorphism was determined in 33/33 patients with available EDTA-blood samples. 8 patients showed the CC polymorphism, 24 the CT and 4 the TT. 103 baseline, 62 at week 4, 4 at week 12, 4 at week 24, 1 at week 24 and 4 at (dual) therapy failure sequences could be obtained. At baseline, 42 samples corresponded to subtype 1a, 30 1b, 2 genotype 1, 2 genotype 2, 21 genotype 3 and 6 unknown. 8/103 sequences showed PI resistance mutation. 5/8 resistant viruses are being treated with PI-containing therapies and were suppressed at week 4; no viral load data at week 8 are available yet.
Conclusion: Baseline PI-resistance mutations were detected at baseline in 7.8% of the patients. Their role in therapy outcome in vivo is under investigation. These results will be used for the update of the geno2pheno\[HCV\] tool to improve the prediction of therapy success.

1205 PRELIMINARY EVALUATION OF A NEW AUTOPHAGY INHIBITOR ANTIVIRAL AGENT FOR HEPATITIS C VIRUS IN AN EX VIVO MODEL OF HUMAN LIVER SLICES CULTURE

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Background and Aim: Host antiviral therapy is an option for the treatment of Hepatitis C virus infection, with a putative lower risk of selecting drug resistance and a pan-genotypic activity. Indeed, autophagy machinery is required to initiate HCV replication inducing in the host cells, the accumulation of autophagosomes which uses membranes for its RNA replication. Our aim was to test and evaluate the antiviral potency of a new antiviral agent (GNS 396) targeting the cellular autophagy machinery in the ex vivo model of human liver slices culture.

Methods: Non-infected liver slices, obtained from human liver resection and cut in 350-μm-thick slices (2.7x10^6 cells per slice) were infected with HCVcc Con1b/C3 supernatant (MOI = 0.1) cultivated for up to ten days. HCV infected slices were treated at day 4 post-infection with GNS396 for 6 days at different concentrations. HCV replication was evaluated by strand-specific RT-qPCR. The infectivity titers of supernatants were evaluated by foci formation upon inoculation into naïve Huh-7.5.1 cells. The cytotoxic effect of the drugs was evaluated by lactate dehydrogenase leakage assays. Gene expression profiling was performed on GNS396 treated JFH-1-infected Huh7 cells to identify the host cellular genes that are transcriptionally regulated by infection, and silenced by GNS396 based treatment.

Results: Our assays evidenced a dose-dependent inhibition of HCV infection associated with GNS396 on HCVcc Con1b/C3 infected liver slices. At day 6 post-treatment, the inhibition of infectious particles production (infectivity titer) was 160 nM without cytotoxic effect. GNS396 reduces the expression of genes induced by the viral infection such as those encoding autophagic key factors triggering the turn-off of mTOR activity.

Conclusion: In this new ex vivo model, we demonstrated that a new agent GNS 396, which inhibits the autophagy proteolysis, is a potent inhibitor of HCV replication and could constitute an attractive alternative to the direct antiviral agent for the treatment of HCV infection.
Background and Aims: Naturally occurring resistant-variants to directly acting antiviral agents (DAAs), including protease inhibitor and polymerase inhibitor, may be present in treatment-naive patients. The preexisting-resistance has not been reported in China. The aims of this study were to detect natural polymorphisms and illustrate the prevalence of such mutations in different HCV genotypes among DAAs-unnaed patients in China.

Methods: The study analyzed resistant-mutations by direct sequencing in 103 HCV DAAs-naive patients infected with HCV genotype 1b (N = 41), 2a (N = 13) and 6a (N = 49). Several nested PCR assays with genotype-specific primers were performed to amplify the HCV viral regions of NS3, NS5A and NS5B.

Result: The research successfully amplified 91.3% (94/103) in NS3, 90.3% (93/103) in NS5A as well as 92.2% (94/103) in NS5B. In HCV NS3 sequences, at least one amino acid substitution to resistance was detected in 64 (68.1%) isolates. However, 42 of these only observed Q80K, while 20 of these had only A156S. None of the 94 individuals had the substitution (T54S/A, V55A, R155K/T/Q, D168A/E/G/H/T/Y). Mutation frequency varied with the different genotypes of HCV (M36L present 100% in HCV 2a and 6.12% HCV 6a; Q80K present 91.3% only in HCV6a; Q80G present 100% in HCV 6a). D168A/E/G/H/T/Y. The proportion of four resistance-mutations (M36L, Q80K, A156S, V170I) in different groups were statistically significant (P < 0.05). In HCV NS5A sequences, more variants were observed. Resistant-mutations Q30R was detected in 74 (79.6%) samples with HCV 1b and 6a, L31M was found in 14 (15.1) patients with HCV 1b, 2a and 6a, H58P was discovered in 43 (46.2%) patients with the above genotypes, Y93C was showed in 9 (9.68%) individuals only with genotype 2a. Some other substitution (M281I/F, Q30K, H54T/Q, H58T, Y93A) related to low decreases protease inhibitors sensitivity were common according to different genotypes (from 15.1% to 96.8%). In HCV NS5B sequences, only two resistant-variants were detected, including M28T an C316N, with mutation rate is 12% and 15%, respectively.

Conclusions: Naturally occurring dominant resistance mutations to HCV DAAs do pre-exist in treatment-naive patients with various genotypes in China. Although major DAAs mutations are not very common, other mutations may lead to low level resistance with higher detection. Their influences on treatment to patients are unknown. Further studies are needed to analyze the impact of baseline resistance, and evaluate the benefits of drug resistance test before DAAs therapies in China.
L31M substitution emerged following IDX719 treatment with no other observed changes at suspected resistance loci. In genotype 3, 7/8 subjects receiving IDX719 selected virus with Y93H variants with no other observed changes at suspected resistance loci. In genotype 4, all 8 subjects receiving IDX719 selected virus with Y93H or Y93C variants; 3/8 also showed changes to 28M or 30H.

**Conclusions:** Robust declines in viral load were observed in all subjects receiving 3 days of IDX719 monotherapy except for those with genotype 2 M31 at Baseline. The profile of IDX719 selected NS5A variants varied with HCV genotype. The impact of M31 was highly dependent on genotype. Future clinical trials of IDX719 will screen for the M31 polymorphism in genotype 2-infected patients.

**1210**

**PRE-EXISTENCE, EMERGENCE AND PERSISTENCE OF HCV GENOTYPE 4 NS5A RESISTANCE VARIANTS FROM THE PHASE 2B COMMAND 1 STUDY: DACLATASVIR PLUS PEGINTERFERON-alfa/ RIBAVIRIN IN TREATMENT-NAIVE PATIENTS**

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**Background:** Daclatasvir (DCV) is a first-in-class HCV NS5A inhibitor with pan-genotypic coverage in vitro. In this study, treatment-naive HCV genotype (GT) 4-infected patients received DCV (20 mg or 60 mg QD) plus peginterferon-alfa-2a/ribavirin (alfa/RBV) for 12 or 24 weeks in a response-guided design. SVR12 (HCV RNA <25 IU/mL 12 weeks posttreatment) was achieved by 8/12 (67%) and 12/12 (100%) 20 mg and 60 mg recipients, respectively. We report the NS5A resistance profile through 48 weeks posttreatment.

**Methods:** Plasma samples from DCV recipients were analyzed at baseline, near time of virologic failure, and through 48 weeks posttreatment when HCV RNA was ≥1000 IU/mL. HCV RNA was isolated and the NS5A region amplified and sequenced. NS5A variant susceptibility to DCV inhibition was examined using GT4 hybrid replicons.

**Results:** Three of the four virologic failures, all DCV 20 mg recipients, had HCV RNA levels sufficient for analysis. The linked NS5A resistance-associated variants (RAVs) L28M-L30S (>1000-fold resistance to DCV in vitro) emerged in these failures; baseline sequence analysis revealed no preexisting RAVs. The reference strain and most GT4 sequences analyzed encode methionine at position 31. N55A-L31M confers reduced susceptibility to DCV in GT1a (reference EC50=2 nM in vitro) but no significant resistance in GT4 (reference EC50=-10 pM). Baseline polymorphisms at sites susceptible to resistance included L28M, L30R/S, M31V, H54N/R, P58A/T, and D62E/K/Q. Of note, one patient (DCV 20 mg) had L28M and 100% of treatment-naive HCV GT4 patients receiving DCV 60 mg achieved SVR12; three DCV 20 mg recipients with virologic failure had NS5A RAVs more similar to those reported in GT1a than GT1b. However, the genetic barrier to resistance in this small GT4 cohort appears higher than for GT1a (2 versus 1 nucleotide changes required to confer high level resistance). Baseline RAVs did not appear to influence virologic outcome. Decay of NS5A RAVs was detected but longer-term follow-up studies are required to fully assess persistence.

**1211**

**AMINO ACID CHANGE IN THE POSITIONS ASSOCIATED WITH PROTEASE INHIBITORS RESISTANCE IS LESS FREQUENT IN GENOTYPE 3 THAN IN GENOTYPE 1 HCV NATURAL STRAIN**

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**Background and Aim:** NS3 protease inhibitors (PIs) have emerged as useful drugs in HCV treatment. However, these compounds display a low barrier to virologic escape and a restricted activity across HCV genomes. Next generation PIs with broad genotype coverage including genotype (G) 3 are in different phase of clinical development. Unfortunately, the majority of these new compounds exhibit a pattern of resistance similar to that of first generation PIs. The rate of response to standard anti-HCV therapy is low in pts carrying G3 with advanced liver fibrosis. We analysed the natural mutational pattern and the frequency of natural NS3-PIS resistance in a group 72 pts: 35/43 (78%) HIV/HCV-G1-infected and 18/29 (62%) HIV/HCV-G3 infected. All patients were naive to anti HCV treatment. The comparison of total number aa change and frequency of resistant strain in G1 and G3 was performed using Fisher exact test or Mann Whitney test, when appropriate.

**Results:** Total number of aa change in NS3 protease domain, was similar in G1 and G3 (median number of mutations in G1 =6, IQR 3.5–14.5; median number of mutations in G3 =5, IQR 5–6, p=0.17). On the contrary, the presence of naturally occurring resistant strain at positions associated with NS3-PIS resistance, was more frequent in G1 infected individuals than G3 infected patients (30% vs 3%; p=0.03). Notably, 8/35 (23%) HIV/HCV-G1-infected patients and 5/8 (62.5%) HIV/HCV-G3-infected patients showed HCV-PI resistance mutants. In HIV/HCV-G3-infected patients and HCV-G3-monoinfected 0/18 and 1/11 was found a PI resistant strain.

**Conclusions:** The baseline higher rate of conservation of G3 natural strains for known resistance mutations suggests that the emergence of resistant mutant under drug pressure could be less frequent, probably resulting in a higher susceptibility to PIs. Unexpected high frequency of PI resistance mutations detected in G1 HCV monoinfected individuals may hamper the importance of pre-treatment genotyping for PI resistance also in HIV negative individuals.

**1212**

**PHARMACOKINETIC MODELLING OF THE RELATIONSHIP BETWEEN SUSTAINED ViroLOGICAL RESPONSE AND PLASMA CONCENTRATIONS OF FALDAPREVIR OR BI-207127 IN HCV GT1-INFECTED PATIENTS IN SOUND-C2**

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**Background and Aims:** The SOUND-C2 study assessed faldaprevir (FDV) 120 mg QD plus BI207127 600 mg BID or TID with and without ribavirin for 16, 28 or 40 weeks in over 360 HCV GT1 treatment-naive patients. Outcomes varied depending on viral subtype (GT1a and GT1b) and patient IL28B genotype (CC and non-CC). All GT1b-infected patients, and GT1a-infected patients carrying a CC IL28B genotype (GT1a-CC), had high SVR rates in ribavirin-containing arms (up to 85% and 75%, respectively). Relationships between SVR12 and plasma concentrations of FDV and BI-207127 were investigated in a post-hoc analysis.

**Methods:** Geometric mean trough concentrations of FDV and BI-207127 were investigated as predictors of SVR12 in regression models. Data were analysed for GT1a-CC patients and for GT1b patients (CC or non-CC) for the 16-, 28- and 40-week ribavirin-containing regimens. GT1a-CC patients and all patients in
the ribavirin-sparing arm were excluded because of their high virological failure rates. Separate logistic regression models were obtained for FDV and BI207127 for plasma concentrations obtained during different time windows in the first month of therapy.

Results: Patients with GT1b (any IL28B genotype) had flat or shallow relationships between trough concentrations of FDV and BI207127 and predicted SVR12. The predicted SVR12 was high throughout the concentration ranges examined. For both drugs there was a significantly greater effect of trough concentration on SVR12 in GT1a-CC patients compared with GT1b patients; GT1a-CC patients had predicted SVR12 >70% only at higher concentrations.

Conclusions: GT1b-infected patients carrying any IL28B genotype are predicted to achieve high SVR rates across broad plasma concentration ranges of FDV and BI207127 given in combination. The results suggest that the daily doses tested in SOUND-C2 give adequate plasma concentrations in GT1b-infected patients. GT1a-CC patients are predicted to require higher levels of FDV and BI207127. Further investigations of plasma concentration-response relationships in various patient subgroups, factors contributing to low plasma levels, and potential confounders, should be performed. Phase III trials of FDV 120 mg QD plus BI207127 600 mg BID plus RBV for 16 or 24 weeks are ongoing in GT1b-infected patients.

1213 CYCLOPHILIN INHIBITOR EDP-546 IS A POTENTIAL CORNERSTONE DRUG FOR USE IN COMBINATION WITH NSSA AND PROTEASE INHIBITORS DUE TO ITS HIGH BARRIER TO HCV RESISTANCE
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Background: Current HCV therapies can be classified into two categories: those that present a high barrier to the development of HCV resistance and those that present a low barrier. Only cyclophilin (Cyp) inhibitors and nucleotide inhibitors have proven to present a high barrier to resistance. EDP-546 was evaluated for its ability to select for resistance as well as its capacity for favorable combination effects with other direct acting antivirals (DAA’s) having a lower barrier to HCV resistance.

Methods and Results: HCV genotype 1a (GT1a) and 1b (GT1b) replicon cells were passaged in the presence of the Cyp inhibitor, EDP-546, at sequentially increasing concentrations of drug in the presence of G418 every 2–4 passages starting with the EC50 of the compound followed by 2xEC50, 5xEC50, 10xEC50, and 16xEC50. Resistant colonies were picked by micro-dissection and RNA was subsequently extracted, reverse transcribed, and sequenced. A total of 45 GT1a clones and 37 GT1b clones were interrogated, examining each amino acid variant and subsequently tested for resistance to EDP-546 in transient cell-based replicon assays. In both GT1a and GT1b, a D320E variant within the NSSA protein confers the greatest resistance to EDP-546 (2-fold and 5-fold, respectively, compared to wild-type). Both acute and chronic combination effects were evaluated for EDP-546 in combination with other direct acting antiviral compounds. EDP-546 is additive to synergistic in combination with the protease inhibitors asunaprevir, BI-201335, TMC-435, and MK-5172 as well as the NSSA inhibitor, daclatasvir, in reference to a combination index additivity model in an acute assay. EDP-546, in combination with these DAA’s, suppressed the emergence of resistance as well as demonstrated combination effects following chronic treatment of replicon cells.

Conclusions: Due to its high barrier to HCV resistance, EDP-546 is a potential cornerstone drug for use in combination with other DAA’s having a lower barrier to resistance.
serum of sCD40 and sCD40L levels and CD4 counts or HIV viral load was analyzed additionally among HCV/HIV coinfected.

Materials and Methods: Serum concentrations of sCD40 and sCD40L were determined using ELISA in 60 HCV infected patients HCV, that included 31 HCV monoinfected and 29 HCV/HIV co-infected. Reference values of sCD40 and sCD40L serum concentration was obtained from 15 healthy subjects with similar age and gender distribution. Rs12979860 polymorphism was assessed by sequencing of the PCR product covering this SNP. Blood CD3, CD4 and CD8 count was assessed using flow cytometry.

Results: Serum concentration of sCD40 and sCD40L was significantly higher in HCV and HCV/HIV compared to healthy subjects (sCD40: 25.3, and 25.6 vs. 8.5 pg/ml, sCD40L: 8.3 and 12.7 vs. 0.79 ng/ml). Serum sCD40L level was higher in HCV/HIV with CD4 count ≥350/µl compared to those with CD4 count <350/µl (13.7 vs. 5.6 ng/ml, p < 0.05). Moreover, among patients with CD4 count ≥350/µl we found significantly higher prevalence of CC genotype of rs12979860 polymorphism compared to patients with CD4 count <350/µl (58% vs. of 38%, p < 0.005). Values of sCD40L exceeding by ten times upper limit of norm were associated with significantly higher prevalence of CC genotype CC (46% vs. of 31%, p < 0.02).

Conclusions: We demonstrated that HCV infection stimulate synthesis of both sCD40 and sCD40L irrespectively of possible HIV coinfection. HCV/HIV coinfection resulted in additional increase of sCD40L particularly in immunocompetent patients with CD4 count above 350/µl. These patients demonstrated significantly higher incidence of CC genotype of rs12979860 polymorphism. Therefore elevated sCD40 and sCD40L should be considered as a possible predictor of HCV virologic response in HCV/HIV coinfected.

1216 ACCESS TO HCV TRIPLE THERAPY WITH TELAPREVIR OR BOCEPREVIR WITH PEGINFERON RIBAVIRIN IN REAL LIFE SETTING IN HIV–HCV CO-INFECTED PATIENTS – ANRS CO13 Hepavih COHORT

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Background: Data concerning the access to HCV direct-acting drugs (DAD) in HIV/HCV co-infected patients are sparse. Access to therapy and efficacy, which may be over-estimated in clinical trials compared to observational settings, were evaluated in a prospective French cohort of HIV/HCV co infected patients ANRS CO13-HEPAVIH.

Methods: All HCV patients chronically infected with genotype 1 and who had a study visit from 01/2011 to 06/2012 were included. We estimated the rate of treatment uptake with an HCV DAD-based therapy and compared patients who did and did not initiate HCV triple therapy. We defined virological response as undetectable HCV RNA at week 4 (RVR) and at week 12 (W12) (EVR) post-initiation of DAD.

Results: Among 226 HCV eligible patients in the cohort, 65 (28.8%) initiated an HCV DAD-based triple therapy (telaprevir n = 46, boceprevir n = 14, other n = 5) in combination with peg IFN/Ribavirin. When compared to non-treated patients, those who initiated a DAD-based triple therapy had a more severe fibrosis score as assessed by elastometry (8.7 vs 6.8 Kpa, p < 0.03), were more frequently F3/F4 (43.4% vs 21.1%, p < 0.02) and more often cirrhotic (30.2 vs 13.8%, p < 0.008). At least one contra-indication to treatment initiation was found in 37/161 (30%) of non-treated patients: decompensated cirrhosis or hepatocellular carcinoma (2.5%), cardiovascular disease (4.3%), renal insufficiency (creat >200 mmol/l) (1.2%), anemia <10g/dl (1.3%), thrombocytopenia <50,000/mm³ (2.6%), CD4 <100/mm³ (2.6%), psychiatric disorders (9.9%), current active IV drug use (7.9%), excessive alcohol consumption (>5 units/d) (2.1%). Among patients for whom a follow-up was available at W4 and W12 after the initiation of triple therapy, a RVR and an EVR were obtained in 56% (15/27) and 83% (15/18) in telaprevir-treated patients and in 0% (0/6) and 60% (3/5) of the boceprevir-treated patients, respectively.

Conclusions: One year after the first anti-HCV DAD approval in France, one fourth of genotype 1 patients included in a French cohort of HCV/HCV co-infected patients have initiated an anti-HCV triple therapy. Access to triple therapy may still increase substantially, as contra indications to therapy were only found in 30% of the non-treated patients.

1217 EXPERIENCE OF HCV RESISTANCE AFTER 1.5 YEARS CLINICAL PRACTICE; DETECTION OF RESISTANCE MUTATIONS AFTER LONG PERIOD OF UNDETECTABILITY

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Background and Aims: Telaprevir and boceprevir are hepatitis C virus (HCV) NS3/4A protease inhibitors recently approved for treatment of HCV genotype 1 infection. During clinical trials NS3 resistance mutations were selected in patients with treatment failure. Their detection before treatment or when virological breakthrough occurs could be important for patient follow-up and still needs to be explored. The aim of the present study was to evaluate the characteristics of resistance associated variants (RAV) selected by linear antiproteases during the first 1.5 years after the introduction of triple therapy combining pegIFN, ribavirin and one linear antiprotease in clinical practice.

Methods: Resistance genotyping was assessed by Sanger population sequencing of the NS3 gene in 99 genotype 1 patients upon physicians’ demand.

Figure: Repartition of RAV according to genotype 1 subtype.

Results: Forty-seven were of genotype 1a, forty-seven 1b, two 1c and three 1a/1b with discordant genotypes with Versant Lipa and NS3 sequencing. 60% of the patients were sequenced upon treatment failure, 40% before treatment. At baseline, one 170A/T was observed in a genotype 1b, 15% of genotype 1a had a 80K and one a 80L; other RAVs were not detected. Upon treatment, the majority of the patients with RAVs were of genotype 1a (63%) and only 32% of genotype 1b. All but three G1a had two or more RAVs, half G1b had one, half two RAVs. Repartition of the mutations is shown in figure 1 and will be further discussed. Noteworthy low-level RAVs were detected in two genotype 1b patients relapsing after end of treatment and >40 weeks plasma HCV-RNA below quantification threshold. Another relapse with persistent low-level
of A156S RAV was observed after liver transplantation performed when HCV RNAemia was undetectable after triple therapy. **Conclusions:** Real-life experience is in accordance with data observed in clinical trials regarding the higher rate of selection of resistance with linear antiproteases for patients infected with subtype 1a versus 1b. In addition, cases suggest the existence of reactivation reservoirs that might currently not be reached with sufficient concentration by current treatments and where HCV might steadily replicate without contributing significantly to plasma viral loads.

**1218** **THE ADDITION OF NITAZOXANIDE TO PEGYLATED INTERFERON AND RIBAVIRIN DOES NOT IMPROVE SVR RATES IN CHRONIC HCV GENOTYPE 4**

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**Background and Aims:** Nitazoxanide – a thiazolide derivative – has been proposed as a novel therapeutic agent potentiating the effect of interferon and improving SVR rates to up to 80% in genotype 4. This is an independent randomized trial not supported by the industry to confirm the efficacy of nitazoxanide in the treatment of chronic HCV genotype 4.

**Methods:** This was an open-label randomized trial that took place between December 2010 and October 2012. Treatment-naive genotype 4 HCV patients were recruited and randomized equally into 2 groups: Group 1 received weekly subcutaneous pegylated interferon 160ug in addition to weight-based ribavirin (1200mg if ≥75kg and 100mg if <75kg) for 48 weeks. Group 2 received 4 weeks lead-in therapy by nitazoxanide alone (500mg orally twice daily) followed by triple therapy including nitazoxanide, pegylated interferon and ribavirin for a further 48 weeks.

**Results:** 50 patients were recruited in each group. Baseline characteristics were similar in both groups except for a higher BMI in group 1 (28.5 vs. 26.5, p = 0.01). SVR rates were similar in both groups (24/50 (48%) vs 25/50 (50%) in groups 1 and 2 respectively, p: 0.99). RVR, cEVR and ETR rates were also similar in both groups (61% vs 53% – p: 0.42, 70% vs 72% – p: 0.5 and 62% vs 58% – p: 0.68 in groups 1 and 2 respectively). Biochemical response as defined by normalized ALT at week 12 was also similar in both groups (57% vs 46% in groups 1 and 2 respectively, p: 0.26). The occurrence of complications was similar in both groups except for a higher rate of dyspepsia in the group receiving nitazoxanide (32% vs 14%, p = 0.03). Stepwise logistic regression revealed the following as predictors of dyspepsia in the group receiving nitazoxanide: BMI > 25, female, and diabetes (OR: 6.05, 95%CI: 1.2–29.6, P: 0.027. ALT: OR: 0.2, 95%CI: 0.06–0.68. P: 0.01).

**Conclusion:** The addition of nitazoxanide to pegylated interferon and ribavirin does not improve the virological or biochemical response rates in chronic HCV genotype 4. (clinicaltrials.gov identifier: NCT01276756).

**1219** **VX-135, A POTENT SINGLE DIASTEREOMER OF ALS-2200, FOR THE TREATMENT OF CHRONIC HEPATITIS C**


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**Background and Aims:** ALS-2200, a novel uridine nucleotide analog, has demonstrated potent antiviral activity with a 4.5 log10 drop in HCV RNA over 7-days at 200mg QD in patients infected with chronic hepatitis C (CHC). VX-135, a single diastereomer of ALS-2200, is currently advancing in Phase 2 clinical trials. Both ALS-2200 and VX-135 are activated to the identical nucleoside triphosphate (NTP). In this study we expand on and highlight the data supporting the advancement of VX-135 into Phase 2 trials.

**Methods:** The antiviral activities of ALS-2200 and VX-135 were evaluated in vitro using the HCV genotype 1b replicon system. In multiple cell lines, ALS-2200 was assessed for its ability to inhibit mitochondrial protein synthesis. The ALS-2200/VX-135 NTP was evaluated for its ability to be incorporated by human mitochondrial RNA polymerase. Studies were performed to determine the formation of the ALS-2200/VX-135 NTP in human hepatocytes and in the liver of non-rodent species.

**Results:** In the replicon assay, both ALS-2200 and VX-135 exhibited potent antiviral activity with an EC50 of 150 nM and 117 nM, respectively. ALS-2200 did not inhibit mitochondrial protein synthesis, and the ALS-2200/VX-135 NTP was not incorporated into RNA by the human mitochondrial RNA polymerase. Both compounds were rapidly activated to the NTP in vitro and in vivo. Following incubation at a concentration of 50 μM of each compound, ALS-2200 formed 609 pmol/million cells NTP and VX-135 formed 1174 pmol/million cells NTP in primary human hepatocytes. Following identical oral doses, comparable, high and sustained levels of NTP were formed in dog liver for both ALS-2200 (4.89 mM at 24 hours) and VX-135 (5.38 mM at 24 hours).

**Conclusions:** ALS-2200 is a potent nucleotide analog polymerase inhibitor that has demonstrated a promising preclinical and clinical profile for the treatment of CHC. Based on available data, VX-135, a single diastereomer of ALS-2200, demonstrates a similar preclinical profile to ALS-2200 and is currently advancing in multiple interferon free Phase 2 combination studies for the treatment of CHC.
of a lower mean HCV-RNA at the time of starting triple therapy with BOC than with TLV (4.6 [2.7–5.5] vs 6.3 [5.9–6.7] log IU/mL; p < 0.0001), RVR was achieved by 84% of patients on TLV vs 60% on BOC (p < 0.0001). A multivariate logistic regression analysis (OR [95% CI] p) confirmed that TLV use was the strongest predictor of RVR (3.3 [1.6–6.8], p = 0.001). Other independent predictors of RVR were baseline HCV-RNA (0.42 [0.2–0.7], p = 0.002) and absence of advanced liver fibrosis (4.8 [1.6–14], p = 0.006). HIV coinfection did not influence the chances of RVR.

Conclusion: Compared with TLV, triple combination therapy with BOC produces lower RVR rates, potentially favoring selection of drug resistance. Thus, TLV might be the best option in more difficult-to-treat patients, such as those with high baseline HCV-RNA and/or advanced liver fibrosis. HIV coinfection does not influence early HCV-RNA responses.

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NO S282T MUTATION DETECTED BY DEEP SEQUENCING IN A LARGE NUMBER OF HCV PATIENTS WHO RECEIVED GS-7977 WITH RBV AND/OR GS-0938: THE QUANTUM STUDY

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Background: A Phase 2b study (Quantum) evaluating the efficacy of the uridine nucleotide analog sofosbuvir (SOF; GS-7977) in combination with ribavirin (RBV) or the guanidine nucleotide analogue GS-0938 was conducted in treatment-naïve patients with chronic HCV infection. Due to hepatotoxicity, dosing of arms containing GS-0938 were halted with these patients discontinuing all treatment after 2 to 9 weeks of therapy. In this report, development of S282T NS5B resistance mutant was evaluated in patients from all sofosbuvir-containing treatment arms who relapsed due to early drug discontinuation or after completion of dosing in the SOF + RBV arm.

Methods: The HCV NS5B gene was amplified from patient serum samples at baseline and viral relapse time points. PCR products were analyzed using standard population sequencing at baseline and at virologic relapse time point by Illumina MiSeq deep sequencing with an assay cut-off at 1%. PCR products from selected patient samples were also cloned into a replicon vector and tested for susceptibility to sofosbuvir.

Results: Across all sofosbuvir-containing treatment arms of the Quantum study (SOF + GS-0938, SOF + RBV, or SOF + GS-0938 + RBV for 12 or 24 weeks) there were 97/154 patients who qualified for resistance testing due to early drug discontinuation or virologic relapse post treatment. All 97 were sequenced by population sequence at baseline (BL) and 92/97 were deep sequenced at the virologic relapse timepoint. No S282T mutations were observed at BL or virologic relapse by either method. Consensus sequences were generated from the deep sequencing results and were compared to corresponding baseline sequence to assess development of mutations at other NS5B positions. Drug susceptibility of variants developed in more than two patients is currently being evaluated by phenotypic analyses.

Conclusions: Across all patients who received sofosbuvir-containing regimens for 2–24 weeks and experienced virologic relapse, no S282T NS5B resistance mutation was observed by deep sequencing further supporting the high resistance barrier of sofosbuvir.

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IN VITRO RESISTANCE TO ALS-2200, A POTENT NUCLEOTIDE POLYMERASE INHIBITOR FOR THE TREATMENT OF CHRONIC HEPATITIS C

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Background and Aims: Hepatitis C virus (HCV) NS5B polymerase inhibitors are emerging as important components of combination therapy for chronic hepatitis C (CHC). ALS-2200, a novel uridine nucleotide analog, is a potent inhibitor of NS5B-directed HCV RNA replication and has recently demonstrated activity in the clinic. In this study, the generation of in vitro resistance and cross-resistance to ALS-2200 was characterized in the HCV replicon system.

Methods: The inhibitory activity of ALS-2200 was determined using a transient Firefly luciferase genotype 1b (GT-1b) HCV replicon engineered to encode NS5B proteins from clinical isolates or containing nucleoside inhibitor-associated NS5B mutations. Huh-7 cells were treated for 72 hrs and inhibition of HCV replication measured by luminescence. Replication capacity was determined at 72 hours standardized to the 4 hour input signal. Resistance development was evaluated using a stable GT-1b replicon after sequential passaging in increasing concentrations (up to 16μM) of ALS-2200 by population and clonal sequencing.

Results: ALS-2200 inhibits the wild-type (WT) GT-1b replicon with an IC50 of 0.13μM. The IC50 of ALS-2200 versus a panel of 14 replicons containing NS5B coding regions from GT-1a or GT-1b clinical isolates ranged from 0.12 to 0.2μM. ALS-2200 inhibited a GT-1b NS5B S96T replicon with a similar potency (IC50 = 0.21μM) as the WT replicon. ALS-2200 also inhibited GT-2b WT and GT-2b NS5B C223H/V321I replicons with similar IC50 values (0.09μM vs. 0.07μM). The GT-1b NS5B S282T mutation conferred a >38-fold reduction in the activity of ALS-2200 but reduced replication capacity to ~8% of WT levels. Passaging of a GT-1b replicon in ALS-2200 for >5 months resulted in the selection of a replicon with the NS5B amino acid substitutions, S282T/T344N/E440G. Comparison of the S282T/T344N/E440G replicon to the S282T replicon revealed no differences in replication capacity or resistance to ALS-2200. In addition, ALS-2200 retained potency against >20 replicon variants resistant to NS3/4A, NS5A and non-nucleoside NS5B inhibitors.

Conclusions: ALS-2200 is a potent nucleotide analog that demonstrates broad activity versus HCV clinical isolates, a high barrier to the development of viral resistance and no cross-resistance to other classes of HCV inhibitors.

1223

HEPATITIS C VLPS DELIVERED TO DENDRITIC CELLS BY A TLR2 TARGETING LIPOPEPTIDE RESULTS IN ENHANCED NEUTRALIZING ANTIBODY AND CELL-MEDIATED RESPONSES

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Introduction: Although many studies provide strong evidence supporting the development of HCV virus-like particle (VLP) vaccines it is necessary to improve their immunogenicity. We
have evaluated an anionic lipopeptide/TLR2 agonist (E8Pam2Cys) to enhance the immunogenicity of HCV VLPs.

**Methods:** We utilised an adenviral system to express HCV genotype 1a core, E1 and E2 proteins in Huh7 cells to produce HCV VLPs. Mice (BALB/c and HLA A2 transgenic) were immunised with purified HCV VLPs alone, with alum or E8Pam2Cys and assessed for; (1) innate immune responses (2) Neutralisation (NAB) of HCV VLPs and JFH1 HCV (3) T cell responses (IFN-γ ELISPOT). Also, the ability of IgG purified from the sera of patients chronically infected with HCV genotypes 1a and 3a to block the binding and entry of the HCV VLPs into Huh7 cells was tested.

**Results:** HCV VLPs had EM morphology, buoyant density (1.2 to 1.28 g/cm²), biochemical and glycosylation properties characteristic of HCV. Immunoprecipitation with anti-ApoE and anti-ApoC confirmed the incorporation of apoproteins E and C in to HCV VLPs. Binding of HCV VLPs to Huh7 cells was blocked by anti-CD81 antibody. FITC-labelled HCV VLPs mixed with E8Pam2Cys bound to 99% of bone marrow-derived murine dendritic cells (CD11c+ BMDC) and matured 83% of BMDC (MHC class IIhigh). Immune sera from mice immobilised with HCV VLP/E8Pam2Cys neutralised binding of FITC-HCV VLP to Huh7 cells by 90%, compared to 35% with non-immune sera (p < 0.001). HCV VLP/E8Pam2Cys induced strong HCV VLP-specific IFN-γ producing cells in the spleens of HLA-A2 transgenic mice compared to mice inoculated with HCV VLPs alone or in CFA. Finally, IgG from sera HCV genotype 1a and 3a infected patients inhibited FITC-labelled HCV VLP entry into Huh7 with a 50% inhibition of binding (IC50) of 50 ng/ml and 260 ng/ml for genotype 1a and 3a IgG respectively compared to >20000 ng/ml for negative control IgG.

**Conclusion:** These results suggest overall that the immunogenicity of HCV VLPs can be significantly improved by the addition of this novel adjuvant by targeting their delivery to DCs and could therefore constitute a viable vaccine strategy for HCV.

**1224 TIMING AND CHARACTERISTICS OF DRUG RESISTANCE MUTATIONS (DRMs) IN CHRONIC HEPATITIS C PATIENTS DURING AND AFTER TREATMENT WITH PROTEASE INHIBITOR THERAPY AT A SINGLE CENTRE**

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**Methods:** Triple therapy based in pegylated interferon (PEG-IFN), ribavirin (RBV) and first-generation NS3/4A protease inhibitors (PI) is actually the standard treatment for immunocompetent patients infected by Hepatitis C Virus (HCV) genotype 1. In liver transplant recipients, the use of triple therapy is very recent and only preliminary results are available. Characterizing tolerability to antiviral treatment with dual therapy (PEG-IFN + RBV) is essential in order to understand where we are when starting to use triple therapy.

**Introduction:** We report the prevalence and characteristics of DRMs associated with virological failure (VF) in a prospective cohort of 59 patients with chronic HCV infection treated with telaprevir (TVR) or boceprevir (BOC) combined with pegylated interferon–alfa and ribavirin (PEG/R).

**Methods:** All patients had baseline HCVRNA levels >10,000 IU/ml and evidence of at least bridging fibrosis. VF was defined as HCVRNA >1000 IU/ml at week 4 or 12 for TVR, or HCVRNA >100 IU/ml at week 12 for BOC, or an increase of >1 log₂ VL from nadir. Genotypic resistance was detected using nested RT-PCR to amplify viral RNA and the first 181 amino acids of the NS3 protease were sequenced by population sequencing.

**Results:** Forty seven patients (80%) were treated with TVR and 12 (20%) with BOC. Nineteen patients (32%) had VF (18 TVR, 1 BOC) of which DRMs were detected in 16 (84%). All patients who developed VF with DRMs had HCV genotype 1a and were treated with TVR. Mutations evolved at NS3 positions 155 and 36; Arginine to Lysine at 155 and/or Valine to Methionine, Leucine, Alanine or Valine/Methionine at 36, none of which were detected at baseline. Eight of fifteen DRMs were detected during TVR therapy, the remainder during PEG/R ‘tail’ of therapy (4 at week 4; 2 at week 8; 2 at week 12; 1 at week 15; 3 at week 24; 1 at week 36; 1 at week 48 and 1 at week 60). Those patients with VF without identifiable DRMs had breakthrough after week 12 with TVR (1 at week 22; 1 at week 24) and at week 8 with BOC. Of the VF/no DRM patients, all TVR patients had subtype 1b and severe fibrosis. The BOC treated patient had subtype 1a with moderate fibrosis.

**Conclusion:** In this cohort treated with TVR, VF was associated with development of DRMs which were identified both before and during the PEG/R tail of therapy. Given the potential impact of DRMs on success of future therapy with protease inhibitors we suggest HCV protease sequencing for DRMs for all patients presenting with VF.
A HIGH RISK OF CHRONICITY IS THE MAJOR CONCERN OF HCV INFECTION AND CHRONIC INFECTION OFTEN LEADS TO LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA. ALTHOUGH PROPORTION OF PATIENTS ACHIEVING A SUSTAINED ViroLOGICAL RESPONSE HAS BEEN INCREASED BY THE INTRODUCTION OF COMBINATION THERAPY OF pegylated-IFN-alpha AND ribavirin, still half of the patients exhibits no response to the therapy. ONE OF THE MECHANISMS FOR THE ESTABLISHMENT OF PERSISTENT INFECTION OF HCV IS THE ESCAPE FROM THE HOST IMMUNE SYSTEM. TO ELIMINATE HCV FROM THE HEPATOcyTES OF THE PATIENTS WITHOUT POSSIBLE CYTOToXITY DUE TO AN OVER INDUCTION OF HOST IMMUNITY, WE GENERATED A THERAPEUTIC CONSTRUCT, cMR3, BY WHICH TYPE I IFN IS SELECTIVELY INJECTED IN INFECTED CELLS.

METHODS: The cMR3 is composed of the N-terminal part of the interferon regulatory factor 7 (IRF7) possessing a dominant active function, the sequences specifically cleaved by HCV NS3/4A protease and an ER anchor, and expresses a potent IFN inducing activity in HCV infected cells. After cleavage by the HCV protease, the processed cMR3 migrates into the nucleus and activates various IFN promoters including IFN-alpha6, IFN beta, and IFN stimulated response element. Furthermore, a recombinant baculovirus cMR3-expressing vector was constructed.

RESULTS: The specific activation of the IFN promoters was observed in both HCV replicon cells and JFH1 virus infected cells upon introduction of the cMR3 but not in cells infected with JEV or DENV. Expression of viral protein and viral replication were also impaired by the introduction of cMR3 into the HCV replicon cells. Furthermore, the recombinant baculovirus can delivery cMR3 into both HCV replicon cells and JFH1 virus infected cells efficiently. And the specific activation of the IFN promoters and suppression of viral replication was also observed.

CONCLUSIONS: These results suggest that the selective expression of type I IFN in the hepatocytes infected with HCV by the introduction of the cMR3 might be feasible to eliminate HCV from the chronic hepatitis C patients without liver damage and baculoviral cMR3-expressing vectors have the potential for therapeutic use against HCV infection.

A NEW THERAPEUTIC CONSTRUCT OF HCV THROUGH A SELECTIVE INDUCTION OF IRF7
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Background: A high risk of chronicity is the major concern of HCV infection and chronic infection often leads to liver cirrhosis and hepatocellular carcinoma. Although proportion of patients achieving a sustained virological response has been increased by the introduction of combination therapy of pegylated-IFN-alpha and ribavirin, still half of the patients exhibits no response to the therapy. One of the mechanisms for the establishment of persistent infection of HCV is the escape from the host immune system. To eliminate HCV from the hepatocytes of the patients without possible cytoxicity due to an over induction of host immunity, we generated a therapeutic construct, cMR3, by which type I IFN is selectively injected in infected cells.

Methods: The cMR3 is composed of the N-terminal part of the interferon regulatory factor 7 (IRF7) possessing a dominant active function, the sequences specifically cleaved by HCV NS3/4A protease and an ER anchor, and expresses a potent IFN inducing activity in HCV infected cells. After cleavage by the HCV protease, the processed cMR3 migrates into the nucleus and activates various IFN promoters including IFN-alpha6, IFN beta, and IFN stimulated response element. Furthermore, a recombinant baculovirus cMR3-expressing vector was constructed.

Results: The specific activation of the IFN promoters was observed in both HCV replicon cells and JFH1 virus infected cells upon introduction of the cMR3 but not in cells infected with JEV or DENV. Expression of viral protein and viral replication were also impaired by the introduction of cMR3 into the HCV replicon cells. Furthermore, the recombinant baculovirus can delivery cMR3 into both HCV replicon cells and JFH1 virus infected cells efficiently. And the specific activation of the IFN promoters and suppression of viral replication was also observed.

Conclusions: These results suggest that the selective expression of type I IFN in the hepatocytes infected with HCV by the introduction of the cMR3 might be feasible to eliminate HCV from the chronic hepatitis C patients without liver damage and baculoviral cMR3-expressing vectors have the potential for therapeutic use against HCV infection.

AN ANALYSIS OF RESPONSE RATES BY FIBROSIS STAGE IN PATIENTS TREATED WITH FALDAPREVIr, BI 207127 AND RIBAVIRIN IN THE SOUND-C2 STUDY
S. Zeuzem1, V. Soriano2, T. Asselah3, J.-P. Bronowicki4, A.W. Lohse5, RIBAVIRIN IN THE SOUND-C2 STUDY
F. Voss13, P. Baum14, R. Lohse6, R. Lohse6, V. Soriano2, T. Asselah3, J.-P. Bronowicki4, A.W. Lohse5, pacientes categorizados como no/leve/mild/advanced liver fibrosis (F3–F4) based on liver biopsy or a liver stiffness score of ≥9.5 kPa by transient elastography. Patients were categorised as having advanced liver fibrosis or cirrhosis (F3–F4) based on liver biopsy or a liver stiffness score of ≥9.5 kPa by transient elastography. If patients had baseline data from biopsy and transient elastography, the biopsy score was used. SVR rates were calculated based on the intent-to-treat population for each subgroup (F0–F2 or F3–F4) within each treatment arm.

Results: Among all patients treated in the SOUND-C2 study, 253 patients were categorised as F0–F2, 107 patients were categorised as F3–F4 and two patients were excluded from the analysis due to the absence of reliable biopsy or transient elastography data. The SVR rates for patients with F0–F2 and F3–F4 are shown in the Table.

Table: SVR rates (%) by treatment group and stage of fibrosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F0–F2</th>
<th>F3–F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>faldaprevir</td>
<td>67/107</td>
<td>67/107</td>
</tr>
<tr>
<td>BI 207127</td>
<td>67/107</td>
<td>67/107</td>
</tr>
<tr>
<td>ribavirin</td>
<td>67/107</td>
<td>67/107</td>
</tr>
<tr>
<td>total</td>
<td>131/214</td>
<td>134/214</td>
</tr>
</tbody>
</table>

Conclusion: Patients treated with the interferon-free combination of faldaprevir, BI 207127 and ribavirin achieved high SVR rates whether they had no/mild/moderate liver fibrosis or advanced liver fibrosis/cirrhosis. Thus, with this interferon-free combination the degree of liver fibrosis appears to have limited impact on viral cure rates.

HepDirectTM IMPROVES LIVER-TARGETING OF HCV NUCLEOTIDE NS5B POLYMERASE INHIBITOR LG-7501 VERSUS PHOSPHORAMIDATE PHONUCLEOTIDE TECHNOLOGY
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Background: Nucleotide prodrugs targeting NS5B polymerase are key components of next generation therapies against HCV because of their excellent efficacy and anti-resistance profile. However, development of the compound class faces significant challenges due to the safety concerns of nucleosides in non-targeted organs. We report the liver-targeting profile of LG-7501, a HepDirectTM nucleotide prodrug, in comparison with BMS-986094 (INX-189) and IDX184, the phosphoramidate prodrugs of the same nucleoside (2′-C-MeG).

Methods: The three prodrugs were orally administered to Sprague-Dawley rats at nucleoside equivalent doses. Blood and tissues were harvested and snap-frozen in liquid nitrogen. Nucleoside and nucleoside mono-, di- and triphosphate (NMP/NDP/NTP) concentrations were measured by LC-MS/MS to compare liver-targeting efficiency.

Results: At equivalent doses, LG-7501 achieved NTP levels in the liver over 130-fold higher than that of IDX184 and over 3-fold higher than BMS-094 (see Table 1 below). The relative NMP/NDP levels among the compounds are comparable to relative NTP levels in the tissues. Since LG-7501 is mainly metabolized by CYP3A4 levels among the compounds are comparable to relative NTP levels in the tissues. Since LG-7501 is mainly metabolized by CYP3A4 and phosphoramidate bonds of BMS-094 and IDX184 are cleaved by histidine triad nucleotide-binding protein 1 (HINT1), one can expect that exposure levels of the nucleotides in other target tissues will be largely dependent on the tissue expression levels of these enzymes. CYP3A4 is expressed at particularly high levels in the liver, making it ideal for enhanced liver targeting of LG-7501. The metabolite
09. VIRAL HEPATITIS A & E

1229
COMPARISON OF VIROLOGICAL RESPONSE BETWEEN RIBAVIRIN AND PEG-INTERFERON MONOTHERAPY DURING AUTOCHONOUS CHRONIC HEPATITIS E VIRUS INFECTION

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Aims: It has been previously reported that both peg-interferon (Peg) and ribavirin monotherapy are effective treatments for a chronic HEV infection. The aim of this study was to compare HEV load kinetic and adverse effects during Peg-IFN or ribavirin monotherapy.

Patients and Methods: 23 patients with chronic HEV infection defined by the presence of persisting serum HEV RNA and elevated enzyme level at least 3 months after acute hepatitis. These patients were treated with 600 to 800 mg of ribavirin/day/12 weeks (riba group, n=18) or with Peg-IFN 180 mg/week/12 weeks (Peg group, n=5). Serum HEV RNA load, ALT, gGT, Hemoglobin, Platelet, leucocytes) were determined in both groups every 2 weeks.

Results: 23 patients, (18 men and 5 women; 48.3±7 years), infected with HEV for 7±3 months were included. Among them, 20 patients were solid organ recipients and 3 have a haematological malignancy. At baseline no significant difference was observed between patients from Riba group or Peg group: RNA HVE load 5.79±1 vs 6.04±1 Log10 IU/mL; ALT 257.2±20 vs 274±58 IU/L. After 4 weeks of ribavirin treatment HEV viral load was lower 3.09±0.5 than in Peg group 3.91±0.8 Log10 IU/mL. At week 4, HEV RNA was negative in 11/18 (61.1%) patients from ribavirin group and 3/5 (60%) from Peg group. At week 8, all the patients from ribavirin group had negative HEV RNA. A complete virological response was observed in both groups after 12 weeks of treatment. After treatment withdrawal a virological relapse occurred in 5/18 (27.7%) patients from riba group and in 1/5 (20%) patients from Peg group. Treatment tolerance was good in both groups but the decrease of hemoglobin level was higher in patients from ribavirin than in Peg group.

Conclusions: Ribavirin as well as Peg-interferon during 12 weeks induce a sustained virological response in patients with chronic HEV infection. Ribavirin therapy seems to be associated to a faster HEV RNA drop than Peg-IFN therapy.

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CLINICAL AND LABORATORY FEATURES AND NATURAL HISTORY OF SERONEGATIVE HEPATITIS (SNH)

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Background: SNH is a recognised cause of liver failure requiring transplantation. The aetiology is unknown, but might relate to an unidentified virus or immune dysregulation. The diagnosis is based on exclusion of other causes of hepatocellular injury. There are few data on SNH presenting to non-transplant centres.

Aims: To describe the clinical/laboratory features and natural history of SNH, and compare these with viral/autoimmune hepatitis in a non-transplant centre.

Methods: Cases of SNH, viral, and autoimmune hepatitis were identified from 2080 consecutive patients attending a rapid-access jaundice clinic over a 14-year period. SNH was defined as ALT >500 IU/L with no cause determined, including no evidence of viral, autoimmune, or drug-induced hepatitis.

Results: Of 881 (42%) patients with hepatocellular jaundice, 27 (3%) had SNH, 44 (5%) autoimmune, and 58 (7%) viral hepatitis (HAV, HBV, HEV). 15/27 (56%) patients with SNH were male, median age 60 years (range 14–74). Symptoms included nausea (56%), vomiting (44%), abdominal pain (41%). Peak bilirubin was 63 µmol/L (range 9–363), ALT 932 IU/L (range 503–3807). 11 (41%) had an ALT >1,000 IU/L at presentation. Duration of illness was 7 weeks (range 4–12 weeks), LFTs normalised after 6 weeks (2–18 weeks). No patients developed liver failure and none had further bouts of hepatitis during 62 months follow-up (range 5–128 months). One patient developed acute lymphoblastic leukaemia shortly after presentation.

There was no difference in age (p=0.18) or sex (p=0.108) of the patients with SNH and viral hepatitis. Compared with autoimmune hepatitis, patients with SNH were younger (age 65 years, range 15–91; p=0.002) and more likely to be male (p=0.004). A past medical history of autoimmune disease was more common in autoimmune hepatitis, compared to SNH (p=0.035) and viral hepatitis (p=0.027). Patients with autoimmune hepatitis were more likely (p<0.0001) to have an albumin <35 g/L, INR >1.2, raised IgG, and positive anti-nuclear/smooth muscle antibody, compared to SNH.

Conclusions: SNH presenting to a non-transplant centre appears to be a self-limiting illness. The association between PNH and ALL is rare, but previously well documented. The comparative data suggest that the aetiology is more likely to be viral than autoimmune.
Materials and Methods: ACLF was defined as per the definition proposed by Asian Pacific Association for the Study of Liver (2008). The study included 150 patients (93 males and 57 females) with liver disorders admitted to Lok Nayak Hospital, New Delhi in the year 2011 out of which 100 were identified as ACLF. These cases were evaluated on the basis of history, clinical examination, liver function profile, and serological test of hepatitis A, B, C, and E using commercially available ELISA kits. Hepatitis E virus (HEV) RNA was detected using primers based on the Open reading frame 2 (ORF 2) region of HEV by RT-PCR.

Results: Mean age of the patients was 34 (range: 10–66) years. The majority of the cases among the ACLF were patients with cirrhosis (40/100 (40%) with high jaundice, ascitis and portal hypertension followed by patients with alcoholic liver disease 20/100 (20%). HEV IgM was positive in 30/100 (30%) of the ACLF group. Other co-infections included HEV with HCV – 10/100 (10%) and with HBV – 25/100 (25%), HAV 12 (12%). HEV RNA was detected in the sera of 24/30 patients (80%) of the ACLF group. Presence of HEV infection in 24/30 (80%) with cirrhosis and patients without HEV in 30/70 (42.85%) (p < 0.001) with ACLF were significantly associated. Mortality was observed in 15% (150) of the patients.

Conclusion: HEV viremia was one of the causes of acute on chronic liver failure in India. The danger of the HEV in ACLF should not be ruled out.

1232 INCREASED HEV SEROPREVALENCE IN PATIENTS WITH AUTOIMMUNE HEPATITIS

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AUTOIMMUNE HEPATITIS WITHIN GENOTYPE 4

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Patients with autoimmune hepatitis but not RA or HBV/HCV patients showed a higher anti-HEV seroprevalence. Previous contact to HEV could be confirmed by an independent immunological readout. Future studies need to investigate if this phenomenon can be explained by misdiagnosis of AIH or specific induction of AIH by HEV infection. As HEV viremia may occur, HEV RNA testing is recommended for AIH patients not responding to immunosuppressive therapy.

1233 TO FIND OUT MATERNAL AND FOETAL OUTCOME IN PREGNANT WOMEN INFECTED WITH ACUTE VIRAL HEPATITIS 'E' (AVH-E) DURING THIRD TRIMESTER OF PREGNANCY – A RETROSPECTIVE ANALYSIS

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Introduction: Acute Viral Hepatitis E is self limiting disease with very low rate of fulminant hepatic failure (FHF) and mortality. However various studies in past has suggested that if pregnant females during third trimester of pregnancy gets AVH-E, it leads to severe disease with very high maternal and foetal mortality.

Objectives: To find out the maternal and foetal outcome in pregnant women infected with AVH-E during third trimester of pregnancy – A retrospective Analysis.

Material and Methods: Records of all pregnant females from Oct 2007 to Sept 2012 with liver disease were analysed. All pregnant women having third trimester of pregnancy with serology confirmed AVH-E were recruited. All patients having hepatitis due to other viruses were excluded. Other exclusion criteria were patients with AFLP, toximia of pregnancy, HELLP syndrome, cholestatic hepatitis of pregnancy underlying CLD. Patients delivering outside and then referred to us were also excluded. All patients were seen during antenatal period and delivered in our institute.

Results: There were 27 pregnant females (Age 19–39 yrs) who fulfilled inclusion and exclusion criteria. All patients had serology confirmed AVH-E and was diagnosed in antenatal period during third trimester. 14/27 (51.85%) were primi. Only 5/27 (18.5%) developed hepatic failure and three of them (11%) died. Early preterm labour was seen in 7/27 (25.92%) while late preterm labour was seen in 17/27 (62.96%) and only 3 having term delivery. Only 2 patients had IUD while overall fetal mortality was seen in 44%.

Conclusions: 1. AVH-E in third trimester is associated with higher rate of FHF 2. However overall maternal mortality is not as high as reported in literature.

1234 REEMERGING HEPATITIS E EPIDEMIC IN SAPPORO, JAPAN, CAUSED BY A MONOPHyletic AND VIRULENT HEV STRAIN WITHIN GENOTYPE 4

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Background and Aims: Acute sporadic hepatitis E can occur indigenously even in the industrialized countries, but not likely for epidemic or outbreak. Sapporo has been considered as one of the most prevalent regions for hepatitis E virus (HEV) in Japan. During the regional surveillance for hepatitis E, we have encountered an epidemic of hepatitis E two times in Sapporo with a two-year interval. The aim of this study is to clarify the clinical and molecular epidemiological characteristics of these epidemics.
Methods: From 2007 until June 2012, 105 patients were prospectively diagnosed as autochthonous hepatitis E out of 451 patients with non A, non B, non C acute liver injury registered from 44 hospitals covering the whole regions of Hokkaido prefecture. HEV infection was diagnosed by HEV RNA and/or anti-HEV antibodies (IgM and/or IgA) in sera. Acute liver failure (ALF) was defined to be a case with prothrombin time INR more than 1.5. A phylogenetic analysis was performed based on a 326 nt sequence within ORF1 of HEV genome.

Results: Out of 105 cases of hepatitis E thus collected in Hokkaido, 68 were from Sapporo and its suburbs. Among them, 21 sporadic cases (14 males, median age 54 years) emerged consecutively: 11 from September to October 2009, 10 from late December 2011 to April 2012. In the both epidemics, 11 developed ALF, and among them, 1 presented fulminant progression. In the phylogenetic analysis, HEV sequences from all the patients segregated to a compact cluster in genotype 4, “New Sapporo strain”, suggesting a single-source transmission responsible for the epidemics. The common infection route of HEV was not found, in spite of minute analysis, HEV sequences from all the patients segregated to a compact cluster in genotype 4, “New Sapporo strain”, suggesting a single-source transmission responsible for the epidemics. The common infection route of HEV was not found, in spite of minute research for the patients and their families. Sapporo epidemics showed higher incidence of ALF in comparison with other sporadic cases (57.1% vs. 23.5%, p < 0.0005).

Conclusions: This study demonstrates hepatitis E epidemics including serious disease progression have reemerged repeatedly in Sapporo, and the third epidemic would occur as long as the transmission routes of the virulent HEV, New Sapporo strain, remain obscure.

1235 HIGH PRE-TRANSPLANT HEV SEROPREVALENCE IN HCV INFECTED LIVER TRANSPLANT RECIPIENTS; EVIDENCE FOR HCV TREATMENT PROTECTION AGAINST DEVELOPMENT OF CHRONIC HEV INFECTION

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Background and Aims: Recent reports have shown that HEV infection in transplant recipients can lead to chronic progressive hepatitis. However, the cross sectional studies that have been reported to date, miss data on HEV seroconversion after transplantation. Chronic HEV infection can be treated with ribavirin (RBV), a compound also used for the treatment of HCV. We studied seroconversion and active HEV infection in liver transplant (LT) recipients with HCV up to 5 years post-LT, including those treated with RBV for HCV recurrence post-LT.

Methods: A total of 489 samples were available in 207 HCV patients transplanted from January 1995 to July 2010 at the Mayo Clinic in Rochester, MN, USA. Samples at baseline (pre-LT) and 1, 3 and 5 years post-LT were tested for HEV serology (Wantai assay). All last available samples and all HEV IgM positive samples were tested for HEV RNA.

Results: Overall seroconversion in the total cohort of 207 patients was 37.7% (n = 78). Baseline samples were available in 146 patients of which 53 (36.6%) were positive for HEV IgG. Five patients (3.4%) tested positive for HEV IgM and IgG antibodies, but negative for HEV RNA at baseline and were most likely recently infected. One patient experienced HEV IgM seroconversion 5 years post-LT, while being positive for HEV IgG from baseline up to 5 years of follow up. This patient had been treated for HCV with RBV for over 3 years at IgM seroconversion. Post-LT samples were available in 126 patients with baseline samples. IgG seroconversion after LT was seen in 8 patients (6.8%) of which 7 received HCV treatment with (pegylated) interferon and/or RBV. No HEV RNA was found in any of the 207 patients’ last follow up sample.

Conclusions: A considerable proportion of LT recipients with HCV infection have evidence of HEV infection, with IgG seroconversion mostly occurring before LT. No patients had ongoing HEV infection at last follow up, suggesting efficacy of HCV treatment in the prevention of chronic HEV development, given the high frequency of interferon/RBV treatment in newly HEV seroconverted patients. Non-HCV treated LT recipients are still at risk of developing chronic HEV.

1236 EXPERIMENTAL INFECTION OF CYNOMOLGUS MACAQUES WITH THE NEWLY IDENTIFIED RABBIT HEPATITIS E VIRUS

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Background and Aims: Hepatitis E virus (HEV) is the causative agent of acute hepatitis E. At least four genotypes of HEV have been isolated from humans, swine, wild boar and deer. The recent discovery of rabbit HEV in China, USA and France revealed that rabbits harbor another important reservoir of HEV. However, it is unclear whether rabbit HEV can infect humans. The aim of the present study is to determine the zoonotic potential and pathogenesis of rabbit HEV.

Methods: Two juvenile cynomolgus macaques and two rabbits were inoculated with a rabbit HEV strain isolated from Beijing, China. Sera and feces were collected twice a week after inoculation. Anti-HEV antibody, HEV RNA and alanine aminotransferase in sera and HEV RNA in feces were detected. Further, the two infected rabbits were necropsied when serum and feces were tested simultaneously positive for HEV RNA and nine different types of tissues and organs including liver, kidney, small intestine, spleen, stomach, heart, brain, bladder and lung were collected during necropsy subject to positive and negative strand HEV RNA detection.

Results: Both monkeys developed typical hepatitis, with liver enzyme elevations, viremia, fecal virus shedding and seroconversion. Comparison of the complete genome sequence of rabbit HEV passed in the macaques with that of the inoculum revealed 99.8% nucleotide identity. 18 nucleotide mutations resulting in nine non-synonymous amino acid substitutions were found over the entire genome of HEV, with 11 present in the helicase domain and the RNA-dependent RNA polymerase domain. Positive and negative strand HEV RNAs were detectable in six of the nine kinds of tissues including liver, bile, kidneys, small intestines, spleens and stomachs from the experimentally infected rabbits.

Conclusions: Our data showed rabbit HEV is transmissible to cynomolgus macaques and negative-strand RNA, which is an intermediate product during HEV replication, was detected in five non-hepatic tissues, suggesting that rabbit HEV appears to be a new source of human HEV infection and extrahepatic replication may be a common feature of rabbit HEV like human and swine HEV.

1237 COMPARISON OF IMMUNOGENICITY BETWEEN INACTIVATED AND LIVE ATTENUATED HEPATITIS A VACCINES: A DOUBLE-BLIND, RANDOMIZED, PARALLEL-GROUP CLINICAL TRIAL AMONG CHILDREN IN XINJIANG UIGHUR AUTONOMOUS REGION, CHINA

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Objectives: To compare immunogenicity among an inactivated hepatitis A vaccine (Healive®) with one-dose and two-dose regimens, and three kinds of live attenuated vaccines in children.
Methods: A double-blind, randomized, parallel-group clinical trial was conducted among healthy children aged 1.5–6 years in Xinjiang Uighur Autonomous Region, China. Subjects were randomly assigned to 5 groups. Two groups were administered one-dose or two-dose inactivated vaccine and the remaining three groups were immunized with one of three kinds of live attenuated vaccines, respectively. Serum samples were collected at 6- and 12-month follow-ups for immunogenicity evaluation. Anti-HAV IgG was measured with a microparticle enzyme immunoassay.

Results: The geometric mean concentration (GMC) of anti-HAV IgG was significantly higher in the two-dose Healive® group than in the one-dose Healive® group and the attenuated vaccine groups at 12 months (932.4 vs. 212.8, 203.3, 212.8 mIU/ml, respectively, p < 0.05). In the one-dose Healive® group, the GMC was significantly lower than that in the attenuated vaccine B and C groups at 6 months (152.6 vs. 212.4, 204 mlU/ml, p < 0.05) and at 12 months (112.7 vs. 203.3, 212.8, p < 0.05), but was similar to the attenuated vaccine A group at 12 months (112.7 vs. 135.8 mlU/ml, p > 0.05). No significant differences were observed in seroconversion rates among the five groups at 6 or 12 months (p > 0.05).

Conclusions: A higher GMC of anti-HAV IgG was induced in the two-dose Healive® group than in the one-dose group and the attenuated vaccine groups at 12 months. The attenuated vaccine B or C produced higher GMCs than the one-dose Healive® at attenuated vaccine groups at 12 months. The attenuated vaccine ORF1 supports a papain-like cysteine protease (PCP) domain.

1238

MOLECULAR CHARACTERIZATION OF HEPATITIS E VIRUS (HEV)-ORF1 SUPPORTS A PAPAIN-LIKE CYSTEINE PROTEASE (PCP) AND IDENTIFIES A PUTATIVE ‘GLY–GLY’ SUBSTRATE

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Background and Aim: The HEV-ORF1 encodes a non-structural polyprotein essential for viral RNA replication wherein the functionality of a putative PCP-domain still remains contested. In this study, we intended to characterize the HEV protease.

Methods: GeneBank HEV a.a. sequences (n = 77), including 9 representative strains as well as those of closely-related RNA viruses were analyzed in silico. A total of 32 ORF1 (PCP-domain: n = 19; X-domain: n = 13) mutants of HEV-sar55 genomic-replicon (pSK-GFP) were constructed. Replicons were in vitro transcribed and transfected into hepatoma S10–3 cells, followed by monitoring GFP expression, an indicator of RNA replication. An expression vector (pTriEx-ORF1) with full-length ORF1 gene with ‘His’ (N-terminal) and ‘His’ (C-terminal) tags was engineered and tested in vitro (TNT coupled transcription-translation). The in vivo synthesis of ORF1 and its processing in S10–3 cells was analyzed by immunoprecipitation (IP)/Western-blot (WB).

Results: Of the PCP constructs, six ‘Cys’ (C457A, C459A, C471A, C472A, C481A, C483A) and three ‘His’ (H443L, H497L, H590L) mutants completely abolished RNA replication. Alignment of a.a. sequences mapped a highly conserved ‘Gly’-triplet (G815, G816 and G817) in the downstream X-domain, homologous to Rubella virus protease substrate (G1299, G1300 and G1301). Two (G816V and G817V) of the X-‘Gly’ constructs abrogated viral replication whereas the G815V mutant did not, similar to Rubella virus. All seven PCP-domain (nts. 1340–1801) and ten X-domain (nts. 2396–2910) constructs with each and every base mutated without a.a. changes, produced GFP. This showed the regulatory roles of PCP- and X-domains on viral replication at protein level but not at mRNA level. WB analysis of in vitro translation yielded the expected ~191 kDa band, comparable to full-length ORF1 (~182 kDa). IP/WB analyses of in vivo synthesized ORF1 showed two supposedly processed fragments of ~60 kDa (anti-His) and ~35 kDa (anti-HA), previously reported in insect cells. However, we could not detect either a full-length ORF1 or other forms. Based on these molecular evidences and the close homology with Rubella virus and FMD virus proteases, a 3-D model of ‘HEV protease’ was proposed.

Conclusion: The dispensability and functional effects of ORF1 ‘PCP-catalytic’ and ‘X-substrate’ residues on HEV replication, supports a viral protease.

1239

HEV INFECTION POST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION MISDIAGNOSED AS GRAFT VERSUS HOST DISEASE OR DRUG TOXICITY


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Background and Aims: Hepatitis E virus (HEV) is the causative agent of acute and chronic hepatitis and gastro-enteritis. It is an emerging health issue in industrialized countries, particularly in the immunocompromised. Since little is known of HEV infections in allogeneic hematopoietic stem cell transplantation recipients (alloHSCT), we studied HEV infection in alloHSCT.

Materials and Methods: Patients receiving alloHSCT between January 2006-July 2011 were included. Anti-HEV serostatus (Wantai assay) before alloHSCT and presence of HEV RNA after alloHSCT was assessed. Additionally, all plasma samples from episodes characterized by common toxicity criteria grade 2–4 liver function abnormalities were screened for HEV RNA. From confirmed cases, the course of HEV infection and clinical implications were studied retrospectively and phylogenetic analysis was performed.

Results: 328 Patients were included in the study (Table 1). In total, eight HEV infected cases (2.4%) were identified, of which five developed chronic HEV. These were misdiagnosed before as hepatic Graft versus host disease (GVHD, n = 5), or drug-toxicity (n = 3). HEV-ORF1 sequences classified all cases as genotype 3 and ruled out a common source. Seroprevalence prior to alloHSCT was 12.9%, 2 patients (0.6%) were anti-HEV-IgM positive, though HEV viremia could not be confirmed by PCR. The median time from alloHSCT to infection was 4.6 months (range −2–18 months).

Table 1. Cohort characteristics (n = 328)

<table>
<thead>
<tr>
<th>Age of transplantation (years)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>50.4</td>
<td>17–66</td>
</tr>
<tr>
<td>Female</td>
<td>178 (54%)</td>
<td>150 (46%)</td>
</tr>
<tr>
<td>Diagnosis (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML – acute myeloid leukemia</td>
<td>142 (43%)</td>
<td>49 (15%)</td>
</tr>
<tr>
<td>ALL – acute lymphoid leukemia</td>
<td>49 (15%)</td>
<td>31 (9%)</td>
</tr>
<tr>
<td>NHL – non-Hodgkin lymphoma</td>
<td>24 (7%)</td>
<td>18 (5%)</td>
</tr>
<tr>
<td>CLL – chronic lymphoid leukemia</td>
<td>24 (7%)</td>
<td>31 (9%)</td>
</tr>
<tr>
<td>MM – multiple myeloma</td>
<td>16 (5%)</td>
<td>18 (5%)</td>
</tr>
<tr>
<td>MDS – myelodysplastic syndrome</td>
<td>49 (15%)</td>
<td>49 (15%)</td>
</tr>
<tr>
<td>Other</td>
<td>16 (5%)</td>
<td>16 (5%)</td>
</tr>
<tr>
<td>Type of allogenic transplantation (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB – cord-blood</td>
<td>48 (15%)</td>
<td>141 (43%)</td>
</tr>
<tr>
<td>MUD – matched-unrelated donor</td>
<td>49 (15%)</td>
<td>141 (43%)</td>
</tr>
<tr>
<td>SIB – sibling</td>
<td>186 (57%)</td>
<td>141 (43%)</td>
</tr>
</tbody>
</table>

Four of eight cases died with HEV viremia, signs of ongoing hepatitis (n = 4) and neurologic disease (n = 1), after a median period of infection of 4.1 (range 2–12) months. The four living patients...
cleared HEV after a median period of 8.8 (range: 2–42) months, supported by ribavirin treatment (n = 1) and reduction of immune suppression (n = 3). Two of four living patients were diagnosed with chronic hepatitis and fibrosis by liver biopsy. One HEV patient presented with recurring episodes of viremia, characterized as viral reactivation.

Conclusions: Although HEV is a relatively infrequent opportunistic pathogen after alloHSCT, a differential diagnosis including hepatitis E is mandatory given the clinical impact. Future alloHSCT recipients should be screened for HEV RNA prior to transplantation and should be monitored after alloHSCT, especially during episodes of intensive immunosuppressive therapy and if liver abnormalities occur.

1240 CHRONIC HEPATITIS E IN LUNG TRANSPLANT RECIPIENTS
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Chronic courses of hepatitis E virus (HEV) infection have been described in various groups of transplant recipients. We identified a particular high anti-HEV-IgG-seroprevalence in heart transplanted patients (Pischke et al., Am J Transplant 2012). However, the relevance of HEV infections in lung transplant (LuTx) recipients is unknown.

We therefore studied sera of 95 LuTx outpatients (including 44 patients with liver transaminase values of at least 2 times upper the limit of normal) for the presence of HEV RNA and anti-HEV-IgG (MP-Assay). Anti-HEV seroprevalence rate was compared to previously published data (537 healthy individuals). Subsequently, a prospective HEV screening of LuTx pts. was initiated.

Results: Anti-HEV-IgG was detected in 5/95 LuTx pts (5.3%) which was slightly higher than in healthy controls (2%, 11/537; p = 0.07).

Conclusion: Chronic hepatitis E virus infection is potentially a cause of recurrent viremia during pulmonary HSCT and should be monitored after LuTx.

1241 MATERNAL NUTRITIONAL STATUS AND HEPATITIS E VIRUS DURING PREGNANCY
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Background: Hepatitis E virus infection (HEV) is fatal and fulminant especially during pregnancy resulting in potential disaster for both mother and fetus. Pregnant women in developing countries suffer from malnutrition which causes reduced immunocompetence leading to greater risk of multiple viral infections.

Aim: The preliminary study was aimed for evaluation of the nutritional factors and its correlation with HEV in Pregnancy.

Methods: The study included 65 jaundice pregnant patients (33 AVH and 11 FHF) with HEV infection admitted to LNJP, New Delhi in the year 2011. ELISA was used for serological testing for detection of various hepatotropic viruses. Nutritional factors were evaluated by measurement of anthropometric parameters – triceps skin fold thickness, mid arm circumference, height, weight, BMI, total protein, albumin, and globulin. Biochemical factors were also analyzed.

Results: HEV was found to be most common form of hepatitis during pregnancy. Maternal mortality was found in 5 (45.45%) pregnant women. The patients with FHF had significantly low BMI and tricep skin fold thickness as compared to AVH group. It was observed that there was significant correlation between arm span and alanine aminotransferase in pregnant women with HEV infection in FHF group (p < 0.01) but no correlation was observed in AVH group. Albumin and globulin in FHF group (2.26 ± 0.310 g/dl, 1.69 ± 0.380 g/dl) was slightly lower than AVH group (2.66 ± 0.815, 1.76 ± 0.035 g/dl). The Mean difference of proteins between group AVH and FHF were not found to be significant (p = 0.121 and p = 0.290) respectively.

Table 1: Maternal nutrition parameters in pregnancy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AVH (N=33)</th>
<th>FHF (N=11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>16.02±1.00</td>
<td>14.89±0.064</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Arm span (m)</td>
<td>1.54±0.24</td>
<td>1.54±0.027</td>
<td>0.068</td>
</tr>
<tr>
<td>Mid arm circumference (m)</td>
<td>0.226±0.014</td>
<td>0.210±0.013</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tricep skin fold thickness (mm)</td>
<td>13.54±1.24</td>
<td>11.63±1.64</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Conclusion: Nutritional parameters might affect the course of HEV during pregnancy.

1242 ELECTRON MICROSCOPY IDENTIFICATION OF A NOVEL HEPATOTROPIC VIRUS ASSOCIATED WITH TISSUE EXPRESSION OF THE NV-F ANTIGEN
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Background: By performing random amplification of serum derived DNA, followed by elimination of chromosome-derived sequences, a non-human DNA fragment had been obtained from a patient with non-A-E fulminant hepatitis. This sequence, named NV-F, was detected in 24.6% non-A-E hepatitis patients and 2.8% healthy individuals. Physiological characterization showed that the NV-F sequence was associated with a virus-sized particle (<200 nm). The sequence encoded a putative complete open reading frame. We performed random amplification of serum derived DNA, followed by elimination of chromosome-derived sequences, a non-human DNA fragment had been obtained from a patient with non-A-E fulminant hepatitis. This sequence, named NV-F, was detected in 24.6% non-A-E hepatitis patients and 2.8% healthy individuals. Physiological characterization showed that the NV-F sequence was associated with a virus-sized particle (<200 nm). The sequence encoded a putative complete open reading frame. We conducted an electron microscopy experiment to search for new viral particles in liver biopsy tissues.

Methods: Two approaches were undertaken. Serum samples from 347 patients with severe hepatitis activities (ALT >5-fold ULN) were retrospectively included for identification of viral etiology. Liver biopsy samples from 23 patients with severe hepatitis were prospectively included for immunochemical examination of the hepatitis NV-F antigen (HnFag) expression as well as electron microscopy.

Results: Of the 347 patients with severe hepatitis, acute HAV, acute HBV, chronic HBV infection with acute flare, acute or chronic HCV, and acute NV-F associated virus (HnFV) infection were found in 5, 11, 147, 41 and 9 patients, respectively. Multiple viruses infection including HnFV+HBV, HnFV+HCV, HBV+HCV, and HBV+HDV were found in 18, 7, 10, and 5 patients, respectively. In 81 of the 347
(23.3%) patients, no known viral etiology could be identified. Of the 23 patients receiving liver biopsy and electron microscopy examination, 7 showed positive tissue Hnfag expression. Electron microscopy examination revealed a novel viral particle in all 7 Hnfag-positive liver tissues but not in 10 Hnfag-negative liver tissues. The novel hepatotropic viruses were enveloped, 100–110 nm in size, and icosahedral in shape. They were located in the cytoplasm. One of the 7 patients had hepatocellular carcinoma and one had active cirrhosis.

**Conclusion:** We identified a novel hepatotropic virus associated with tissue expression of the NV-F antigen.

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**10a. FATTY LIVER DISEASE: EXPERIMENTAL**

**1243**

**INCREASED NEUROLIGN-4 RECEPTOR EXPRESSIONS IN NK CELLS IS ASSOCIATED WITH IMPAIR KILLING ACTIVITY IN LEPTIN-DEFICIENT MICE (ob/ob)**

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**Background and Aim:** Neuriligin-4 (NLGn4) is involved in the neuronal synapse formation and remodeling to control vesicular release. NK cells lose their original anti-fibrotic properties in insulin resistance and cirrhosis; our gene-array analysis revealed NLGn4 over expression. We investigated a potential NLGn4 role to mediate NK responses in leptin-deficient mice (ob/ob) as a model for insulin resistance.

**Methods:** Isolated liver NK cells from ob/ob mice and wild-type (WT) littermate were assessed for NLGn4 expressions by real time PCR. Additionally, isolated liver NK cells from both strains were co-cultured with freshly isolated WT primary hepatic stellate cells (pHSCs). NLGn4 silencing or nonsilencing control siRNAs were prepared prior to co-culture with pHSCs. Following 24 hr of co-cultures, cells were trypsinized, washed and analyzed by flow cytometry for NK cell and pHSC’s activities. Therefore, anti-NK1.1 (NK cell marker) and anti-α-SMA (Smooth Muscle Actin, a HSCs activation marker) were used. Annexin-V and propidium iodide were used to determine cell apoptosis and viability, respectively.

**Results:** Naïve liver NK cells showed a significant up-regulation of NLGn4 mRNA in ob/ob mice as compared to WT mice. In co-cultures, the ob/ob NK cells had significantly decreased lysosomal-associated membrane protein-1 (CD107a, NK activation marker). NLGn4 silencing significantly increased CD107a activity (from 9% to 25%, P=0.01). WT pHSCs co-cultured with siRNA NLGn4 silencing over expression. We investigated a potential NLGn4 role to mediate NK responses in leptin-deficient mice (ob/ob) as a model for insulin resistance.

**Conclusions:** The anti-fibrotic NK ability was impaired in ob/ob NK mice; associated with NLGn4 over expression. NLGn4 silencing activates NK cells to promote anti-fibrotic effects through increased HSCs killing. NLGn4 modulation of CD107a activity of NK cells extend the understanding and therapeutic strategies in fatty liver disease.

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**1244**

**NLGn4 AND NMDA RECEPTORS MEDIATE NK CELL ACTIVITY VIA mTOR PATHWAY IN NAFLD**

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**Background and Aims:** Neuriligin-4 (NLGn4) is involved in the neuronal synapse formation. NLGn4 interacts with NMDAR (an insulin responding N-Methyl-D-Aspartate Receptor) to encode PSD-95 (Post Synaptic Density-95 proteins) and to control synaptic vesicular release through F-Actin. NK cells lose their original anti-fibrotic properties in cirrhosis; our gene-array analysis of NK cells revealed NLGn4 over expression in cirrhosis. We investigated a potential NLGn4 role to modulate NK responses in Nonalcoholic-Fatty-Liver-Disease (NAFLD) progressions.

**Methods:** Flow cytometry analysis was performed in peripheral blood lymphocytes from healthy and histology documented NAFLD cases with low and advanced fibrosis.

**Results:** The NK cytolytic CD56DimCD16+ showed to be the dominant population than the CD56BrighCD16− in all studied populations. These 2 sub-populations showed different expressions of the NLGn4 receptor. NAFLD CD56Dim had lower insulin receptors, F-Actin and mTOR expressions but NLGn4 and PSD-95 were increased, mainly in advanced fibrosis (P<0.05). CD107a (NK granzymes activation marker) tend to increase in low fibrosis NAFLD cases (P=0.06) but was unchanged in advanced fibrosis.

NLGn4 silencing, NMDAR antagonist (Ketamine) and agonist (Alanine), Insulin or Rapamycin incubations of isolated NK cells were assessed to understand the interactions of the NLGn4 receptor with its protein complex. The overall downstream alterations suggest that CD107a is lessened with the F-Actin to inhibit killing. F-Actin is maintained by increased activities of mTOR in healthy or PSD-95 in NAFLD cases. The NLGn4 is up-regulated by insulin to stimulate mTOR pathway and by low mTOR activity as a feedback. On the other hand, insulin up-regulation of the NMDAR depresses mTOR activity. Low mTOR activities up-regulate PSD-95 which down regulate the NMDAR as a feedback. Therefore, insulin exposure or NLGn4 silencing unleashed the CD107a to increase NK killing. However, insulin resistance of NAFLD NK cells showed lower responses and decreased killing ability.

**Conclusions:** NAFLD NK cells exert insulin resistance, mainly the CD56Dim cytolytic population. The NLGn4 and NMDAR unit regulate NK activity as a result of metabolic modifications; via an mTOR dependent pathway. It involves PSD-95, F-Actin and NK granzymes complex; indicate a new molecular pathway through which NK cells contribute to the NAFLD progression.

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**1245**

**ROLE OF INNATE AND ADAPTIVE IMMUNITY IN THE DEVELOPMENT OF NON ALCOHOLIC FATTY LIVER DISEASE IN MORBIDLY OBESE PATIENTS**


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Obesity has been characterised as a state of chronic inflammation. Signalling through toll-like receptors (TLR) has been recognized as an activator of obesity-induced inflammation as well as systemic depletion of regulatory T cells (Treg). Their role in non-alcoholic fatty liver disease (NAFLD) pathogenesis in morbidly obese (MO) patients has not yet been established.
Aim: To study the TLR expression profile and function in peripheral blood mononuclear cells and the percentage of Treg in peripheral blood and liver of MO patients.

Methods: 16 patients prior to bariatric surgery and 12 healthy controls (HC) were analysed. Peripheral blood TLR expression in mononuclear cells (PBMCs) and Treg frequencies (CD4+CD25+FOXP3+) were assessed by flow cytometry and TLR function was determined by stimulating PBMCs with specific ligands. In 10 liver biopsies a mechanical cell extraction was performed and the percentage of Treg was determined by flow cytometry. The diagnosis of NAFLD was established according to Brunt classification.

Results: According to liver histology, 7 patients (44%) showed an inflammatory infiltrate in a variable grade (NASH), 8 (50%) simple steatosis and one (6%) normal liver histology. MO patients displayed a significant lower percentage of peripheral Treg when compared to HC (1.66±1.17 SD vs 3.00±0.97 SD; p=0.002). Although no significant differences were observed in peripheral Treg between NASH and simple steatosis, the percentage of Treg in liver biopsies was significantly higher in NASH patients in contrast to simple steatosis (3.12±1.72 SD vs 1.08±0.96 SD; p=0.04). Significant differences were observed in the expression of different TLRs in B cells of MO patients compared to HC. TLR4 expression was lower in MO (p=0.03) whereas expression of TLR7 was higher (p=0.03). TLR4 expression was lower in NASH patients compared to simple steatosis (p=0.05). Despite these different expression profiles, circulating monocytes from MO patients showed no significant differences according to in vitro response to TLR4 and TLR7 agonists, although there was a trend towards a decrease in TNF production after specific TLR4 stimulation.

Conclusions: Our data support a role for TLR and Treg in NAFLD pathogenesis in MO patients.

Funding: FIS-ISCIII (PI12/02026). IFIMAV

1246

NUCLEAR–MITOCHONDRIAL GENOME INTERACTIONS IN NONALCOHOLIC FATTY LIVER DISEASE SUSCEPTIBILITY

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Background and Aims: Genetic variation in the mitochondrial genome has been linked to components that comprise the cardiometabolic syndrome. However, the role of mtDNA in the pathogenesis of nonalcoholic fatty liver disease (NAFLD); the hepatic manifestation of the cardiometabolic syndrome, is not clear. Herein, we compared the hepatic response to an atherogenic diet (Ath) diet in WT C57BL/6j and C3H/HeN mice, which are more and less susceptible to NAFLD, respectively. In order to determine the role of nuclear and mitochondrial genome interactions in disease susceptibility, Mitochondrial-Nuclear eXchange (MNX) mice (C57*:C3Hm and C3H*:C57m) were used for comparison.

Methods: Two groups of rats were fed a high cholesterol (1.25%) or a chow diet. After six weeks, animals were sacrificed to analyse liver pathologist, routine biochemistry, reduced/oxidised glutathione (GSSG/GSH), mitochondrial bioenergetics and H2O2 flux. Targeted lipidomics of oxysterols in liver specimens was analysed by state of the art isotope dilution mass spectrometry. HepG2 cells and isolated liver mitochondria were challenged with oxysterols that were found significantly modified by the CHO diet.

Results: CHO diet was associated with dyslipidemia and steatohepatitis. In addition, we found an increased hepatic GSSG/GSH, and a large alteration in mitochondrial function – including enhancement of uncoupled respiration, decrease in membrane potential based on increased proton leak, and increased H2O2 production rate. Oxysterol profiling revealed a significant increase in 7α- and 7β-hydroxycholesterol in the liver of CHO fed rats, compared to control rats. In vitro experiments, these two oxysterols impaired mitochondrial respiration both in isolated liver mitochondria and HepG2 cells.

Conclusions: We report that high cholesterol diet causes steatohepatitis in rats. The biochemical signature of this process can be found in the upregulated oxidative stress status centered on mitochondrial dysfunction, which is eventually initiated and propagated by cholesterol oxidation products. Oxysterols are potential pharmaceutical targets to prevent NASH progression.
Background and Aims: Evidence from clinical and laboratory studies have accumulated indicating that the activation of the cannabinoid system is crucial for non-alcoholic fatty liver disease. However, the association between hepatic cannabinoid receptor with mitochondria function has not been well investigated in obesity-induced fatty liver. In this study, we examined the role of a cannabinoid receptor (CBR) and the lipid accumulation in primary hepatocytes and for it’s ability to modulate pathways implicated in obese mice that develop hyperglycemia and fatty livers.

Methods and Results: We challenged hepatocytes with high concentrations of a mixture of oleate and palmitate (HFHA) as a model of hepatic lipogenesis and impairment of mitochondria function. Both cannabinoid receptor-1 antagonist AM251 and silencing of the CB1R gene prevented HFHA-stimulated change in intracellular triglyceride levels, the generation of ROS, and mitochondria membrane potential (MMP). On the other hand, AM251 suppression of steatosis was reflected by a lowering in liver triglyceride contents as compared with obese (ob/ob) controls. In addition, AM251 simultaneously decreased hepatic fatty acid synthesis (SREBP-1c, FAS) and increased b-oxidation (CPT-1, CPT-2) genes expression of ob/ob mice. Reduction of SREBP-1 protein level in livers of ob/ob mice by AM251 treatment modestly improves fat drop deposition. This was accompanied by a marked reversal in the expression of mitochondria biogenesis-related factors (PGC-1a and NRF1 and 2) as reflected by the elevated expression of mitochondrial complex I and ATP levels.

Conclusions: These data suggest that up-regulated CB1-mediated signaling by increasing SREBP-1 proteins, leading to up-regulation of fatty acid synthesis expression and increased oxidative stress in liver. Thus, AM251 may play a modulatory role in pathogenesis of the hepatic steatosis and suggest that CB1R antagonist has potential clinical applications in obesity-induced hepatic lipogenesis and that should be explored.

1249 NAFLD AND CVD: MAY A SUFFERING LIVER BE USEFUL TO PREDICT CARDIOVASCULAR RISK?

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Background and Aims: Nonalcoholic Fatty Liver Disease (NAFLD) is the most common form of chronic liver disease worldwide, ranging from simple steatosis to Nonalcoholic Steatohepatitis (NASH) combined with inflammation, fibrosis, cirrhosis which may eventually lead to hepatocarcinoma (HCC). NAFLD shares key features of the Metabolic Syndrome (MS), such as obesity and insulin resistance, being considered the hepatic manifestation of the MS, one of the main cardiovascular disease (CVD) risk. There are many indications in literature about the putative relationship between liver steatosis and CVD. So, our focus was on investigating the possibility to identify hepatic prognostic targets useful to predict cardiovascular risk.

Methods: Our study was performed both in a mouse model of liver steatosis, obtained feeding the animals with a High Fat Diet (HFD) for 3, 6 and 12 months, and in Peripheral Blood Mononuclear Cells (PBMCs) from NAFLD Patients. By qRT-PCR and WB we analyzed a panel of genes and proteins potentially involved in lipid metabolism and CVD both in mice and patients.

Results: Immediately after 3 months of diet, mice livers displayed an up-regulation of 66 genes related to lipid metabolism, which was later present in the hearts (after 12 months of HFD). In PBMCs from NAFLD patients the results were similar to that in mice livers. Our attention was focused on Krüppel-like Factor 15 (KLF15) and Tafazzin (TAZ) genes, which have never been evaluated as possible predictors of damage. Both of these genes might be considered novel markers of cardiovascular risk.

Conclusions: Our work, addressed in understanding if cardiovascular damage can be evaluated by studying liver damage, supports a possible strict correlation between liver damage and cardiovascular system, and, by our data, liver seems to be the “primum movens” of the sequel of pathological events related to MS. Furthermore, we appreciated a similar modulation of gene expression profiles both in mice livers and PBMCs from NAFLD patients. The same genes modulation observed in PBMC of NAFLD patients could be useful to evaluate the CVD risk in NAFLD patients.

1250 HUMAN IN VITRO MODEL OF FATTY LIVER AND THE INFLUENCE OF ANTI-LIPEMIDIC DRUGS ON METABOLISM AND SIGNAL PATHWAYS IN STEATOTIC HEPATOCYTES

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) caused by an excessive caloric intake is already the most common chronic liver disease in Western countries. Therefore fatty liver diseases have become pivotal in surgical interventions and organ transplantation. Measures to effectively lower hepatic lipid content at an early, still reversible stage are prerequisite. Drugs, such as Fenofibrate (FEN positive control) and GEN were then applied for 24h. Lipid accumulation was displayed by Oil-Red-O staining. Lipotoxicity was investigated by measurement of cell viability (AST, LDH and XTT assay). Real time-PCR and Western blot analysis were used for investigation of known targets in insulin resistance and energy metabolism.

Conclusions: These data suggest that up-regulated CB1-mediated signaling by increasing SREBP-1 proteins, leading to up-regulation of fatty acid synthesis expression and increased oxidative stress in liver. Thus, AM251 may play a modulatory role in pathogenesis of the hepatic steatosis and suggest that CB1R antagonist has potential clinical applications in obesity-induced hepatic lipogenesis and that should be explored.
Conclusion: In summary, our data show that the human in vitro model of NAFLD is a reliable reproduction of in vivo conditions regarding fat accumulation, lipotoxicity and insulin resistance. Testing of anti-lipidemic drugs revealed that FEN and GEN were able to improve some of pathophysiological changes of steatotic PHH and showed a beneficial influence on signaling pathways of the lipid metabolism.

1251 DYSBIOSIS CONTRIBUTES TO FIBROGENESIS IN THE COURSE OF LIVER DAMAGE
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Background and Aim: Non-alcoholic fatty liver disease may lead to hepatic fibrosis. Dietary habits affect gut microbiota composition, while endotoxins produced by GRAM− bacteria stimulate hepatic fibrogenesis. However, the mechanisms involved in the process of liver damage and the potential effect of microbiota in the liver is unknown. Thus, we seek to analyze whether microbiota composition and bacterial translocation may interfere with liver fibrogenesis.

Material and Methods: Mice were treated with control (CTRL) or high fat diet (HFD) for 1 month and subsequently subjected to either BDL for 2 weeks or i.p. injection of CC14 for 3 weeks. Microbiota transplantation was obtained by oral gavage of previously gut-sterilized mice by antibiotic cocktail (Neomycin, Ampicillin, metronidazole, vancomycin). Fibrosis degree, hepatic stellate cell activation, intestinal permeability, bacterial translocation and serum endotoxia were measured. Inflammasomes components were evaluated in the gut and in the liver. Microbiota composition (dysbiosis) was evaluated by pyrosequence analysis.

Results: mRNA for collagen α1 (I) and αSMA, collagen deposition (Sirius Red), hydroxyproline content, were increased in the HFD-BDL mice versus CTRL-BDL mice, while no differences were observed between CTRL-CC14 mice and HFD-CC14 mice. Culture of mesenteric lymphnodes showed higher density of infection in HFD-BDL mice respect to CTRL-BDL mice, suggesting higher bacterial translocation rate. No evidence of bacterial translocation was observed in CC14-treated mice. Pyrosequence analysis revealed an increase in percentage of GRAM− vs GRAM+ bacteria, a reduced ratio between Bacteroidetes and Firmicutes and, more specifically, a dramatic increase of the GRAM− proteobacteria in HFD-BDL vs CTRL-BDL mice. Inflammasomes expression was increased in the liver of fibrotic mice, but significantly reduced in the gut. Furthermore, microbiota transplantation revealed more liver damage in the chimeric mice treated with CTRL diet but receiving the microbiota of HFD-treated mice.

Conclusions: By increasing the percentage of GRAM− producing endotoxins, different dietary habits lead to a pro-fibrogenic effect in the course of chronic hepatic liver damage. Bacterial translocation is needed by hepatic steatosis to act as a co-factor in chronic liver diseases.

1252 EFFECT OF BARIATRIC SURGERY ON SERUM LEVELS OF GASTROINTESTINAL HORMONES IN OBESE NAFLD PATIENTS
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Background: Food intake and energy homeostasis are regulated by gut release of humoral peptides signaling to brain, as peptide YY (PYY), glucagon-like peptide (GLP)-1, GLP-2, ghrelin (GHR), orexin (ORE) and cholecystokinin (CCK). Over the last years, bariatric surgery have provided interesting results, not only in achieving and maintaining appropriate weight loss, but mostly in modulating gut peptides, contributing to the improvement of diabetes, appetite sensations, and the metabolic changes associated.

Aim: To evaluate dietary intake, nutritional assessment as well as the plasma levels of some gastrointestinal peptides regulating food intake in obese nonalcoholic fatty liver disease (NAFLD) patients before and after bariatric surgery.

Patients and Methods: We enrolled 25 obese patients with NAFLD (M/F 11/14; age M±SD 42±16 years; BMI 30–39), 19 severely obese with NAFLD (M/F 4/15; age M±SD 47±11; BMI ≥40) submitted to bilio-intestinal bypass, compared to 16 healthy normal weight controls (M/F 8/8; age M±SD 39–8 years; BMI 20–25). In all subjects food intake was evaluated by electronic program (WinFood, Medimatica s.r.l.). Plasma levels of PYY, GLP-1, GLP-2, GHR, ORE and CCK were determined by enzymatic immunoassay, Blood composition was evaluated by impedentiometric analysis (BIA 1015). In severely obese patients all parameters were evaluated at 0 time and after 6 months after bariatric surgery.

Results: In obese patients we found:
1. a higher intake of nutrients, both as calories and as macro and micronutrients in respect to controls (p<0.05); 2. a decrease of free fat mass (p<0.01) and an increase of BMI (p<0.01), fat mass (p<0.01) and trunk fat (p<0.01) in respect to controls;
3. a significant decrease of GLP-1 and an increase of GLP-2, GHR and PYY in respect to controls (p<0.05);
4. further increase in GLP-2, GHR and PYY, and increase over control values of GLP-1 after bariatric surgery (p<0.05 versus pre-surgery).

Conclusions: A crucial role for gastrointestinal peptides may be considered (i.e. low level of GLP-1 may be involved in the pathogenesis of insulin-resistance in NAFLD). The bariatric surgery modifies the plasma levels of gastrointestinal peptides, thus changing the metabolic pattern of obese NAFLD patient.

1253 ATTENUATION OF ADIPOSITY AND HEPATIC STEATOSIS BY GROUP VIA PHOSPHOLIPASE A2 DEFICIENCY IN OB/OB MICE
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Background and Aims: Group VIA PLA2 also named iPLA2b, hydrolyzes fatty acyl bond at the sn-2 position of phospholipids recognized as a key process on homeostatic membrane metabolism. iPLA2b and its product lysophospholipids also play a key role in cellular signalling and lipid synthesis under stress and activated states. We found that global iPLA2b−/− double-knockout (PKO) mice exhibited lower body weight, liver and visceral fat weights as well as serum and hepatic lipids when compared with control littermates (WT). We herein tested whether global deficiency of iPLA2b would attenuate adiposity and hepatic steatosis in leptin-knockout Ob/Ob (OB) mice.

Methods: We performed cross-breeding between leptin−/− and iPLA2b−/− mice to generate leptin−/−iPLA2b−/− double-knockout (PKO-OB) mice. Male and female mice at 6–7 months old (n = 5–7) were used. Serum transaminases, lipids, glucose and insulin were determined. HOMA-IR was calculated by the formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5. Formalin-fixed liver sections were stained with hematoxylin-eosin (H&E). Hepatic gene expression was studied by quantitative TaqMan RT-PCR.

Results: Compared with WT. OB mice demonstrated marked obesity with fatty liver and insulin resistance. Compared with PKO-OB mice had reduced body, liver, visceral, subcutaneous fat weights.
as well as reduced serum transaminases (AST, ALT, LDH) as well as serum triglycerides and phospholipids. Compared with OB, PKO-OB mice had reduced HOMA-IR values (OB: 3.1±0.6 vs PKO-OB: 1.8±0.3, n=5), indicating improvement in insulin sensitivity. Histology data revealed that PKO-OB mice exhibited decreased hepatic steatosis, this was concomitant with significant reduction of hepatic mRNA expression of transcription factors PPARγ and SREBP1c as well as inflammatory CC chemokine ligand MCP-1. 

Conclusion: Deficiency of iPLA2β in obese mice decreased adiposity and elicited protection against hepatic steatosis, liver injury, and insulin resistance. Our data shed lights on the role of iPLA2β on adipose and hepatic lipid syntheses during obesity and insulin resistance development, and that this gene may be used as a therapeutic target.

1254 LIVER OXIDATIVE STRESS-SUPPRESSION BY NOVEL NANOPIRATES FOR TREATMENT OF FIBROSIS ASSOCIATED WITH NONALCOHOLIC STEATOHEPATITIS

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Oxidative stress (OS) is largely thought to be a core abnormality responsible for liver damage and disease progression in nonalcoholic steatohepatitis or NASH. Moreover, OS, caused by increase in reactive oxygen species (ROS) has been closely associated with fibrosis. The aim of our study was to determine the therapeutic efficacy and safety for OS suppression using novel redox nanoparticles (RNP).

Methods: RNPs were prepared by self-assembling amphiphilic block copolymers composed of a hydrophilic polyethylene glycol (PEG) segment and hydrophobic poly(4-methylstyrene) segment possessing nitroxide radicals via amine linkage. C57BL/6 mice were placed on choline-deficient L-amino acid defined (CDDA) diet to generate NASH mice model. After 16 wks of CDDA diet, a time point that results in significant NASH and fibrosis, mice were treated with RNP, control NP, control NP, control NP, or vehicle for 4 weeks via gavage. Plasma and liver tissue were then collected for determination of histopathology, inflammation by real time PCR and serum ALT levels. Hepatic stellate cell (HSC) activation was determined by real time PCR and liver fibrosis quantitated by digital image analysis of Sirius-red stained sections.

Results: The polymer derived from RNP was delivered to the liver after disintegration of nanoparticle in the stomach and absorption into the bloodstream through the mesentery. After 4 weeks of oral administration in CDDA-treated mice, liver fibrosis was significantly improved in RNP-treated group as compared to control NP or vehicle as assessed by Sirius-red quantitation as well as mRNA expression of fibrosis genes such as COL1, a-SMA and TIMP-1 (p <0.05). The mRNA expression of inflammatory cytokines including IL-6 and serum ALT level were slightly decreased with RNP, whereas no significant changes were detected on hepatic steatosis and inflammatory foci in the CDDA-treated groups.

Conclusion: This study demonstrates that oral administration of RNP reduces HSC activation and improves liver fibrosis associated with experimental NASH. These findings uncover RNP as novel potential anti-fibrotic therapy for NASH.
to partial hepatectomy (70%) under 60 min of ischemia (PH+I/R). Retinol levels were measured and altered pharmacologically and their effects on hepatic damage and regeneration were studied after reperfusion. Retinol levels in steatotic livers from sham group were significantly higher than the levels in non-steatotic livers, indicating that the presence of fatty infiltration in and of itself induce changes in retinol metabolism. Thus, the effects of retinol were dependent on the type of liver. In non-steatotic livers, retinol levels in animals subjected to PH+I/R were similar to those of the Sham group. However, retinol levels in in steatotic livers undergoing PH+I/R were reduced in comparison with the Sham group. The administration of retinol increased hepatic injury and impaired liver regeneration in non-steatotic livers. In steatotic livers, retinol reduced damage and improved regeneration after surgery. These benefits of retinol were associated with reduced hepatocellular fat accumulation. Retinol did not modify oxidative stress or PPARs in steatotic livers, but did increase PPAR-γ. Retinol actions could be mediated through PPAR-γ since inhibition of this latter signaling pathway abolished retinol benefits on regeneration and damage. In contrast to the key role of PPAR-γ in these actions of retinol, the effects of retinol on lipid accumulation were PPAR-γ-independent. Thus, in terms of clinical applications, retinol pre-treatment might open new avenues for liver surgery that specifically benefit the steatotic liver.

1257 THE ACCUMULATION OF LIPID DROPLETS IN KUPFFER CELLS DISTURBS THEIR PHAGOCYTOSIS AND CLEARANCE FUNCTIONS
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Background and Aims: Obesity is one of the first causes of liver disease in Western countries. Non-alcoholic steatohepatitis (NASH) is characterized by steatosis associated with liver inflammation. Resident macrophages of the liver, Kupffer cells (KC), accumulate toxic lipids in steatotic liver. This phenotype is associated with a pro-inflammatory phenotype participating to the lymphocyte recruitment into the liver. One of the main KC functions is the clearance of pathogens and apoptotic cells through phagocytosis. We therefore aimed to study whether susceptibility to Listeria monocytogenes infection in obesity was related to a decreased hepatic clearance of L. monocytogenes.

Methods: Steatosis was induced in mice by a high fat diet. KC were isolated from the liver and cultured. We assess by in vitro studies phagocytosis and clearance dysregulation of KC in obese mice. KC were differentially incubated/infected with aggregates (PKH-26), E. Coli or Listeria monocytogenes. Phagocytosis was quantified by flow cytometry and bacterial clearance was determined by the plate count method.

Results: We evaluated phagocytosis of aggregates labelled by PKH-26 in KC of normal and obese mice. We observed an increase of phagocytosis of these particles by KC in obese mice. Conversely, we showed that KC of obese mice phagocitized less intra and extracellular pathogens, such as L. monocytogenes and E. coli, respectively. We also showed that the ability of KC in obese mice to eliminate L. monocytogenes was decreased.

Conclusions: Lipid droplets accumulation in KC of obese mice is correlated to a dysfunction of phagocytosis and bacterial clearance. KC of obese mice showed a higher efficiency to eliminate aggregates but were less efficient to phagocitize and clear bacteria.

1258 FLUVASTATIN AMELIORATES LIVER DISEASE IN HCV MOUSE MODEL WITH IMPROVEMENTS OF LIPID METABOLISM, MITOCHONDRIAL DYSFUNCTION AND INTRAHEPATIC CARDIOLIPIN COMPOSITION
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Background and Aims: Hepatic steatosis and mitochondrial electron transfer system (ETS) dysfunction are the hallmark of liver disease caused by hepatitis C virus (HCV) infection. In this study, we assessed the effects of fluvastatin, one of the lipid-lowering agents, on HCV-associated metabolic disorders.

Methods: We used HCV core gene transgenic male mice (CoreTg), which develop hepatic steatosis, increase of monounsaturated fatty acids (MUFAs), ETS dysfunction, and hepatocellular carcinoma (Moriya K, J Gen Virol 1997). C57BL/6J male mice were used as controls (NTg). CoreTg or NTg were fed an ordinary chow diet, cared for according to the institutional guidelines. Fluvastatin containing diet (0.004%) was administered for 3 months beginning at 3 months of age. At least five mice were used in each experiment. Triglycerides in the liver were measured by enzymatic method and fatty acid compositions were analyzed by gas chromatograph. Morphologic alteration of mitochondria was analyzed by electron microscopy. Cardiolipin, which is essential phospholipid for mitochondria, was analyzed by liquid chromatography mass spectrometry.

Results: Fluvastatin treatment led to a significant reduction in the amounts of lipid and content of MUFAs in the liver (p<0.01). The expression of stearoyl-CoA desaturase, which may be a key molecule for the pathogenesis in HCV infection, was significantly decreased in fluvastatin-treated CoreTg than in non-treated CoreTg (p=0.0113). The expression of ETS complex I and IV were restored in fluvastatin-treated CoreTg. The double structured mitochondrial membrane was disrupted in CoreTg, which was ameliorated with fluvastatin administration. In contrast, mitochondrial morphology was exacerbated in fluvastatin-treated NTg. The hepatic content of tetralinoeoyl-cardiolipin, which is the most essential cardiolipin for mitochondrial function, tended to be higher in fluvastatin-treated CoreTg than in fluvastatin-treated NTg.

Conclusions: Fluvastatin ameliorates the disorder of lipid metabolism and ETS disorder caused by HCV core protein. Fluvastatin also recovers the mitochondrial structures and improves the composition of cardiolipin. These results may provide new therapeutic tools for not only chronic hepatitis C, but also other metabolic liver diseases such as nonalcoholic steatohepatitis, in which steatosis and mitochondrial dysfunction contribute to liver pathogenesis.

1259 EFFECTS OF OESTROGEN DEFICIENCY IN PLASMATIC PARAMETERS AND HEPATIC MITOCHONDRIAL FUNCTION
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Aims and Background: Menopause, a physiological loss of ovarian hormone production, can be associated with hepatic steatosis and related metabolic pathologies. The aim of the present study was to evaluate the effects of oestrogen deficiency in plasmatic parameters and hepatic mitochondrial function.
Methods: 7-weeks-old ovariectomized swiss CD-1 mice (20–25 g) were used in this study. The results were compared to those obtained with control (sham-operated) mice. After 10 weeks, mice were fasted for 12 h, blood was taken by cardiac puncture and tissues were immediately harvested. Plasma glucose, total cholesterol, triglycerides, HDL-cholesterol, VLDL-cholesterol and LDL-cholesterol concentrations were analysed by standard methods. Hepatic mitochondrial function was measured by assessing oxygen consumption in the presence of fatty acids, succinate and β-hydroxybutyrate, as well as, the maximal respiration in the presence of FCCP, and reactive oxygen species (ROS) production. Results: Fasting plasma glucose levels were significantly elevated in the OVX mice (+31.5% ±0.71, n=8), as well as, total cholesterol (+86% ±1.65, n=8) and LDL cholesterol levels (+200% ±0.63, n=8). Average fasting triglyceride and HDL cholesterol levels were not significantly different between the groups. Oxygen consumption by intact mitochondria in β-oxidation was inhibited irrespective of the fatty acid used. Octanoyl-CoA (-56% ±8.5, n=6), palmitoyl-CoA (-45% ±1.16 n=6), and palmitoyl-carnitine (-45% ±1.18 n=6) oxidations were inhibited in OVX mice. With β-hydroxybutyrate and succinate as substrates the rates of oxygen consumption in the presence of ADP (state III) were decreased (-25.2% ±3.1 and -55.2% ±12.6, n=8) in OVX mice. State IV of respiration was also significantly lower in OVX group with both substrates (-17.2% ±0.84 and -27.6% ±1.69, n=8), as well as, the respiratory control ratio. In the presence of FCCP the rates of oxygen consumption were decreased in OVX mice with both substrates, β-hydroxybutyrate (-19.7% ±2.4, n=8) and succinate (-18.09% ±3.46, n=8). The OVX group exhibited increased (+94.42% ±4.0 n=6) ROS production compared to the SHAM group. Conclusion: These data indicate that OVX resulted in significant impairment in the mitochondrial carbohydrate and lipid oxidations. So, in a condition of oestrogen deficiency, these could be contributing effects to divert hepatic lipid metabolism from fatty acid oxidation to FFA synthesis, thereby favouring liver triglyceride accumulation. Financial support: CAPES.

1260 URI HEPATOCYTE-SPECIFIC OVEREXPRESSION IS SUFFICIENT TO INDUCE NON ALCOHOLIC STEATOHEPATITIS (NASH)

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Background and Aims: Liver is the most essential metabolic organ. Nutrients overload, through elevated levels of insulin and related insulin-like growth factor (IGF) dysregulate hepatic function affecting whole body energy metabolism balance leading to severe hepatic disorders which can ultimately progress to hepatocellular carcinoma (HCC), supporting epidemiological studies indicating the strong correlation existing between obesity, type 2 diabetes (T2D) and HCC development. The well-established nutrient sensing mTOR circuitry has been described to be a critical signalling pathway downstream of IGF and deregulated in both obesity-associated T2D and HCC and therefore linked to poor prognosis. However, downstream mTOR effectors remain elusive. In this regard, URI, identified as a downstream effector of mTOR/S6K1 pathway, was described to be an additive oncogene amplified in human ovarian cancer. Moreover, data from our lab show URI is frequently up-regulated in human HCC and correlates with tumour cell proliferation and decreased patients survival. Additionally we gather evidence that URI is increased in livers from obese patients and high-fat diet-treated C57Bl6J mice present high hepatic URI expression, contrariwise to fasted mice, placing URI as a nutrient sensor. Finally, mice generated in our lab and expressing URI in hepatocytes display hepatic metabolic dysfunctions and spontaneous liver tumours. However, the underlying molecular mechanisms remain unknown. We therefore aim to a better understanding of the role of URI in hepatic metabolic dysfunctions that may progress to HCC.

Methods: Transgenic mice have been generated in which, FLAG tagged hURI transgene is targeted to the Col1A1 locus and the rTAT transactivator is expressed under the LAP promoter. To follow disease progression, mice were sacrificed and analysed at different time points of hURI expression. Liver histopathological characterization, body densitometries, cytokine profile and glucose and insulin tolerance tests were performed.

Results: Increased fibrosis, inflammation and finally hepatosteatosis were detected at early time points of hURI expression. Physiological mechanisms indicate insulin resistance (IR) of the white adipose tissue (WAT) leading to increased serum circulating free fatty acids accumulating into the liver as triglycerides and defining the presence of a NASH.

Conclusions: URI induces NASH pinpointing URI to be a new therapeutic target in NASH treatment.

1261 DELETION OF CASPASE-8 IN HEPATOCYTES IMPROVES HEPATIC INFLAMMATION AND INJURY BY MODULATING THE EXPRESSION OF LIVER METABOLISM-RELATED GENES IN A MURINE MODEL OF NASH

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Background: Hepatocyte injury in form of apoptosis is a prominent feature in human disease and murine models of non-alcoholic steatohepatitis (NASH). Caspase-8 (Casp8) is essential for the activity of death receptor signalling pathway and thus for the modulation of apoptosis. However, little is known on non-apoptotic functions of Casp8 in liver disease with the view to find a novel therapy for the treatment of NASH.

Aim: To dissect the role of hepatocyte Casp8 in murine models of steatohepatitis.

Methods: We generated hepatocyte-specific Casp8 knockout (Casp8loxP) mice by crossing Casp8loxP with transgenic Alb-cre mice. Animals were then fed with either a methionine-choline-deficient (MCD) diet, a high fat diet (HFD) or a Chow diet for 10 weeks. Liver injury was evaluated by histo-pathological analysis, serum markers, apoptotic cell-death, FACS analysis of liver infiltration and inflammation and assessment of reactive oxygen species (ROS). Moreover, microarray analysis was performed on animals treated with or without TNFα.

Results: Expectedly, MCD and HFD feeding triggered hepatosteatosis, lipid storage and accumulation of free fatty acids (FFA) in wildtype (WT) livers. In contrast, Casp8loxP livers elicited significant reductions in these NASH-related hallmarks. Additionally, deletion of Casp8 in hepatocytes caused a reduction in apoptosis and decreased hepatic infiltration and expression of proinflammatory cytokines such as TNFα and IL-6 in the MCD feeding model. However, no differences in hepatocyte injury and inflammation were observed in HFD-fed mice. Furthermore, microarray analysis of animals challenged with TNFα revealed a profound change in gene expression of liver enzymes related to lipid metabolism (e.g. MCAD, ELOVL3). In order to further prove these results we analyzed these genes in the NASH-diet models at mRNA and protein levels and confirmed our previous findings in MCD and HFD fed mice.

Conclusion: Our results demonstrate that selective ablation of Casp8 in hepatocytes ameliorates development of NASH by...
modulating hepatic lipid metabolism, hepatic inflammation and liver injury aside its apoptotic function. Consequently, targeted inhibition of Casp8 in hepatocytes might provide a novel therapeutic approach to prevent lipid deposition and liver fibrosis.

1262 HEMATOPOIETIC CASPASE-1 DEFICIENCY REDUCES HEPATIC INFLAMMATION AND CHOLESTEROL CRYSTALLIZATION IN HYPERLIPIDEMIC MICE

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Background and Aims: While non-alcoholic steatohepatitis (NASH) is characterized by hepatic steatosis combined with inflammation, the mechanisms triggering hepatic inflammation are unknown. Consequently, therapeutic options are poor, and non-invasive markers to detect NASH do not exist. In Ldr−/− mice, we have previously shown that lysosomal cholesterol accumulation in Kupffer cells (KCs) correlates with hepatic inflammation and cholesterol crystallization. Cholesterol crystals activate inflammasomes; protein complexes that induce the processing and release of pro-inflammatory cytokines IL-1β and IL-18 via caspase-1 activation. We therefore hypothesized that caspase-1 deletion in KCs leads to reduced hepatic inflammation.

Methods: Ldr−/− mice were transplanted (tp) with wild-type (Wt) or caspase-1−/− bone marrow and fed either regular chow or a high-fat, high-cholesterol (HFC) diet for 12 weeks. Additionally, plasma concentrations of IL-1 receptor antagonist (IL-1Ra) were measured in severely obese patients with varying degrees of fatty liver disease.

Results: After dietary intervention, no differences in plasma and liver lipid levels were found between Wt- and caspase-1−/−-tp mice. In line with our hypothesis, caspase-1−/−-tp mice had less severe hepatic inflammation than Wt-tp animals, as evident from liver histology to detect inflammatory cells and gene expression analysis in isolated KCs. Remarkably, KCs from caspase-1−/−-tp mice showed reduced cholesterol crystallization. Finally, we confirmed the relevance of IL-1-dependent inflammation to human NASH by demonstrating that plasma IL-1Ra, which is known to reflect IL-1 activity, is higher in NASH patients than in control patients, either with or without steatosis.

Conclusions: These data indicate that caspase-1 activation in KCs is an important factor in NASH development. Caspase-1 activation in hematopoietic cells results in cholesterol crystallization, thereby contributing to a vicious cycle of inflammation by further activating the inflammasome. Our findings also stress the potential use of plasma IL-1Ra as a biomarker for NASH.

1263 ADENOSINE A2a RECEPTOR STIMULATION REDUCES INFLAMMATORY Th CELLS SUBSETS IN STEATOTIC MICE LIVER AND PREVENTS PROGRESSION TO NASH

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Background and Aims: The mechanisms responsible for steatosis evolution to non alcoholic steatohepatitis (NASH) are still unclear and no ultimate therapeutic options are available. This study evaluated the involvement of the adaptive immune response in this process and investigated the effect of adenosine A2a receptor (A2aR) activation on NASH-associated inflammatory and immune responses.

Methods: C57BL/6 mice were fed either a normal diet or a methionine/choline deficient diet (MCD) diet for 4 weeks. The A2aR agonist CGS21680 [2-p-(2-carboxyethyl)phenethlaminio-5-Nethylcarboxamidoadenosine] was administered i.p. (0.5 mg/kg of body weight). Liver Th1 and Th17 lymphocytes were analyzed by flow cytometry.

Results: Four weeks on the MCD diet caused macrovesicular steatosis, liver-cell necrosis and lobular inflammation increasing ALT release and liver TNF-α expression. CGS 21680 treatment was started after 1 week on the MCD diet, when livers showed extensive steatosis, without major evidence of parenchymal damage or inflammation. Three weeks of CGS 21680 treatment protected against liver injury and inflammation without affecting steatosis. The development of NASH was associated with an increase in the liver content of CD3+ T-lymphocytes. Flow cytometry revealed that Th-1 (IL-17+ CD4+ and IL-22+ CD4+) T-cells markedly increased in the liver following the 1st and 2nd week on the MCD diet, whereas a significant increase of Th1 (IFN-γ+ CD4+) T-cells was evident at 4th week. CGS21680 treatment entirely prevented liver infiltration by both Th-17 and Th-1 T-cells.

Conclusions: Steatosis progression to NASH is associated to an early increase of Th-17 T-cells and by a subsequent Th1 cells recruitment. A2aR activation inhibits T-lymphocyte infiltration and ameliorates NASH, suggesting a possible use of A2aR agonists as immunosuppressive and hepatoprotective agents for NASH therapy.

1264 THE IMMUNOLOGICAL RESPONSE OF INNATE CELLS IS ALTERED REGARDLESS THE SEVERITY OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) PROGRESSION IN CHILDREN AND ADULTS

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Background and Aims: Obesity increases the risk for metabolic diseases. NAFLD ranges from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH). Reactive oxygen species (ROS) and cytokines production by Kupffer Cells (KC), monocytes (Mo) and T cells are associated with liver damage. We aimed to evaluate alterations of the immunological response during NAFLD progression.

Patients and Methods: Peripheral blood mononuclear cells (PBMC) were obtained from adults (AD) with NAFLD (NAFLDA), n=60, healthy controls (Co, n=30), obese children (OB, n=30) with/without NAFLD (n=70) and Co (n=30), and liver biopsies from NAFLDA. To evaluate ROS production, PMBC or chemically/mechanically obtained liver cell suspensions were incubated with dichlorofluorescein-diacetate (5μM), stimulated with Lep (10nM), palmitic and linoleic acids [PA (500μM), LA (200μM)], stained with anti-CD14 (Mo) or anti-CD14/CD11b mAbs (KC) and analyzed by flow cytometry. A stimulation index (SI) results from the mean fluorescence intensity (MFI) in stimulated/non-stimulated cells. Intracellular TNFα was evaluated in Lep-stimulated PBMC and a fold of increase index (FI) calculated for CD14+ TNFα+ cells. Production of IFN-γ after PMA stimulation (25 ng/ml) was evaluated in CD4+ or CD8+ cells. Mann–Whitney (p) and chi-square association tests were conveniently used.
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Results: LA induced higher production of ROS in Mo from NAFLDAd (p=0.032) and OBch (p=0.015) vs. controls. LA, PA and Lep stimulated the oxidative burst in KC from NAFLDAd (p=0.001, 0.010 and 0.045 respectively). Leptin induced a higher production of TNFα in NAFLDAd (p=0.009) and OBch (p=0.009) vs. controls. IFNγ-producing CD4 and CD8 subpopulations were larger in NAFLDAd (p=0.007 and 0.08) and OBch (p=0.07 and 0.005) vs. controls. Significant associations were found between NASHAd and NASHCh with CD4+ IFNγ (p=0.029 and 0.013) and CD8+ IFNγ (p=0.036 and 0.039) but not between ROS and TNFα production in Mo/KC with NASHAd or SSAd, or OBch with/without NASH by chi-square association tests.

Conclusions: Given the immunological alterations found in innate cells, SS should not be considered a benign condition. Furthermore, immunological alterations may be involved in NAFLD progression.

1265 FEMALE MOUSE LIVERS ARE MORE SUSCEPTIBLE TO OXIDATIVE STRESS THAN MALE LIVERS IN CAFETERIA DIET-INDUCED OBSESE MICE

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Background and Aims: Changes in the cellular oxidative status are involved in the pathogenesis of obesity-associated hepatic steatosis. Whether there are gender differences in this process is unknown. This possibility was examined by comparing parameters of oxidative status and the gene expression of related enzymes in the livers of female and male mice with obesity induced by a western high-fat and high-carbohydrate cafeteria diet.

Methods: 4 Groups of 6 Swiss CD1 mice (21 d old) received either cafeteria diet (cafeteria male CafM; cafeteria female CafF) or balanced diet (control male CM; control female CF) for 14 weeks. Hepatic hydrogen peroxide (H2O2), thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and the activity and/or gene expression of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), hypoxia inducible factor (HIF-1-α) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) were measured.

Results: Cafeteria-fed mice presented higher body fat gain, steatosis, and higher plasmatic levels of LDL, VLDL, total cholesterol and glucose. Higher levels of TBARS and mitochondrial H2O2 were found in livers from cafeteria-fed animals of both genders (CafM and CafF), with higher levels in females than in males, independent of diet. The level of GSH was lower in cafeteria-fed mice of both gender; females (CF and CafF) showed higher levels of GSH than males (CM and CafM). CAT and GPx activities were reduced in cafeteria-fed mice of both sexes; the activities in females (CF and CafF) were lower than those of males (CM and CafM). SOD activity and the gene expression of CAT, GPx and SOD were not significantly altered when compared by gender or dietary treatment, but there was a significant increase in the expression of HIF-1-α and Nrf2 in female cafeteria-fed mice (CafF).

Conclusions: The female animals exhibited a higher susceptibility to cellular oxidative stress in cafeteria diet-induced obesity in comparison to males. The molecular mechanisms seem to be, in part at least, post-transcriptional, since the mRNA expression of the antioxidant enzymes was not different with regard to gender or dietary treatment. The higher mRNA expression of HIF-1-α and Nrf2 suggests more complex regulatory mechanisms.

Acknowledgements: CAPES, CNPq, Fundação Araucária.

1267 PERFORMANCE OF NEW ULTRASOUND METHOD FOR ASSESSING LIVER STIFFNESS – SHEAR WAVE™ ELASTOGRAPHY IMAGING IN RATS WITH EXPERIMENTAL OBESITY

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Background: NAFLD is the most important cause of chronic liver disease and is considered the hepatic manifestation of the metabolic syndrome associated with type 2 diabetes. The prevalence of NAFLD in the general population reaches 15–20% and it goes up to 76 to 90% in the obese population. The current study aims to evaluate changes in liver stiffness (LS) measured by ShearWave™ Elastography (SWE) in rats with obesity and assess its diagnostic efficacy in noninvasive steatosis assessment.

Materials and Methods: We studied changes of LS measured by SWE in animals with experimental obesity. Rats were divided into 3 groups: I – intact control (n=10), II – animals with high fat diet-induced obesity (DIO) (n=15), III – animals with glutamate-induced obesity (GMO) (n=10), and IV – animals with high fat diet and glutamate deficiency (DIO-GMO) (n=10). All animals were sacrificed at 7 weeks post treatment. The LS was measured on the right liver lobe of anesthetized rats. The LS was evaluated by transverse B-mode ultrasonography using a high-resolution ultrasound scanner (Hitachi, Japan) and placed in a water bath to reduce acoustic impedance mismatch between the transducer and the rat’s skin. The SWE technique was applied to measure the LS, and the raw stiffness values were recorded. The LS values were then calculated using the following formula: 1−tan(θ) = (1−tan(β))/tan(β), where θ is the angle of the shear wave and β is the angle of the shear wave to the direction of the ultrasound beam. The shear wave angle (θ) was measured by an automatic shear wave calculator (Hitachi, Japan) based on the acoustic impedance mismatch between the SWE probe and the rat’s liver.

Results: The LS measured by SWE was significantly higher in animals with high fat diet-induced obesity (DIO) compared to the intact control group (n=10). The LS values in the DIO group were significantly higher than in the intact control group (n=10) and the DIO-GMO group (n=10). The LS values in the DIO-GMO group were significantly lower than in the DIO group. The LS values in the intact control group were significantly lower than in the DIO-GMO group. The LS values in the DIO-GMO group were significantly lower than in the intact control group.

Conclusions: The SWE technique is a noninvasive method for assessing liver stiffness and has the potential to be used as a diagnostic tool for the early detection of NAFLD and its progression.

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obesity (GIO) (n = 15), which induced by subcutaneous injection of monosodium glutamate (4 mg/g) in the neonatal period (2, 4, 6, 8, 10 day of life). To assess morphological changes in liver we used NAS (NAFLD activity score). We performed 10 valid measurements of LS in every rat, and a median value was calculated, the result being measured in kPa. Significance was tested using Student's **t** test. Evaluation of diagnostic accuracy of SWE performed using ROC-analysis.

**Results:** A valid LS determination (success rate of at least 60%) was observed in 15/15 (100%) rats with GIO and 14/15 (93.3%) with DIO. We noted significant increase of LS on 63.6% in rats with GIO, and respectively on 52.2% in animals with DIO. We noted significant increase of LS on 63.6% in rats with GIO and 14/15 (93.3%) with DIO. The AUROC of SWE for in animals with GIO was 0.983 (95%CI 0.955–0.999; p < 0.001) and 0.994 (95% CI 0.981–1.0; p < 0.001) respectively with DIO. The optimal LS cutoff point for the prediction of steatosis were >6.25 and >6.1 kPa, with sensitivity, specificity, PPV and NPV 86.6%, 100%, 100% and 93.7% respectively for GIO and 93.3%, 100%, 99.7% and 96.7% for rats with DIO.

**Conclusion:** Shear Wave™ Elastography can be used as noninvasive method for the detection of non-alcoholic steatosis.

**1268**
**HEME OXYGENASE-1 LEVELS AND OXIDATIVE STRESS-RELATED MARKERS IN FATTY LIVER, INDUCED BY HIGH FRUCTOSE DIET**

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**Background and Aims:** Excessive dietary fructose intake may have an important role in the current epidemics of fatty liver disease, obesity and diabetes – features of metabolic syndrome. The mechanisms associated with development of the fatty liver disease appear to involve multiple cellular adaptations to the oxidative stress occurring when fatty acid metabolism is altered. We evaluated the relationship between lipid peroxidation and other oxidative stress biomarkers with changes in expression of heme oxygenase-1 (HO-1) in rat fatty liver, induced by high fructose diet (HFD) and the effect of S-adenosylmethionine (SAMe).

**Methods:** Twenty-one male rats were randomly assigned to three groups of seven animals each: HFD (35% fructose in drinking water for 16 week) group, HFD + SAMe (20 mg/kg in drinking water for 16 week) group and control group. HO-1 expression, MDA (marker of lipid peroxidation), triglycerides (TG), glutathione (GSH) levels and histological (H&E) studies were assayed in liver.

**Results:** HFD group showed microvesicular steatosis without inflammation and fibrosis. In HFD+SAM group microvesicular steatosis was not established. The HO-1 expression was significantly increased in HFD rats (p < 0.001). SEMe augmented the increase in expression of HO-1 (p < 0.01). The levels of MDA and TG were elevated in HFD group (p < 0.01). In HFD rats with lower levels of GSH exhibited higher expression of HO-1. SAMe inhibited the elevation in lipid peroxidation and TG levels and prevented the decrease in GSH levels (p < 0.05).

**Conclusions:** The induction of HO-1 is an adaptive response against oxidative damage elicited by lipid peroxidation and it may be critical in the pathogenesis of the high fructose-induced fatty liver in rats. SAMe has hepatoprotective effect and its protection likely exerted by increased expression of the antioxidant enzyme HO-1 to prevent the development of fatty liver.

**Figure 1. Dose–response curve to phenylephrine.**

**Results:** Maximum contraction was significantly lower in models of PHT (steatosis, CBDL and PPVL) compared to controls (Sham):
143.89±12.86%, 133.70±7.41% and 137.50±8.73%, respectively compared to 181.67±11.86 (p<0.0001, Figure 1). Piroxicam significantly reduced vascular response in sham and PPVL, modestly in CBDL, but not in steatosis. L-NAME restored responses in PPVL. Also in steatosis L-NAME restored vascular contractility to values comparable to controls: 160.91±6.65% vs. 166.36±7.94% (p=0.1732).

In CBDL L-NAME only slightly ameliorated vascular response. L-NAME/piroxicam combination did not significantly alter arterial contraction.

Conclusions: This study confirms vascular hyporeactivity in non-cirrhotic NAFLD-associated PHT comparable to what is observed in classical PHT. In normal conditions vascular reactivity is regulated by both NO and vasoconstrictor COX activity. Hyporesponsiveness in steatosis and CBDL (both models of sinusoidal PHT) is in part caused by absence/reduction of COX-mediated vasoconstriction and by NO overproduction. By contrast, COX-mediated vasoconstriction is maintained in PPVL, in which hyporesponsiveness is solely caused by NO overproduction.

1270 ALTERED FATTY ACID PROFILE IN LIVERS OVEREXPRESSION THE Igf2 mRNA BINDING PROTEIN p62: INDUCTION OF FATTY ACID ELONGATION ELOVL6 VIA Igf2-DEPENDENT SREBP1 ACTIVATION

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Background: The dysfunctional lipid metabolism in steatosis is not only reflected by the increased amount but also by an altered composition of fatty acids. Recently, the overexpression of elongation of very long chain fatty acids 6 (ELOVL6), which catalyses elongation of fatty acids, has been shown to promote nonalcoholic steatohepatitis in mice and humans (Matsuzaka et al., Hepatology 2012). Liver-specific overexpression of the insulin like growth factor 2 (Igf2) mRNA binding protein p62 promotes lipogenesis by decreased hepatic expression of IFN-γ (decreased IFN-γ receptor expression and IFN-γ expression in WT and KO mice). Piroxicam replaced with fish oil to create iso-caloric diet with decreased ω-6:ω-3 ratio to 2.7:1 (HF7ω). After 15 weeks on the respective diets, we assessed insulin action, adipose and liver pathophysiology and inflammatory gene expression in WT and KO mice.

Methods: Soybean oil rich HF with 45% kcal from fat was used as model of high dietary ω-6:ω-3 ratio of 11:1 (HFω). Soybean oil was replaced with fish oil to create iso-caloric diet with decreased ω-6:ω-3 ratio of 0.78:1 (HFω-LP). In addition, L-NAME/piroxicam combination did not significantly alter arterial contraction.

Results: WT mice fed HFω exhibited reduced liver but not adipose tissue inflammation compared to WT mice fed HFω-LP, as evidenced by decreased hepatic expression of IFN-γ (P<0.01), TNF-α (P<0.05), IL-12p40 and 18 (P<0.05), and C-C chemokine receptor type 7 (P<0.05). Fatty liver, adipose tissue inflammation, and adiposity were not different between HFω and HFω-LP-fed groups. However, KO mice were protected from fatty liver and from liver inflammation (decreased IFN-γ, P<0.05) and lymphocyte homing (decreased C-C motif chemokine 19, P<0.05) induced by HFω in WT mice. Only the KO mice were protected from HFω-LP-induced insulin resistance as determined by hyperinsulinemic euglycemic clamp.

Conclusions: Nonalcoholic steatohepatitis is characterized by increased ω-6:ω-3 hepatic ratio. High dietary ratio of ω-6:ω-3 commonly found in Western diet may exacerbate this proinflammatory microenvironment. Our study concludes that diet rich in fish oil significantly reduces HF-induced hepatic inflammation. In addition, we conclude that inhibiting the activity of 12/15-lipoxygenase may protect from diet-induced steatohepatitis, as well as enhance insulin action.

1272 n-3 PUFA AND UDCA CO-TREATMENT REDUCES INFLAMMATION AND FIBROSIS BUT NOT STEATOSIS AND BALLOONING CHANGE IN MURINE NASH MODEL

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Introduction: Hepatic steatosis, ballooning change, inflammation and fibrosis are hallmarks of nonalcoholic steatohepatitis (NASH). For the treatment of NASH, improvement of fibrosis is important to prevent the development of liver cirrhosis.

Aim: We evaluate the effects of n-3 polyunsaturated fatty acid (PUFA) and/or ursodeoxycholic acid (UDCA) on a murine model of NASH.

SS14 Journal of Hepatology 2013 vol. 58 | 5409–5566
Material and Methods: C57BL/6 mice, fed a high-fat diet (HFD) for 24 weeks, were confirmed to result in NASH, and divided into four groups for additional 24 weeks treatment. HFD group was fed HFD for 48 weeks, UDCA group was fed with HFD for 24 weeks, and then HFD+UDCA at a dose of 20 mg/(kg.d) via gavages for additional 24 weeks, Pufa group was fed with HFD for 24 weeks, and then HFD+PUFA at a dose of 70 mg/(kg.d) via gavages for following 24 weeks, and UDCA+PUFA group was fed with HFD for 24 wk, followed by HFD+UDCA+PUFA for 24 weeks. At the end of the experiment, body weight, serum biochemical index, and hepatopathologic changes by CRN scoring system were examined.

Results: Liver showed hypertrophic and yellowish color change and all mice developed steatohepatitis after 24-week-HFD. The mean CRN scores by their liver histology as follows: 2.9 ± 0.3 in steatosis, 2.65 ± 0.7 in lobular inflammation, 1.8 ± 0.4 in ballooning and 1.9 ± 0.3 in fibrosis. After additional 24 weeks of HFD, only fibrosis score was increased significantly to 2.4 ± 0.3. Treatment with UDCA and/or PUFA reduced liver/body weight ratio. Compared with the HFD group, significant improvement of steatosis was noted only in the UDCA group. Lobular inflammation was improved significantly both in UDCA and UDCA+PUFA groups compared with the HFD group. However, significant improvement of fibrosis was seen only in the UDCA+PUFA group.

Conclusion: UDCA might play a role in the improvement of steatosis and lobular inflammation. Co-treatment of UDCA and PUFA reduces inflammation and finally fibrosis. For clinical application for NASH, UDCA and PUFA co-administration can be beneficial to prevent progression into cirrhosis.

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OBESITY-INDUCED NAFLD: IDENTIFICATION OF TRANSCRIPTIONAL MASTER REGULATORS CONTROLLING METABOLIC AND INFLAMMATORY RESPONSES TO HIGH FAT DIET IN LIVER AND ADIPOSE TISSUE

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Background and Aim: Obesity can be induced with high fat diets (HFD) and is associated with inflammation in white adipose tissue (WAT) and liver. The factors that control the early metabolic responses to HFD and that trigger inflammatory gene expression are only poorly understood. The aim of this study is to identify the master regulators that control the early metabolic and inflammatory responses in adipose tissue during obesity and to examine whether these master regulators also have a role in the liver.

Methods and Results: Time-resolved analysis of differentially expressed genes in expanding adipose tissue of mice (12 weeks HFD feeding) identified specific clusters of lipid metabolism-related genes and inflammation-related genes with similar time expression profiles. Subsequent promoter analysis of the clustered genes revealed that specific master regulators (among which Fos, Esr1, Hnf4a, Jun, Ppara, Pparg, Nr1h2/Lxrβ, Nfkβ, Srebfl and 2, Sfp1, Smad2, Sp1) orchestrate metabolic adaptations and early inflammatory responses in WAT. Some of these transcription factors (Esr1, Jun, Fos, Pparg, Sp1) have a dual role and regulate the adjustment of lipid metabolism as well as expression of inflammatory genes such as Cxcl1/KC, Ccl5/Rantes, complement factors, ASC, granzyme A Ccl5/Rantes, Ccl5, Ccl7/Mcp3. Subsequent analysis of corresponding livers revealed comparable molecular responses on the level of transcription factors. More specifically, many of the master regulators identified in WAT were also involved in the liver response to HFD as demonstrated by analysis of hepatic target gene expression in conjunction with transcription factor binding activity analysis.

Conclusions: Our findings support the view that metabolic and inflammatory processes are interlinked in WAT and liver, and that responses to HFD are controlled in a similar way on the transcription factor level. Distortions of the mechanisms which control metabolic homeostasis in these organs may thus also affect their inflammatory tone.

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URSODEOXYCHOLIC ACID IMPROVES HEPATIC STEATOSIS AND INFLAMMATION BY THE FARNESOID X RECEPTOR IN OBES Model with Nonalcoholic fatty liver disease

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Background and Aim: Hepatic fat accumulation and changes in bile acids composition are hallmarks of nonalcoholic fatty liver disease (NAFLD). The farnesoid X receptor (FXR) is not only a key regulator of hepatic lipid biosynthesis but also a transcription factor regulated by bile acids such as ursodeoxycholic acid (UDCA). UDCA, initially introduced as a therapeutic approach to exhibit an anti-inflammatory property. A comprehensive study addressing the role of UDCA in the coordinated regulation of FXR-dependent manner of lipid homeostasis and together with inflammatory response is lacking.

Methods and Results: We therefore fed ob/ob mice with 1% (w/w) UDCA. Bile acid synthesis and transporter were assessed by immunohistochemical study and real-time RT-PCR for cytochrome P-450 (Cyp7a1, BSEP and Mrp2, and HPLC for determination of bile acids composition. UDCA repressed Cyp7a1 in FXR-dependent manner. FXR induction in the liver not correlated with bile acid levels and was observed in UDCA-fed obese animals. Accompanying the induction in FXR levels, we found a decreased in SrebP-1c, FAS and ACC in mRNAs suggesting a decreased lipogenesis and a reduced hepatic triglyceride production, respectively. As for metabolic parameters, UDCA reduced elevated serum triglyceride and free fatty acids values in ob/ob mice. Liver histology showed improvement of steatosis in obese mice concomitant with reductions in hepatic triglyceride and CD36 levels. Administration of the UDCA could attenuate hepatic inflammatory response, reduce transaminase activities, suppress inflammation mediators (TNF-α, IL-6, and MCP-1) expression and inhibit STAT3 phosphorylation. These protective effects of UDCA were accompanied by an increased expression of suppressor of cytokine signaling 3 (SOCS3), which is a negative feedback regulator of cytokine-STAT3 signaling.

Conclusion: The current study demonstrates that UDCA lowering hepatic lipid overloading as well as susceptibility of hepatocytes toward inflammatory stimuli, the modulation of SOCS3 expression may represent a novel mechanism through which FXR activation could selectively affect cytokine bioactivity in inflammation response. Thus, UDCA could be a promising tool for the treatment of liver inflammatory in NAFLD.

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1.03–5.39, p=0.043), with greatest risk in those homozygous for the I148M minor (G) allele in NAFLD was significantly associated with increased risk of developing HCC (CG/GG vs. CC: OR 2.35, 95%CI 1.48–3.74, p=0.001). In a multivariate model adjusted for age, diabetes and gender, PNPLA3 I148M genotype remained significant, p=0.009. Carriage of at least one copy of the PNPLA3 I148M minor (G) allele in NAFLD was significantly associated with increased risk of developing HCC (CG/GG vs. CC: OR 2.35, 95%CI 1.03–5.39, p=0.043), with greatest risk in those homozygous for the minor allele (GG vs. CC: OR 4.0, 95%CI 1.44–11.18, p=0.0077). Compared to the UK general population (using the 1958 British Birth Cohort DNA Collection) rather than a NAFLD patient cohort, the risk was even greater (CG/GG vs. CC: OR 4.49, 95%CI 1.85–10.95, p=0.0009; GG vs. CC: OR 14.75, 95%CI 4.0–54.3, p=0.0001).

Conclusions: This gene association study demonstrates that, within a population with histologically confirmed NAFLD, carriage of the PNPLA3 I148M single nucleotide polymorphism is significantly associated with HCC. These data therefore suggest that affected individuals are not only at greater risk of progressive steatohepatitis and fibrosis but also of development of HCC. If validated, PNPLA3 genotyping may offer a tool for patient-risk stratification.

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MACROPHAGE EXPRESSION OF Annexin A1 CHARACTERIZES THE PROGRESSION OF NONALCOHOLIC STEATOHEPATITIS (NASH) IN MICE

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Background and Aims: Annexin A1 (ANXA1), also known as lipocortin 1, is glucocorticoid-regulated anti-inflammatory protein that is expressed by neutrophils and macrophages and inhibits leucocyte recruitment contributing to the termination of inflammatory responses. Although recent studies have implicated ANXA1 in the modulation of the healing process and fibrosis in different tissues, little is known about a role of ANXA1 in the progression of chronic liver injury.

Methods: NASH was induced by feeding four weeks C57BL/6 mice with a methionine-choline deficient (MCD) diet.

Results: We observed that ANXA1 is expressed at low extent in the liver of naïve mice, but its mRNA and protein levels increased steadily after 4 and 8 weeks of feeding with the MCD diet in parallel with the progression of hepatic steatosis, lobular inflammation and the development of fibrosis. At immunohistochemistry ANXA1 was selectively localized in large mononucleated cells containing lipid droplets and apoptotic bodies that were positive for the macrophage marker F4/80, suggesting selective ANXA1 production by macrophages that had scavenged cell debris from dying fat-laden hepatocytes. ANXA1–positive macrophages were particularly evident after 8 weeks on the MCD diet in concomitance with the appearance of hepatic fibrosis. Interestingly, ANXA1 expression in the livers of MCD-fed mice inversely correlates with that of inducible NO synthase (r=−0.62; p=0.01), while it was positively associated with the expression of TGF-β (r=0.77; p=0.0007) as well as with that of fractalkine (CX3CL1) (p=0.59; p=0.008) and its receptor CX3CR1 (r=0.70; p=0.002), these latter implicated in directing survival and anti-inflammatory response in liver macrophages.

Conclusions: Altogether these results suggest that ANXA1 expression by macrophages scavenging apoptotic hepatocytes might modulate macrophage survival and functions during the progression of NASH. This work has been supported by grants from the Fondazione Cariplo (Milan).

The Wnt and Hedgehog (Hh) signaling pathways are part of the morphogenetic program which is essential for multiple processes in mammalian development. Recently, it has become apparent that both pathways fundamentally influence metabolic zonation and lipid metabolism in the adult liver. In the last decade, the Wnt/β-Catenin pathway was identified as a master regulator of liver zonation. For instance, the activation of Wnt/β-Catenin signaling in the liver by deletion of Apc lead to the pericentralization of hepatic functions and, accordingly, to a shift in the balance between anabolic and catabolic functions. Recent work has shown that the Hh signaling pathway regulate the lipid metabolism in adipose tissue in adult drosophila and mice. The morphogens in both pathways are usually poorly mobile signaling molecules that spread in the tissue to form concentration gradients. This was clearly shown for the gut, where both pathways act antagonistically and need mutual activation in a minimal dose-dependent manner, crucial for tissue homeostasis.

To investigate the crosstalk of Hh and Wnt signaling in metabolic zonation and lipid metabolism as well as the zonal distribution of Hh and Wnt components in the liver lobule we analysed the expression of Hh and Wnt signaling components in isolated perportal and pericentral hepatocytes. Beside this, transgenic mice with deletion of Smo (SMO-KO) or adenomatous polyposis coli (APC<sup>neo</sup>) where used to activate Wnt/β-Catenin or inactivate Hh signaling in the liver. Cell culture samples were analyzed using GC-MS and LC-MS-MS to determine metabolite concentrations in the supernatant. Changes in lipid classes were quantified in isolated hepatocytes using the MALDI-TOF/TOF analyses tool. With qRT-PCR technique changes in Wnt and Hh components were analyzed.
Our findings demonstrate a novel role of Hedgehog signaling in lipid metabolism which dominates with a more periporal distribution, whereas depletion of APC leads to a pericentralization of hepatic functions, associated with reduced urea production and glutamine uptake. The increase in pyruvate uptake with simultaneously decreased citrate and alpha-ketoglutarate production implicates its use for fatty acid synthesis. Investigations on Hedgehog signaling have demonstrated a prominent role in lipid metabolism, which is also subject to liver zonation.

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**URSODEOXYCHOLIC ACID COUNTERACTS LIPOTOXICITY AND INDUCES UNFOLDED PROTEIN RESPONSE IN MORBIDLY OBESE PATIENTS**


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**Background and Aim:** Expansion of white adipose tissue (WAT) with elevated fatty acid (FA) flux to the liver as a result of insulin resistance plays a key role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Treatment with taurine-conjugated UDCA (TUDCA) improved ER stress and steatosis in mice. Although UDCA is used in various liver diseases, its action on unfolded protein response (UPR) in humans remains to be elucidated. We aimed to investigate the impact of UDCA in morbidly obese patients on UPR and hepatic/WAT lipid metabolism as well as lipid partitioning along the WAT-liver-axis.

**Methods:** 20 morbidly obese patients receiving UDCA (20 mg/kg/day) for 21 days prior to bariatric surgery were compared to 20 morbidly obese controls without UDCA. Serum liver enzymes, lipids and bile acids (BAs) were measured before and after treatment. Biopsies from liver and visceral WAT (vWAT) were obtained during surgery to determine expression of genes involved in lipid partitioning by qRT-PCR and investigate total and free FA profiles by gas chromatographic/negative ion chemical ionization mass spectrometry (GC-NICI/MS) and GC-electron impact/MS, respectively.

**Results:** UDCA improved serum liver enzymes, increased triglycerides (TG) and decreased free FA serum concentrations (p ≤ 0.05). In vWAT, UDCA increased oleic acid (OA) in the total FA-pool and lowered free FA concentrations of lipotoxic palmitic acid (PA) and stearic acid by 84.8 ± 19.2% and 94.0 ± 18.4%, respectively. In line, SCD, the enzyme generating OA, was up-regulated in vWAT (p ≤ 0.01). In liver, changes in lipogenesis were reflected by equally up-regulated SCD but unchanged SREBP1c and FAS.

UDCA expanded the BA pool (p ≤ 0.05) with an UDCA enrichment of 87.7 ± 3.7%. Furthermore, hepatic genes involved in BA (CYP7A1, p ≤ 0.05) and cholesterol biosynthesis (SREBP2, p ≤ 0.01; HMGR, p ≤ 0.05) were induced. This was substantiated by increased serum C4 (p ≤ 0.001) and desmosterol (p ≤ 0.05) levels. UDCA stimulated hepatic UPR gene expression (GRP78, CHOP; p ≤ 0.01). Notably, activation of CHOP, a known apoptosis inducer, did not up-regulate apoptosis reflected by stable BAK, BAX and BCL2 expression.

**Conclusions:** UDCA improves hepatic injury, profoundly changes BA/cholesterol synthesis and protects liver from ER stress by inducing UPR. Furthermore, UDCA alters lipid partitioning in vWAT and liver counteracting lipotoxicity in morbid obesity.
to figure out the effect of a high cholesterol diet (HC, 2% cholesterol and 0.5% sodium cholate) in the bile duct ligation (BDL)-induced cholestasis and the involvement of c-Met in the repair process.

**Methods:** C57Bl/6 mice were fed with the HC diet for two days and after that were subject to BDL, parallel animals were fed with regular rodent chow diet (Chow) and BDL was performed. Hepatic enzymes in serum were measured. Western blotting of main survival pathways were analyzed and confocal immunofluorescence for c-Met was performed in primary hepatocytes isolated by the two-step collagenase perfusion.

**Results:** Our data show that HC animals were more susceptible to both insults, all animals in the HC-BDL group (n=6) die during the first 72 h after surgery, while all animals in Chow-BDL (n=7) were alive. We proceeded to sacrifice animals at 24, 48 and 72 h after BDL of all groups. Liver macroscopic inspection of HC mice showed the characteristic pale color in steatosis and changes in gallbladder. Although AST, ALT and ALP were increased as a consequence of BDL, animals fed with the hypercholesterolemic diet increased significantly these values (ranging from 20 to 200-fold), these data were in agreement with an elevation on bilirubin, suggesting an exacerbation of cholestatic damage. Examination of the main signaling pathways involved in repair process, such as Akt, Stat3 and Erk1/2 in HC animals showed a downactivation in Erk1/2 and Akt, but some compensatory effect in Stat3 was observed. In vitro analysis of c-Met activation by Western blotting and immunofluorescence in hepatocytes from HC animals revealed a delayed activation of this receptor after HGF treatment.

In conclusion our data suggest that cholesterol overload in hepatocytes aggravates cholestasis and impairs signaling pathways involved in liver repair as a consequence in c-Met misactivation. Work supported in part by CONACYT 131707, CD-2011–01166042, NNL and scholarship 234219.

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**MILD STEATOSIS AND IMPAIRED GLUCOSE TOLERANCE ARE ASSOCIATED WITH LIVER CANCER DEVELOPMENT, WHILE PROGRESSION TO NASH INCREASES NUMBER/SIZE IN AN ANIMAL MODEL OF NAFLD**

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**Background:** Obesity and T2DM have reached epidemic proportions worldwide, with NAFLD and hepatocellular cancer (HCC) rising as a consequence. The relative contributions of the metabolic syndrome and NAFLD to HCC development/progression are unclear. Our aim was to develop a dietary rodent model of progressive NAFLD, with steatohepatitis (NASH), fibrosis and HCC.

**Methods:** Eight week-old male C57Bl/6 mice were fed ‘American Lifestyle’ (ALIOS: 45% calories fat; high trans-fat, high sucrose/fructose) or nutritionally replete control diet (15% calories fat; low trans-fats) for 9 months ad libitum. Intra-peritoneal glucose tolerance tests were performed before diet initiation and culling. Body/liver weights, number/size of HCC were recorded. Histological features of NASH/fibrosis (Kleiner score) were assessed on H&E and Sirius red sections.

**Results:** All mice gained weight and developed impaired glucose tolerance (IGT) during the study however, ALIOS-fed mice were heavier (50.7±0.53 v. 43.9±0.78mm; p=0.003). Development of small HCC in mice, although none exhibited NASH. In the ALIOS group, marked (S2–3) steatosis was common, with histological NASH and frequent pericellular fibrosis. Similar numbers of mice developed well differentiated HCC in each group (control 8/11; ALIOS 10/11). However, ALIOS-fed mice had more tumours (2.6±0.43 v. 1.0±0.23; p=0.005) and HCC size was greater (total tumour diameter/mouse 12.3±2.6 v. 2.8±0.78 mm; p=0.003). Development of small HCC was independently associated with presence of steatosis (Binary logistic regression, p=0.002) and IGT (p=0.029) rather than body/liver weight or FBG in control-fed mice. Overall, steatosis grade (p<0.009), NAS score (p<0.003) and liver/total weight ratio (p<0.001) were independently associated with increased numbers of tumours, rather than body weight, IGT or FBG. Steatosis grade was an independent predictor of tumour size (p=0.023).

**Conclusions:** We describe a novel dietary model of NAFLD with spontaneous progression to HCC. Weight-gain, IGT and steatosis occur with ageing in control-fed, genetically predisposed, C57Bl/6 mice, associated with development of small HCC. The phenotype was more pronounced in ALIOS-fed mice, accompanied by NASH and increased numbers/size of tumours.
Background and Aims: Excessive accumulation of triglyceride-containing lipid droplets (LDs) within hepatocytes (steatosis) is a potentially reversible process leading, in patients, to Non Alcoholic Fatty Liver Disease (NAFLD) that may evolve into a Non Alcoholic Steato-Hepatitis (NASH), and eventually into cirrhosis and hepatocellular carcinoma (HCC). The NAD+-dependent deacetylase SIRT1 controls metabolic processes in response to restriction calories and administration of the synthetic SRT1720 activator has been shown to protect from diet-induced obesity and its negative consequences on glucose homeostasis by primarily promoting fat consumption in liver, skeletal muscle and adipose tissue in mice. Recent data have also shown that lipid loaded HCC cells up-regulate inflammatory mediators, NF-KB controlled genes and interferon stimulated genes (ISGs). Here, we evaluated the effects of the specific Sirt1 agonist MC2791 in a cellular model of steatosis development in non transformed differentiated human HepaRG cells (dHepaRG) treated with mono-unsaturated oleic acid.

Methods: Neutral lipids accumulation in cytoplasmic droplets and intracellular reactive oxygen species (ROS) generation were quantitated by flow cytometry analysis using a FACs-CAnto (Becton-Dickinson) using the neutral lipid marker BODIPY 505/515 and the non-fluorescent probe H2DCFDA, respectively. The expression of liver-specific markers (Aldolase B, cytochromeP450-CYP2E1, albumin and PPARG) was used to monitor HepaRG cells differentiation.

Results: Oleic acid-treated (4 days) dHepaRG cells showed a significantly higher BODIPY and H2DCFDA fluorescence signals compared to untreated dHepaRG cells, corresponding to an increased lipid accumulation distributed in small to medium size lipid droplets and ROS accumulation. Treatment of dHepaRG cells with the Sirt1 agonist MC2791 alone didn’t affect liver-specific markers expression or basal TG levels in dHepaRG cells. The co-treatment (4 days) with oleic acid together with MC2791 completely inhibited both lipids ROS accumulation in dHepaRG cells whereas a 2 days pre-treatment with MC2791 did not prevent lipid accumulation in response to a subsequent exposure to oleic acid.

Conclusions: Pharmacological modulation of hSirt1 has positive effects on both lipid and reactive oxygen species accumulation in response to excess lipid exposure.

Background and Aims: Hepatic diseases are among the most frequent health problems worldwide. Altered food intake habits, viral infections and lack of physical activity along with genetic background, are the main causes of this threaten. One of the first events triggering the hepatic disease is fat accumulation within hepatocytes, a process mediated by several genes; among the most prominent, are the peroxisome proliferating activation receptors (PPARα, PPARγ) and PPARγ actively regulates adipogenesis through responsive elements present in its target genes. However, the PPARγ transcriptional regulation during early liver disease remains elusive. The zinc finger-containing Krüppel-like transcription factors (KLFs) regulate both, positive- and negatively PPARγ gene expression during early and late adipogenesis, suggesting that KLFs might have a central role in liver disease progress. Here, we aim to demonstrate that KLFs regulate PPARγ gene expression during early liver disease.

Methods: HepG2 cells were treated with either, 10mM fructose or 300mM palmitic acid, in 6 well plates at a 0.5 × 10⁶ cells/well. After steatotic insult, total RNA or whole cell extract, was extracted. Real time PCR or Western-Blot was performed to determine gene expression changes, while PPARγ gene promoter occupancy was determined by Chromatin immunoprecipitation (Chip) experiments, using specific antibodies.

Results: To assess fatty acid storage in HepG2 cells, Red Oil staining absorbance was quantified at 510nm. We determined that fructose treatment slightly increases triglyceride content (F, 1.2±0.1 fold vs FC, 1±0.02); in contrast, palmitic acid treatment induced significantly fatty acid storage in HepG2 cells (P, 1.5±0.10 fold vs PC, 1±0.03; p < 0.001). In accordance, palmitic acid treatment was most effective inducing PPARγ and KLFs gene expression. Our ChIP assays showed that PPARγ is a gene target of KLFs. Under basal conditions, KLFs responsive elements (KEMs) were occupied by KLF6 and KLF9 in three of the four putative KEMs; whereas in palmitic acid-treated cells, this occupancy was replaced by KLF5, KLF6 and KLF9 in three of the four putative KEMs; whereas in palmitic acid-treated cells, this occupancy was replaced by KLF5, specifically at the −773/−764 KEM site.

Conclusions: Taken together, our data strongly suggest that KLFs might contribute to the mechanistic control of triglyceride accumulation within hepatocytes during early liver disease.

Background and Aims: PTEN is a tumour suppressor with a phosphoinositide phosphatase activity antagonizing PI3K signaling. Hepatic PTEN deficiency in mice induces steatosis, inflammation/fibrosis and hepatocellular carcinoma. Supporting a role for PTEN in steatosis development, we previously reported PTEN downregulation in steatotic livers of obese rats and humans. Interestingly, although liver-specific PTEN knockout mice develop steatosis/inflammation/fibrosis, they paradoxically exhibit an improved glucose tolerance. This study aims at understanding the mechanisms by which PTEN deficiency improves glucose tolerance, while promoting steatosis/inflammation/fibrosis.

Materials and Methods: 4-month old AlbCre/PTENlox/lox mice (PTENKO) and PTENlox/lox mice (CTL) were phenotypically characterized and subjected to glucose and pyruvate tolerance tests, as well as euglycemic hyperinsulinemic clamps. Ex-vivo liver and muscle tissues from mice stimulated or not with insulin were analysed.

Results: Liver-specific deletion of PTEN did not modulate body weight, ratio of lean vs total fat mass, food/water intake, locomotor activity, thermoregulation and energy expenditure in mice, but as previously described, it induced hepatomegaly and a marked hepatic steatosis. In the fed state, PTENKO mice
displayed increased ALT/AST serum levels, dyslipidemia, but normal glycermia. Interestingly, PTENKO mice exhibited an improved glucose tolerance that was due in part to a constitutive inhibition of gluconeogenesis. Impaired gluconeogenesis was associated with an increased basal Akt phosphorylation, but an inhibition of insulin receptor and IRS1 expressions/activities and hepatic refractoriness to further insulin signalling. In addition, expression of glycolytic enzymes was increased, whereas that of PEPCK, a rate-limiting enzyme in gluconeogenesis was downregulated. Surprisingly, PTENKO mice exhibited a significant increase in skeletal muscle insulin sensitivity. Indeed, analyses of insulin signaling in ex-vivo muscle tissues revealed increased phosphorylation of the insulin receptor, IRS1 and Akt in insulin-stimulated PTENKO mice. Confirming these data, euglycemic hyperinsulinemic clamps demonstrated a marked increase in the glucose infusion rate and the glucose utilization index in all types of skeletal muscles from PTENKO compared to Ctl mice.

Conclusions: Although liver-specific PTEN deficiency in mice triggers steatosis/inflammation/fibrosis, glucose tolerance is improved through inhibition of hepatic gluconeogenesis and through a liver to muscle crosstalk, promoting muscle insulin hypersensitivity.

1286 HISTONE VARIANT macroH2A1 ISOFORM HAVE DISTINCT EFFECTS ON LIPID ACCUMULATION IN HEPATOCYTES
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Background and Aims: Several epigenetic events including DNA methylation and histone posttranslational modifications have been shown to contribute to the development and progression of hepatic lipid accumulation (steatosis). However, the role of the incorporation of histone variants into the chromatin in liver diseases is poorly defined. MacroH2A1 is a histone variant of histone H2A that exist in two alternatively spliced isoforms: macroH2A1.1 and macroH2A1.2, which differentially bind ADP-ribose and correlate with cell proliferation. Genome-Wide distribution of macroH2A1 in mouse liver chromatin showed genes that cluster in the area of lipid metabolism. The role of the macroH2A1 isoforms in hepatic lipid accumulation is unknown. We hypothesized that macroH2A1 isoforms could be involved in the pathogenesis of steatosis.

Methods: HepG2 hepatoma cells and mouse immortalized hepatocytes (MIH) were used to study the expression of the isoforms. Their role in hepatic steatosis was investigated by plasmid overexpression of cherry-tagged macroH2A1.1 and macroH2A1.2, followed by immunoblotting. Cells transiently overexpressing macroH2A1 isoforms were treated with a mixture of oleic and linoleic acid, then stained with Oil-red-O for lipid accumulation. Image software-assisted lipid quantification was performed. Qualitative assessment of lipid species was determined by high performance lipid chromatography (HPLC) in MIH cells overexpressing macroH2A1 isoforms.

Results: Transient overexpression of macroH2A1.2 increases steatosis in both cell lines, while the overexpression of macroH2A1.1 was protective. Further, HepG2 and MIH cells show higher endogenous protein expression levels of macroH2A1.2 isoform compared to macroH2A1.1. This could be due to a spontaneous basal lipid accumulation. In addition, lipidomics analyses showed that transient macroH2A1.1 overexpression in MIH induce the production of mono-unsaturated fatty acids and omega-3, while macroH2A1.2 overexpression augments specifically cellular saturated fatty acids.

Conclusion: Histone variants macroH2A1.1 and macroH2A1.2 are new players in the development of steatosis in human and mouse hepatic cell lines and could serve as markers. While macroH2A1.1 is strongly protective against steatosis and induces the production of beneficial fatty acids, macroH2A1.2 worsens steatosis and leads to accumulation of detrimental fatty acids. This could be due to a differential effect on chromatin structure and on the expression of key lipogenic genes, which warrants further investigation.

1287 SORTILIN DEFICIENCY LEADS TO IMPROVED METABOLIC PHENOTYPE AND REDUCED HEPATIC STEATOSIS IN DIET-INDUCED OBESITY
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Background and Aims: Sortilin is a trafficking molecule directing newly synthesized molecules from the trans-Golgi network to secretory pathways or cell surface and plays a role in hepatic lipid secretion. One of the molecules trafficked by sortilin is acidic sphingomyelinase (ASMase), whose product ceramide is a major modulator of insulin signaling. Moreover, ASM regulates the turnover of sphingomyelin, and thus the hepatic lipid pool of sphingolipids (SL) and triacylglycerides (TG). We hypothesize that in sortilin−/− mice, reduced hepatic ASMase may improve insulin sensitivity and reduce hepatic TG accumulation in diet-induced obesity.

Methods: C57BL/6 and sortilin−/− male mice were fed regular chow (RC) and high fat diet (HFD) (60% Kcal from fat) for 10 weeks.

Results: Sortilin−/− mice have reduced body weight and visceral fat both after RC and HFD. In addition, sortilin−/− mice have significantly lower serum cholesterol and exhibit better glucose and insulin tolerance tests. In line with our hypothesis, sortilin−/− mice display better insulin signaling, as demonstrated by enhanced expression of phosphorylated Akt. Moreover, hepatocytes from sortilin−/− mice display increased in vivo response to insulin signaling. Hepatic steatosis, lipogenesis and gluconeogenesis are attenuated in HFD-fed sortilin−/− mice. In accordance with the proposed role of sortilin in ASMase trafficking, livers of sortilin−/− mice have reduced ASMase activity. Regarding adipose tissue, sortilin−/− mice display smaller adipocytes, increased expression of adiponectin mRNA, as well as reduced expression of pro-inflammatory cytokines.

Conclusion: Lack of sortilin induces a beneficial metabolic phenotype in diet-induced obesity, with respect to both liver and adipose tissue.

1288 miR-21 DEFICIENCY IMPROVES GLUCOSE TOLERANCE AND HEPATIC LIPID CATABOLISM IN MICE FED A HIGH-FAT DIET
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Background and Aims: miR-21 is a potent oncogenic microRNA overexpressed in many human cancers. In the liver, miR-21 is among the most constitutively expressed microRNAs and is further highly upregulated in HCC. However, whether miR-21 expression regulates also metabolic processes in the liver is unknown. This study aims at investigating the role of miR-21 in the hepatic lipid and glucose metabolism.

Methods: 2-months old Gdf-9-icre/miR-21flox/flox mice (miR-21KO) and miR-21flox/flox mice (CTRL) were subjected to glucose and pyruvate tolerance tests following a 3 weeks high-fat diet (HFD). Lean and
fat masses were evaluated by EchoMRI analysis. Serum and ex-vivo isolated liver tissues were then analysed.

**Results:** Under chow diet, no phenotypic differences or alterations of the hepatic lipid/glucose metabolism were observed in mir-21KO mice, except a decreased glucose output in response to a pyruvate challenge. In contrast, when fed a HFD, mir-21KO mice displayed an enhanced glucose tolerance associated with a reduced hepatic glucose production, and exhibited a decreased body and liver fat content as compared to CTL mice. In the liver, IRS2 expression and Akt phosphorylation were upregulated suggesting enhanced insulin sensitivity. Consistent with a decreased hepatic glucose output, G6Pase expression was significantly downregulated although PEPCK expression was increased. Regarding lipid metabolism, mir-21KO mice displayed a drastic reduction of the fatty acid transporter FAT/CD36, but normal expression of lipogenic enzymes. However, expressions of the intracellular liver-fatty acid binding protein FABP-1 and of rate-limiting enzymes promoting fatty acids oxidation in mitochondria and peroxisomes (CPT-1a and Acox1) were significantly upregulated, whereas the GPAT enzyme catalysing the first step of triglycerides synthesis was reduced in mir-21KO mice. Finally, circulating and hepatic levels of cholesterol were also significantly decreased in mir-21KO mice.

**Conclusions:** miR21 deficiency improves glucose tolerance and the hepatic lipid metabolism in mice fed a high-fat diet by enhancing hepatic insulin sensitivity, inhibiting gluconeogenesis and promoting fatty acids oxidative catabolism. Preventing a fine but choral modulation of miR21 targets involved in the regulation of the hepatic lipid and glucose metabolism (e.g. PPARα, CPT1a) might be of relevance to treat metabolic hepatic disorders associated with obesity and the metabolic syndrome.

**1289 INHIBITION of microRNA-21 ATTENUATES NON ALCOHOLIC STEATOHEPATIS IN MALE LOW-DENSITY LIPOPROTEIN RECEPTOR KNOCKOUT (LDLR−/−) MICE**
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**Introduction:** MicroRNAs (miRNAs) are a family of noncoding RNAs that regulate gene expression at the posttranscriptional level. Using microRNA microarray assay, miRNA-21 overexpression was observed in the liver in nonalcoholic steatohepatitis (NASH) patients. This study aimed to investigate the role of miRNA-21 in NASH.

**Methods:** Effect of miRNA-21 inhibition, using an antagonim, was investigated in Ldlr−/− mice under high fat diet for 14 weeks. Mice received two intravenous injection of antagonim-21, antagonist control (without specific target) or PBS. Wild-type mice served as control.

**Results:** Liver miRNA-21 expression was increased in Ldlr−/− as compared to wild-type mice (p<0.002; n=6 per group). Treatment of Ldlr−/− mice with antagonim-21 reduced liver miRNA-21 expression (9.7 fold decrease vs. antagonist control treated Ldlr−/− mice; p=0.002; n=6 per group). As expected, as compared to wild-type mice, Ldlr−/− mice treated with PBS or antagonist control had increased liver/body weight ratio, liver triglyceride and cholesterol levels, liver steatosis at histology, serum AST levels, liver expression of inflammatory molecules (Tnfa and Mcp1; mRNA and protein), liver CD3 cell staining and liver fibrogenic-related genes expression (Tgfb, collagen-Iα2). As compared to antagonist control, antagonim-21 did not change fasting serum glucose and lipid profile, blood arterial pressure or body weight (n=10–12 per group). Antagonim-21 had also no effect on liver/body weight ratio, liver triglyceride and cholesterol levels, and liver steatosis at histology (n=10–12 per group). Conversely, as compared to antagonist control, antagonim-21 decreased serum AST levels (p=0.016; n=11–12 per group), liver Tnfa (mRNA: p=0.046; protein: p=0.0006; n=7 per group), and Mcp1 expressions (mRNA: p=0.0006; protein: p=0.002; n=7 per group). Collagen Iα2 staining (p=0.028; n=11 per group), and Tgfb (p=0.004; n=7 per group) and collagen Iα2 (p=0.007; n=7 per group) gene expressions.

**PPARα, a known target of miRNA-21, is implicated in NASH. PPARα expression was decreased in Ldlr−/− mice as compared to wild-type mice (p=0.009; n=6–4 per group). Antagonim-21 restored liver PPARα expression in Ldlr−/− mice (p=0.019; n=6–4).

**Conclusion:** MiRNA-21 regulates key steps of NASH development: liver cell injury, liver inflammation and fibrogenesis. This effect likely implicates PPARα, one of miRNA-21 targets. Thus, antagonim-21 may be a new therapeutic strategy in NASH.

**1290 SULFATASE-2 (SULF2) EXPRESSED IN HEPATOCYTES AND STROMAL CELLS CONTRIBUTES TO THE DEVELOPMENT OF STEATOSIS, THE PROGRESSION OF NAFLD TO FIBROSIS AND THE ADVANCEMENT OF CANCER**
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**Background:** SULF2 is a target of Kruppel-like factor 6, involved in NAFLD progression to cirrhosis and HCC. SULF2 desulfates heparin sulfate proteoglycans (HSPGs) such as glypicanc-3 (GPC3) and Syndecan-1, modulating ligand activated FGF and Wnt signalling, as well as lipoprotein uptake in hepatocytes. We aimed to characterise SULF2 in murine and human tissues, exploring a role in NAFLD progression and cancer.

**Methods:** SULF2 mRNA was analysed in livers from CD1 mice (n=40) after 3 months normal/control; high sucrose; high fat or high sucrose/fat diets. These were compared to expression in C3H/He mice with NASH, pericellular fibrosis and HCC after 9 months on the American lifestyle (ALIOS) diet. Immunohistochemistry for SULF2, GPC3 and α-SMA was performed on needle core biopsies from 61 patients with HCC, graded according to Edmondson (n=17/29/15 grades 1/2/3).

**Results:** SULF2 mRNA increased 3–4 fold in CD1 murine liver tissues after 3 months high fat or sucrose diets (p<0.001) and increased stepwise in C3H/He mice at 1yr, in association with ALIOS-induced steatosis 0–3 (Relative Quantity (RQ) 0.78±0.09; 0.88±0.09; 0.92±0.19; 1.30±0.07; p=0.006 ANOVA; Pearson correlation 0.684; p=0.001). NASH, perisinusoidal fibrosis and well differentiated HCC (2–15 mm) were common in ALIOS mice. SULF2 was independently associated with perisinusoidal fibrosis (p=0.012, linear regression adjusted for steatosis, weight, liver weight and NAS score). In ALIOS associated HCC, SULF2 mRNA expression was reduced (0.83±0.22 vs. 1.24±0.07; n=8; p=0.05) rather than increased, as previously reported in advanced human HCC. In our own human biopsies, increased membranous and/or cytoplasmic expression of SULF2 in tumour cells was observed in only 8/61 (13.1%) tumours, although 24/61 (39.3%) tumours showed extensive expression of SULF2 in tumour-associated α-SMA +ve stromal cells. Increased GPC3 expression was observed in 44/61 (72.1%) tumours (cytoplasm or...
membranous). 17/24 (70.8%) of cases with stromal SULF2 were GPC3 positive in adjacent tumour cells. In addition, in five cases there was simultaneous SULF2 and GPC3 expression in tumour cells.

Conclusions: In combination, these murine and human data implicate a role for SULF2 in the development of steatosis, but also in modulation of signalling between the matrix and hepatocytes in both NAFLD progression and HCC.

1291 COMPLEMENT ALTERNATIVE PATHWAY ACTIVATION IS ASSOCIATED WITH SEVERITY OF NONALCOHOLIC STEATOHEPATITIS
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Background and Aims: Activation of the innate immune system plays a major role in the pathogenesis of nonalcoholic steatohepatitis (NASH). Recently, we showed complement activation via classical and lectin pathways in patients with NASH, which was associated with hepatic inflammation, neutrophil infiltration, and increased apoptosis. Here we investigated the role of the complement alternative pathway (AP) in NASH.

Methods: Hepatic mRNA and protein expression of various complement components were investigated in liver biopsies of obese subjects with healthy livers (N=10; NAS score =0) or with severe NASH (N=12; NAS score >=5). Furthermore, immunohistochemistry for properdin and myeloperoxidase (MPO) was performed, and hepatocyte apoptosis was measured.

Results: Although C3 mRNA and protein levels were decreased in subjects with NASH (1.0 vs. 0.5, p=0.04), increased levels of activated C3 (C3c) were found (2.6-fold, p<0.01), which positively correlated with lobular inflammation as assessed by Kleiner classification (r²=0.60, p<0.01). Hepatic properdin expression was not different between the groups (2.7 vs. 3.7, p=0.3). However, properdin expression positively correlated with activated C3 levels (r²=0.69, p=0.01) and increased with higher lobular inflammation scores (p=0.04). Immunohistochemical analysis showed colocalization of properdin and infiltrated MPO+ neutrophils surrounding steatotic hepatocytes. Concomitantly, decay accelerating factor mRNA and protein expression were upregulated in patients with NASH, while mRNA expression of factor H was significantly decreased (p=0.02). Hepatocyte apoptosis, a possible activator of the complement AP was significantly increased in patients with NASH.

Conclusions: Collectively, these data suggest a role for AP activation in driving hepatic inflammation during the progression of NASH, and indicate that alterations in host expression of regulatory complement factors might be involved as well.

1292 RESOLVIN D1 PRIMES INFLAMMATION-RESOLUTION PROGRAMMES DURING MODERATE CALORIE RESTRICTION IN OBESITY-INDUCED NASH
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Background and Aims: Non-alcoholic steatohepatitis (NASH) is a chronic liver disease closely associated with obesity and the metabolic syndrome. Obesity-induced NASH is characterized by aberrant lipid storage in hepatocytes combined with a remarkable unresolved inflammatory component. Both obesity and NASH progression can be effectively attenuated by weight loss following a moderate nutritional intervention aimed to reduce calorie intake. Resolvins of the D-series (RvDs) are a novel family of endogenous anti-inflammatory and pro-resolving lipid mediators that act as agonists of the timely resolution of inflammation and the return to tissue homeostasis. In the current study, we hypothesized that these endogenous autacoids could potentiate and accelerate the inflammation-resolution process in high-fat-fed obese mice with NASH placed on moderate caloric restriction.

Methods: Male C57BL/6 mice (n = 27) were fed with a high-fat diet (HFD) for 12 weeks and then randomly assigned into three groups. One group continued on HFD (n = 13) and the rest were switched to chow diet (calorie restriction) and received either RvD1 (300 ng/daily, i.p., n = 7) or placebo (n = 7) for the last 3 weeks. At the end of the intervention period, hepatic steatosis, inflammation, macrophage accumulation, insulin resistance and serum and tissue adipokine levels were determined. RvD1 (10 nM) actions on liver cells were tested in precision cut liver slices (PCLS). RvD1 gene targets and microRNA networks were screened by real-time PCR and miRNA arrays.

Results: Moderate caloric restriction induced anti-obesogenic and anti-steatotic effects, reduced serum leptin and resistin levels and ameliorated insulin resistance, reflected by decreased JNK phosphorylation. On top of these actions, RvD1 reduced hepatic inflammatory infiltrate and MCP-1, IL-1β, IL-6, TNFα and CCR7 expression, while up-regulating adiponectin and arginase 1. The anti-inflammatory and pro-resolving actions of RvD1 on liver cells were confirmed in organotypic cultures of PCLS, where this lipid mediator attenuated hypoxia-induced COX-2, IL-1β and IL-6 up-regulation. Ingenuity® analysis revealed that miRNAs differentially regulated by RvD1 were intimately related to cytokine signaling pathways.

Conclusion: RvD1 primes the resolution process initiated by moderate caloric restriction through reducing the inflammatory component of obesity-induced NASH.

1293 GENDER DIMORPHISM OF FATTY LIVER DISEASE
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Objective: NAFLD comprises a spectrum of clinical-histological disturbances, ranging from simple lipid accumulation in the cytoplasm of hepatocytes to steatosis combined with inflammation and liver injury (NASH). The disease can potentially progress to more severe forms, culminating in liver fibrosis and cirrhosis. Among patients with NAFLD men are more exposed to NASH and liver fibrosis than women. However, this gender dimorphism of NAFLD evolution was poorly studied and the molecular mechanism is still unclear. The aims of this project are to characterize the gender-specific evolution of liver steatosis and to establish the molecular basis of this gender specificity in mouse.

Methods: Mouse carrying a null mutation in both PPARg alleles was fed with normal chow diet. PPARg null mice have no adipose tissue and develop a massive accumulation of fat in the liver. Characterization of the gender-specific evolution of liver steatosis was assessed and microarray analysis was performed to establish the molecular basis of the gender specificity.

Results: While in females we observed macro and micro-vesicular steatosis at all time points, in males lipid droplets almost disappeared starting from 20 weeks. Furthermore, at 20 weeks the hepatic content of triglycerides is significantly higher in KO females than in KO males, whereas no differences are detected at 7 weeks. Further supporting the different response of the two sexes to lipid loading, the expression of a panel of genes involved in lipid synthesis and sequestration in lipid droplets was increased
only in KO females. Interestingly, several evidences suggest that TG accumulation is less toxic for the liver than gathering abnormal quantities of other lipids, such as ceramide, cholesterol and diacetylglycerol (DAG). Thus, it seems reasonable to hypothesize that KO female livers could be less damaged by steatosis. Microarrays analyses highlighted pathways differently activated during the progression of the pathology in the two genders.

Conclusions: PPARg null mice show a clear gender dimorphism of hepatosteatosis. The comprehension of sex-related difference molecular basis could be important to identify the mechanisms that protect females from a worse progression of the pathology.

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THE ROLE OF CD36 IN THE PATHOGENESIS OF NAFLD
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Non-alcoholic fatty liver disease (NAFLD) is the main cause of chronic liver injury worldwide. Hepatic steatosis, characterized by excess triglyceride accumulation in the cytoplasm of hepatocytes, is the hallmark of NAFLD. While steatosis itself is generally considered an innocent incident, the presence of inflammation can lead to further progression of non-alcoholic steatohepatitis (NASH), resulting in more advanced stages of liver disease. Since CD36 mediates long-chain fatty acid uptake in the liver, we hypothesize that absence of CD36 in the liver will protect against NAFLD/NASH. To investigate this, CD36 knockout (CD36 KO mice) and wild type (WT) mice were fed a low fat (LF; 10% fat from lard) and a high fat (HF; 60% fat from lard) for 12 weeks. Moreover mice were fed a high fat cholesterol diet (HFC; 0.2% cholesterol, 21% milk butter) resulting in more advanced stages of liver disease. Since CD36 depletion neither altered steatosis development nor expression of inflammatory cytokines and chemokines in MCD-fed animals, we propose that CD36 in Kupffer cells may serve as a protective mechanism, dampening liver inflammation and thereby attenuating the progression towards NASH. This research was supported by the Center for Translational Molecular Medicine (CTMM) and the Netherlands Heart Foundation, Dutch Diabetes Research Foundation and Dutch Kidney Foundation (PREDICCt).

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THE INSULIN-LIKE GROWTH FACTOR 2 (IGF2) mRNA BINDING PROTEIN p62/IMP2 ACCELERATES STEATOSIS, INFLAMMATION AND FIBROSIS IN A DIETARY MODEL OF NON-ALCOHOLIC STEATOHEPATITIS (NASH)
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Background: Liver-specific overexpression of p62 induces steatosis. Increased cytoplasmic levels of the NF-kB subunit p65 suggested an increased susceptibility towards an inflammatory stimulus. Aim of this study was to investigate the effect of p62 on inflammation and fibrosis in a dietary model of NASH.

Methods: p62 transgenic mice (tg) and wild type (wt) littermates were fed a methionine-choline deficient diet (MCD) or a methionine-choline supplemented control diet for 2, 4, or 12 weeks (n=10 animals, each). Liver tissue specimens were investigated by histology and immunohistochemistry, real-time RT-PCR, and a fluorimetric TBARS assay. Kupffer cells (KCs) were depleted by clodronate liposomes.

Results: Serum transaminase levels were comparable between wt and tg mice at all time points. MCD-induced steatosis was more pronounced in tg compared to wt animals only at 2 weeks (p=0.02). Most notably, a pronounced ductular reaction was observed as early as 2 and 4 weeks in tg on MCD (p=0.04 and 0.01, respectively). Wt mice showed ductular reactions only after MCD feeding for 12 weeks. Determination of lipid peroxidation revealed increased TBARS levels in tg compared to wt animals upon MCD diet for 4 weeks (p=0.03). This was accompanied by pronounced lobular lymphocytic and neutrophilic inflammation in tg at all time points. We also observed a significantly increased mRNA expression of the chemokine MCP-1 (p=0.03) and the cytokines TNFα (p=0.02) and IL-1β (p=0.02). Activation of the proinflammatory transcription factor NF-kB was increased in hepatocytes of tg mice as assessed by nuclear translocation of p65. Fibrosis development was accelerated in tg animals with increased collagen 1α1 mRNA expression already after feeding for 2 or 4 weeks (p=0.006 and 0.026, respectively), while no expression was observed in wt mice. Histological analysis revealed detectable fibrosis in tg animals at 4 weeks MCD feeding (p=0.03). These data indicated that the proinflammatory effects of p62 in the MCD model are derived from injured hepatocytes rather than from the tissue resident macrophages, the KCs. In fact, KC depletion neither altered steatosis development nor expression of inflammatory cytokines and chemokines in MCD-fed animals.

Conclusion: p62 promotes inflammation and fibrogenesis in the described murine NASH model.

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SAFETY AND EFFICACY OF THE PAN-CASPASE INHIBITOR IDN-6556 IN THE TREATMENT OF NONALCOHOLIC FATTY LIVER AND INSULIN RESISTANCE
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Hepatocellular and extrahepatic cell death are key features of obesity-associated fatty liver disease (NAFLD) and metabolic syndrome. Emerging data suggest that inhibition of caspases may be an attractive therapeutic approach for patients with these conditions. Our aim was to test the hypothesis that the pan-caspase inhibitor IDN-6556 will improve hepatic steatosis and metabolic abnormalities commonly observed with NAFLD, such as insulin resistance and dyslipidemia.

Methods: C57BL/6 mice, aged 6 to 8 weeks at the beginning of the study, were fed either a high fat (HFAT) “western” diet or a low fat control diet for 20 weeks. During the last 5 weeks mice fed the HFAT died were treated with 3mg/kg/day of IDN-6556 (n=12) or with a placebo (n=12), via gavage. Mice were then sacrificed and their livers, adipose tissue (epididymal) and blood were collected. Liver and adipose tissue inflammation and cell death was assessed by histopathology, TUNEL assay, immunoblotting, F4/80 immunohistochemistry, and real time qPCR for pro-inflammatory cytokines (IL-1β, TNF-alpha, and IL-6). Liver injury was further determined by serum ALT levels and alpha-smooth muscle actin (alpha-SMA), collagen 1-alpha (COL1A1), and transforming growth factor-Beta (TGF-beta) by real time qPCR.
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Results: Infiltration of pro-inflammatory macrophages into epididymal white adipose tissue (eWAT), assayed by immunohis-tochemistry staining for F4/80® crown-like structures, was reduced in treated mice compared to placebo mice. Despite the similarity in body weight, treated mice showed a decrease in terminal blood glucose levels compared to placebo mice (P = 0.0037). Interestingly, IDN-6556 treatment resulted in improved insulin function as measured by plasma insulin ELISA versus placebo (0.23:±0.089 vs. 0.97:±0.26 ng/ml, P = 0.023). We examined the inhibition of caspase proteins by IDN-6556 in the eWAT and saw significant reduction in apoptosis in the treated versus the placebo group (P = 0.0072). In addition, IDN-6556 also caused decreased hepatic steatosis in treated mice compared to placebo mice. We concluded that IDN-6556 treatment ameliorated insulin resistance in HFD-fed mice and attenuated inflammation as measured by diminished levels of adipocyte macrophage infiltration. Furthermore, we observed that the pan-caspase inhibitor reduced apoptosis in the eWAT and improved hepatic steatosis in diet-induced obesity.

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IL12 IS DOWN REGULATED IN OBESE CHILDREN WITH NAFLD AND UPREGULATED IN SIMPLE OBESITY
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Introduction and Objectives: Non-alcoholic fatty liver diseases (NAFLD) represents a complex of inflammatory changes in the liver ranging from asymptomatic steatosis with increased aminotransferase levels to non-alcoholic steato-hepatitis (NASH) and cirrhosis. The cellular mechanisms responsible for induction of different forms of liver inflammation are largely unknown. Therefore we tested concentrations of inflammatory mediators in serum (cytokines, soluble cellular receptors) in children with NAFLD, obesity and healthy controls.

Methods: The study included: 53 NAFLD children, 31 aged matched obese children without NAFLD, and 39 age matched healthy children as a control group, with mean age of 13 years. The serum concentrations of inflammatory mediators: MMP-9, TIMP-1, IL-1beta, IL-12p70, IL-6, TNF-alpha, sCD14, (soluble form of CD14 receptor), and TREM (Triggering receptor expressed on myeloid cells-1) were assessed by ELISA. Body mass composition and bone age were evaluated by DEXA (dual-energy X-ray absorptiometry). The Results: Obese children did not differ from the NAFLD group in BMI-Z scores. Obese NAFLD children were characterized by:

a. elevated levels of MMP-9, TIMP-1, IL-1beta and sCD14, decreased TNF-alpha and similar levels of IL12p7-in comparison with healthy children,

b. decreased IL12p70 in NAFLD when compared to obesity group,

c. lack of correlation between mediator level and lipids parameters,

d. negative correlation between TREM-1 and GGTP, and positive correlations between: MMP-9 vs bone age, and IL1-beta vs. total body bone mineral density, lean body mass and fat mass. Additionally, sCD14 correlated positively with the android type of obesity.

Conclusion: NAFLD is associated with upregulation of many parameters of inflammatory response (sCD14, MMP-9, TIMP-1, IL 1beta). Surprisingly however, IL12p70 was down regulated in children with NAFLD when compared to obese age matched controls, remaining similar as in healthy controls. It may indicate that obesity related inflammation in NAFLD children results in monocyte/macrophage inhibition in the course of the disease (antinflammatory response mediated by IL12 blocking fibrotic cytokines?).

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TARGETING PPARS BY BEZAFIBRATE ALLEVIATES OBESITY, NAFLD AND INSULIN RESISTANCE DUE TO MATERNAL OBESITY IN MICE
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Background and Aims: Diet-induced maternal obesity yields an overweight phenotype, with insulin resistance and non-alcoholic fatty liver disease (NAFLD) in the offspring. This study aimed to evaluate whether activation of peroxisome proliferator-activated receptor (PPAR)x and PPARg by Bezafrate (BZ) could attenuate obesity and NAFLD in male offspring from obese C57BL/6 dams.

Methods: Females were fed on a SC (standard chow; 10% lipids) diet or HF (high-fat, 49% lipids) diet for 8 weeks before mating and during gestation and lactation periods. Male offspring were weaned onto a SC diet and subdivided into 4 groups: SC, SC/BZ, HF and HF/BZ. Treatment with Bezafrate started at 12th week and was maintained for 3 weeks. Bezafrate was added to the diets at the dose of 100 mg/kg. Metabolic measurements, biochemical analysis, stereological tools and immunoblotting were performed.

Results: The HF diet yielded an overweight phenotype and an increase in oral glucose tolerance and fasting glucose of dams. The HF offspring presented noticeable body mass gain, high levels of plasmatic and hepatic TG, high levels of pro-inflammatory and low levels of anti-inflammatory adipokines, impairment of glucose metabolism, different fat pad mass distribution, a greater amount of larger adipocytes, hepatic steatosis, high expression of lipogenic proteins concomitant to decreased expression of PPARg in liver, and diminished expression of PPARx and adiponectin in white adipose tissue (WAT). Inversely, treatment with Bezafrate ameliorated hepatic outcomes generated by maternal obesity as well as WAT remodeling, with improvement of structural, biochemical and molecular data of animals' livers and epididymal WAT. NAFLD was completely reversed by the treatment due to decreased PPARx/PPARg ratio, which favours beta-oxidation and counters lipogenesis.

Conclusion: Diet-induced maternal obesity lead to alterations in metabolism, hepatic lipotoxicity and adverse liver and adipose tissue remodeling caused in the offspring. Targeting PPAR by Bezafrate has beneficial effects reducing hepatic alterations. In addition, Bezafrate could be used to reverse the WAT inflammatory state through PPARgamma activation.


1299
PHOSPHOLIPASE A2 CONTROLS THE MEMBRANE FATTY ACID UPTAKE COMPLEX IN HEPATOCYTES: A POTENTIAL THERAPEUTIC TARGET IN NASH
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Background: The mechanism by which fatty acids are taken up, i.e. by hepatocytes, and how this is regulated remains unclear. Uncontrolled fat accumulation within the liver leads to steatosis and non alcoholic steatohepatitis (NASH) which are serious threats to health in Western populations. In mice models of NASH it...
was shown that the bile acid-phospholipid conjugate UDCA-LPE reversed the NASH phenotype which was accompanied by suppression of phospholipase A2 (PLA2) in the liver.

**Methods:** For evaluation of the role of PLA2 in hepatic steatosis the impact of UDCA-LPE and the PLA2 inhibitors MAFP and BEL on fatty acid influx and plasma membrane fatty acid binding proteins was examined in Hep G2 cells. Furthermore, the biosynthesis regulation of the involved transporters was analyzed as a function of lysophosphatidylcholine (LPC), generated by PLA2.

**Results:** PLA2 was shown to be an integral component of a raft plasma membrane associated fatty acid uptake complex further consisting of FABPpm, CD36 and caveolin. Inhibition of PLA2 by UDCA-LPE, MAFP and BEL impaired fatty acid influx by disintegration of the fatty acid uptake complex. At the same time the suppressed LPC generation by low PLA activity inhibited JNK1 activation which blocked biosynthesis of all of the involved transport proteins.

**Conclusion:** It was shown that fatty acid influx is mediated by a four-protein complex within specialized raft plasma membrane structures with phospholipase A2 serving as central regulator.

**Perspective:** This newly discovered metabolic principle opens the door for therapeutic intervention of a yet untreatable disease. A perfect example of such a novel therapeutic strategy is the nontoxic bile acid-phospholipid conjugate UDCA-LPE which can reverse steatohepatitis by PLA2 inhibition, as previously shown in mouse models.

**1300 IMMUNE RESPONSES TRIGGERED BY OXIDATIVE STRESS CONTRIBUTE TO HEPATIC INFLAMMATION IN NONALCOHOLIC STEATO-HEPATITIS (NASH)**


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**Background and Aims:** The mechanisms leading to chronic hepatic inflammation in NASH are still incompletely characterized. Previous studies by our laboratory showed that NASH in both adults and children is often associated with the presence of circulating antibodies against proteins adducted by lipid peroxidation products. From this background, we investigated the possible role of immune responses in NASH pathogenesis.

**Methods:** NASH was induced by feeding four weeks C57BL/6 and Balb/c mice with a methionine-choline deficient (MCD) diet.

**Results:** C57BL/6 and Balb/c mice showed strain difference in the severity of NASH with C57BL/6 displaying more extensive liver injury and hepatic inflammation than Balb/c mice. Despite liver oxidative damage was comparable in the two strains only in MCD-fed C57BL/6 mice we observed increased titres of IgG against malonyldialdehyde (MDA) and 4-hydroxynonenal (4-HNE)-derived antigens that positively correlated with, respectively, TNF-α and IL-12 expression and the frequency of hepatic necro-inflammatory foci (r from 0.62 to 0.81; p<0.0001). Furthermore, NASH in C57BL/6 mice was also associated with an increased liver B- and T-lymphocyte infiltration and higher expression of lymphocyte chemokines CCL5, CXCL9 and CXCL10, suggesting a possible role of adaptive immunity in NASH. To substantiate such a hypothesis Balb/c mice were immunized with MDA-adducted bovine serum albumin (MDA-BSA) and subsequently fed with the MCD diet. MDA-BSA immunization did not cause liver injury or inflammation in control mice. Following MCD diet feeding, MDA-BSA-immunized mice showed higher ALT release, more diffuse lobular inflammation and a two fold stimulation in hepatic TNF-α, CCL2 and IL-12 expression as compared to non-immunized animals. Moreover, the mRNAs for the hepatic fibrosis markers pro-collagen 1α and TGF-β were significantly up-regulated in MDA-BSA-immunized animals with NASH. Similar results were obtained in C57BL/6 mice. In this strain we observed that worsening of NASH by immunization was associated with an increased liver recruitment of CD8+ and CD4+ T-cells and NKT cells and an up-regulation in the production of interferon-γ and osteopontin.

**Conclusions:** Altogether these results indicate that oxidative stress-driven immunity can contribute to hepatic inflammation in NASH. This work has been supported by grants from the Fondazione Cariplo (Milan) and Italian Ministry of Education (Prin 2009).
Conclusions: The characteristic mechanisms of free cholesterol accumulation in hepatic stellate cells aggravate liver fibrosis in nonalcoholic steatohepatitis in mice. These characteristic mechanisms of FC accumulation in HSCs play an important role in the progression of liver fibrosis in NASH.

1303 DIET-INDUCED OBESITY (DIO) AND STEATOSIS MODEL IN ZEBRAFISH: CHARACTERIZATION OF LIVER DAMAGE IN A GENDER AND AGE PERSPECTIVE

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Background and Aims: Several data support the existence of a close relationship between body weight excess and NAFLD even if this correlation is not completely understood. Furthermore, physiologic differences due to gender and age might affect the development, diagnosis, and treatment of NAFLD. Review of the animal literature investigating obesity and NAFLD experimental models revealed a lack of studies in the area of NAFLD, gender and age.

Aim of this project was to set up a Zebrafish model of dietary-induced-obesity that could be used to investigate the development of NAFLD in obesity-associated conditions focusing both on age and gender.

Methods: Zebrafish were fed with a home-made, high-liquid fish food in either high-dose or standard quantity. Once a week zebrafish were weighted and measured to calculate the BMI. Histopathological examination and qPCR on genes involved in lipid metabolism (PPARgamma, PPARalpha), fibrosis (TGBeta), inflammation (IL-6, TNFalpha) and HCC (c-MYC, PSMD10, IGF-2) were carried out on liver samples collected at different time point (weeks 1–6–16–24).

Results: A significant increase in BMI (p < 0.001), was evident in overfed Zebrafish vs. controls. All animals develop obesity indicating that the penetrance of this model is 100%. Steatosis started to develop already from the first week of treatment, reached a peak at week 16 and stabilized thereafter. At week 24, fibrosis started to increase in older females. PPARa and PPARα expression significantly increased both in young and old females (P < 0.0001) as well as in young male Zebrafish (P < 0.0001). IL-6, TNFalpha and TGFbeta expression increased in older female vs. the other groups. c-MYC, PSMD10 and IGF-2 genes became upregulated in the last weeks of treatment especially in young Zebrafish (both males and females).

Conclusion: Our data suggest that Zebrafish DIO is a valuable model to study the development of obesity in a gender and age perspective. Most importantly, the rapid development of steatosis and the activation of pathways similar to humans make Zebrafish DIO an attractive model to study the pathophysiological mechanisms during early phases of development of steatosis.
4. Western blotting using anti-NFκB antibody for inflammatory signaling, anti-Acyl-CoA antibody to estimate the lipid metabolism, and anti-eIF2α, -IRE1 and -ATF6 antibodies to analyse the endoplasmic reticulum (ER) stress.

**Results:**

1. Ratio of liver weight to body weight and blood ALT level were extremely decreased in MCDD with 0.1% WBPs treated group (Gr.3).
2. Blood albumin, total cholesterol and triglyceride levels increased gradually in proportion from MCDD with 0.2% WBPs treated group (Gr.2) to MCDD with 0.05% WBPs treated group (Gr.4).
3. Degree of steatosis, inflammation, ballooning of hepatocytes, Mallory-Denk hyaline bodies, and fibrosis improved in a WBPs-dose-dependent manner, and were significantly decreased in Gr.2 compared with MCDD with normal feed treated group (Gr.1).
4. In Western blotting, the expression of NFκB in liver tissues decreased, but that of Acyl-CoA, PERK, eIF2 and ATF6 increased in proportion from Gr.1 to Gr.4.

**Conclusions:** WBPs suppress ER stress, inflammation and enhance the suppressive effects of other pathogenetic factors in NASH. These peptides, which can be obtained easily, may be a useful therapy in patients with NASH.

1305

**BERBERINE AMELIORATES HEPATIC INJURY IN MICE ACTING ON THE NALP3 INFLAMMASOME PATHWAY**

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**Background and Aims:** The natural alkaloid berberine has been employed in traditional Chinese medicine with a wide range of pharmacological and biochemical effects, including protection against liver injury. Aim of this study was to analyze the effects of BRB administration in two unrelated murine models of liver injury and to investigate the underlying mechanism of action.

**Materials and Methods:** The effects of BRB were tested in a dietary model of steatohepatitis induced by MCD diet and in acute acetaminophen (APAP) intoxication. BRB was administered at 5 mg/kg i.p in both models. Histology was analyzed by H&E staining and semiquantitative scoring. Intrahepatic gene expression was assessed by quantitative real time PCR. The effects of BRB were further investigated in the murine macrophage cell line, RAW 264.7.

**Results:** BRB markedly suppressed ALT serum levels in mice fed a MCD diet for 4 weeks. In addition, the necroinflammatory score was dramatically reduced. In MCD-fed, BRB-treated mice the intrahepatic gene expression of CD11b, CCL2, TGF-beta, type I collagen and TFN was significantly downregulated. MCD diet has been previously found to activate the NALP3 inflammasome pathway. BRB limited gene expression of all components of this pathway, including NALP3, ASC, caspase-1 and IL-1beta. In addition, IL-1beta protein levels in hepatic homogenates were significantly lower in mice treated with MCD and BRB than MCD alone. BRB also reduced the expression of TLR-4. APAP toxicity is another condition associated with inflammasome activation. In mice administered a toxic dose of APAP, BRB administration was associated with significantly lower ALT levels. APAP-induced increase in all components of the NALP3 inflammasome was limited by BRB. In murine macrophages stimulated with LPS, BRB significantly decreased NALP3 inflammasome activation, indicating a direct interference with this pathway.

**Conclusions:** BRB administration ameliorates necroinflammation and liver injury in experimental steatohepatitis and in APAP intoxication. These effects are associated with inhibition of the inflammasome pathway in vivo and in cultured macrophages, identifying a novel mechanism of action for BRB.

1306

**LYSOSOMAL ENZYMES: A NOVEL NON-INVASIVE APPROACH TO DETECT HEPATIC INFLAMMATION IN NASH**

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**Methods:** Liver wedge biopsies from morbidly obese patients (n = 51) were evaluated for histological features according to the Kleiner criteria. Levels of cathepsin D, acid phosphatase and alanine aminotransferase (ALT) were measured in plasma.

**Results:** Whereas ALT was elevated in patients with steatosis and NASH compared to healthy livers, plasma levels of the lysosomal enzymes cathepsin D and acid phosphatase were higher in NASH patients compared to patients with either steatosis or a normal liver. In contrast to ALT, plasma levels of both lysosomal enzymes were significantly higher in patients with portal inflammation versus those without. Interestingly, patients with a mild score for NASH stage and grade demonstrated a rapid increase in plasma cathepsin D levels compared to patients without NASH, whereas acid phosphatase activity is only increased in patients with a severe score for NASH stage and grade.

**Conclusions:** Plasma lysosomal enzymes have the potential to be used as an early non-invasive marker for NASH.

1307

**ANTI-INFLAMMATORY AND DIRECT ANTIFIBROTIC EFFECT OF THE ORAL HEPATOTROPIC DPPIV INHIBITOR LINagliptin in a MODEL of BILiARY FIBROSIS AND NASH**

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**Methods:** Liver wedge biopsies from morbidly obese patients (n = 51) were evaluated for histological features according to the Kleiner criteria. Levels of cathepsin D, acid phosphatase and alanine aminotransferase (ALT) were measured in plasma.

**Results:** Whereas ALT was elevated in patients with steatosis and NASH compared to healthy livers, plasma levels of the lysosomal enzymes cathepsin D and acid phosphatase were higher in NASH patients compared to patients with either steatosis or a normal liver. In contrast to ALT, plasma levels of both lysosomal enzymes were significantly higher in patients with portal inflammation versus those without. Interestingly, patients with a mild score for NASH stage and grade demonstrated a rapid increase in plasma cathepsin D levels compared to patients without NASH, whereas acid phosphatase activity is only increased in patients with a severe score for NASH stage and grade.

**Conclusions:** Plasma lysosomal enzymes have the potential to be used as an early non-invasive marker for NASH.
peptidase IV-inhibitor, on hepatic inflammation and fibrosis in models of biliary fibrosis and of NASH.

**Methods:** Linagliptin was tested in macrophage and myofibroblast cultures, and administered daily by gavage at 0.5, 5, 10 and 50 mg daily per kg BW to Mdr2KO mice from week 7–11 of age, and to 8 week old C57BL/6 mice fed a methionine and choline deficient diet (MCD) for 8 weeks. Wildtype mice fed a supplemented diet served as controls, respectively. Hepatic collagen was measured biochemically, serum biochemistries were determined by an autoanalyzer. Fibrosis and inflammation related transcript levels were quantified from livers by real-time qRT-PCR. Liver histology was assessed by connective tissue staining and histochemistry for inflammation and alpha-smooth muscle actin (alpha-SMA).

**Results:** In Mdr2KO mice, 10 and 50 mg/kg/day of Linagliptin increased putatively anti-fibrotic MMP-9 and -13, but decreased procollagen α1(1) and TGFβ1, TIMP-1, MMP-8 transcript levels. However, in vitro macrophage or fibroblast differentiation, and their production of proinflammatory or profibrogenic transcripts were unaffected. Mice fed the MCD diet showed a rapid induction of hepatic steatosis, inflammation and a 2-fold increase in fibrosis compared to controls, with an up to 10-fold upregulation of procollagen α1(1), TGFβ1, aSMA, MMP-3 and -9, TIMP-1, CCL3 and TNFα mRNA expression. Linagliptin lowered serum ALT, AST, ALP and triglycerides, but did not affect hepatic collagen accumulation in both the Mdr2KO and the MCD model.

**Conclusion:** In experimental biliary fibrosis (Mdr2KO mice) and in a surrogate NASH model (MCD) oral Linagliptin was well tolerated and lowered parameters of (hepatocyte) inflammation. Linagliptin demonstrated only a modest direct anti-fibrotic effect at a higher dose, possibly due to an inhibitory effect on fibroblast activation protein (FAP) rather than via induction of GLP-1.

**1308**

**A ROLE FOR DNA REPAIR PROTEIN KINASE (DNA-PK) IN THE PROGRESSION OF SIMPLE STEATOSIS TO STEATOHEPATITIS (NASH)?**

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**Background:** NAFLD is the commonest cause of cirrhosis in western nations and an increasingly common cause of HCC. In advanced HCC we have previously proposed that rapid DNA repair by DNA-PK is key to cancer progression and treatment resistance. Recently a role for DNA-PK in regulating hepatic fat metabolism has been proposed. DNA-PK phosphorylates and activates USF1 – a transcriptional regulator of fatty acid synthase (FAS) – in response to insulin. FAS may be central to the progression of simple steatosis to NASH. Our aim was to explore a possible role for DNA-PK in NALFD progression to NASH.

**Methods:** We studied hepatic DNA-PK mRNA expression in CD1 mice after 3 months on normal/control; high sucrose; high fat or, high sucrose/high fat diets (n = 40), compared to a longer term model (n = 22), in which C3H/He mice developed NASH and pericellular fibrosis after 9 months on the ‘American lifestyle’ (ALIOS) diet, and human NAFLD liver biopsies. All tissues were staged using the Kleiner score.

**Results:** Hepatic DNA-PK fell in mice fed high sucrose or fat diets for 3 months and was reduced in association with mild (S1) steatosis. In contrast, in older, fatter ALIOS C3H/He mice, with more S2–3 steatosis and features of NASH, DNA-PK expression increased in association with impaired glucose tolerance (IGT), measured as the AUC of intraperitoneal glucose tolerance test (Pearson correlation 0.475; p = 0.034); and body weight (0.484; p = 0.026). In 28 human NAFLD biopsies, there was no significant difference in association with steatosis (stage 1, n = 10; stage 2, n = 7; stage 3 n = 6) or fibrosis (stage 0, n = 13; stage 1 n = 7; stage 2 n = 8). However, with increasing inflammation grade 1 (n = 16) and grade 2 (n = 4), there were highly significant 3.0 fold (p < 0.001) and 3.3 fold (p < 0.001) increases in expression (no inflammation; n = 8). There was no increase or correlation with XRCC5 or XRCC6, which form the DDR DNA-PKcs complex.

**Conclusions:** DNA-PK expression increases in the presence of NASH in murine and human disease. This may be in response to impaired glucose tolerance or oxidative stress. In either case, DNA-PK may be central to up-regulating FAS and promoting NAFLD progression to NASH.

**1309**

**miR-451 INHIBITS FATTY ACID-INDUCED INCREASES IN NF-xB TRANSCRIPTION THROUGH AMPK IN NON-ALCOHOLIC STEATOHEPATITIS (NASH)**

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**Background and Aims:** Accumulating evidence supports that microRNAs (miRNAs) are important gene regulators, which can have critical roles in diverse biological cellular processes including non-alcoholic fatty liver disease (NAFLD). In the present study, we investigated the role of miR-451 which was identified as a target gene for NAFLD, on the mechanism of the inflammatory cytokine production in NAFLD.

**Methods:** Microarray and stem-loop RT-PCR were performed to detect dysregulated miRNAs in mouse models of high fat diet (HFD)-induced NAFLD. After then, we searched the direct miRNA targets through performing pair-wise correlation coefficient analysis on expression levels of miRNAs, and compared the results with predicted miRNA targets from TargetScan5.1. To validate a candidate target gene, real time RT-PCR and Western blot were performed in steatotic HepG2 cells treated with palmitate after transection with control, miR-451 mimic or miR-451 antagonims. We next investigated whether AMP-activated protein kinase and NF-xB were downregulated or not, Western blotting and luciferase reporter assays were carried out in miR-451 mimic-transfected steatotic HepG2 cells.

**Results:** We identified 7 new miRNAs-target gene pairs by bioinformatics analysis and further confirmed their expression by stem-loop RT-PCR (miR-34a, miR-1224, miR-494, miR-453, miR-720, miR-451 and miR-19b) in murine models of HFD-induced NAFLD. Among those genes, we found that miR-451 expression was downregulated in non-alcoholic steatohepatitis (NASH). We also found that Cab39 is the direct target of miRNA-451 in steatotic HepG2 cells. Mechanistically, we demonstrated that AMPK activation through Cab39 as a direct target of miRNA-451 inhibits NF-xB transactivation induced by fatty acid palmitate in HepG2 cells. Consequently, overexpression of miRNA-451 in steatotic HepG2 cells suppressed palmitate-induced proinflammatory cytokine IL-8 expression.

**Conclusions:** These results demonstrated the miRNA/mRNA profiles dysregulated in HFD-induced NAFLD mice model, and suggest that miRNA-451 may play an important role in the pathogenesis of NAFLD.

*This research was supported by grants of the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011–0014620).*
**Results:** Subjects with histologic NASH show down-regulation of CD3E (FC=0.19, p=0.005), FOXP3 (FC=0.29, p=0.01) and CSF1 (FC=0.15, p=0.04) as compared to those without NASH. Subjects with severe hepatic steatosis (grade≥3) compared to mild steatosis (grades 0–2) also show down-regulation of CD3E (FC=0.33, p<0.05). Also, CD3E (r=-0.33, p=0.02) and CSF1 (r=-0.37, p<0.01) are negatively correlated with degree of hepatic steatosis. Interestingly, hepatic steatosis with increased polymorphonuclear leukocyte infiltration (PMN) was accompanied by 0.17 fold down-regulation of CD3E (p<0.01), 0.19 fold down-regulation of FOXP3 (p<0.01) as compared to the hepatic steatosis subjects without PMN infiltration. Correlation analysis supported the trend with negative correlation between PMN infiltration and CD3E (r=-0.40, p=0.006) and FOXP3 (r=-0.41, p=0.005) expression. Significant positive correlation was seen among various lineage specifying factors (data not shown). Interestingly, T-bet specifying factor was negatively correlated with BMI (r=-0.36, p=0.01). Pathway analysis showed enrichment of ‘regulation of lymphocyte activation’, ‘T cell differentiation’ and ‘inflammatory response’ network processes (p=2.02e-32) (Figure 1).

**Conclusions:** In NASH, there is an overall reduction in CD4+ T cell, T cell subset and macrophage specifying markers. This is indicative of an ongoing negative feedback loop among the factors (Figure 1) and interaction between liver and adipose tissue. Further studies exploring the immunomodulatory cytokine milieu can help understand the immune response in NAFLD.
Conclusions: Serum endocan levels seem to be increased in NAFLD patients with CAD. These data suggest that increased serum endocan may be important for detecting endothelial cell damage in NAFLD patients. Additionally, endocan is potentially involved in the pathogenic mechanism of CAD in NAFLD patients.

1312 PIOGLITAZONE UPREGULATES ANGIOTENSIN CONVERTING ENZYME 2 EXPRESSION IN INSULIN-SENSITIVE TISSUES IN RATS WITH HIGH FAT DIET-INDUCED NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: Thiazolidinediones (TZDs) can improve hepatic steatosis in non-alcoholic steatohepatitis (NASH). Angiotensin (Ang) II, the primary effector of renin-angiotensin system (RAS), plays vital roles in the development and progression of NASH. And some AngII-mediated effects can be regulated by TZDs. Angiotensin-converting enzyme (ACE) 2, a new component of RAS, can degrade Ang II to attenuate its subsequent physiological actions. We aimed to evaluate the effects of TZDs on ACE2 expression in insulin-sensitive tissues in NASH rats.

Methods: Forty rats were divided into the normal control, high-fat diet (HFD), pioglitazone control and HFD plus pioglitazone groups. After 24 weeks treatment, we evaluated changes in liver histology, serum concentrations of glucose, insulin, triglyceride (TG), free fatty acid (FFA), aminotransaminase (ALT) and tissue-specific ACE2 expression.

Results: The degree of hepatic steatosis was significantly higher in the HFD group than in normal control group, as were serum concentrations of glucose, insulin, TG, FFA, ALT. ACE2 gene and protein expression was significantly greater in liver and adipose tissue in the HFD group compared with normal control group, while was significantly reduced in skeletal muscle. Pioglitazone significantly reduced the degree of hepatic steatosis compared with the HFD group, as well as serum concentrations of glucose, insulin, TG, FFA and ALT. Pioglitazone significantly increased ACE2 protein expression in liver, adipose tissue and skeletal muscle compared with the HFD group.

Conclusions: Pioglitazone improves hepatic steatosis in the rats with HFD-induced NASH and upregulates ACE2 expression in insulin-sensitive tissues.

10b. FATTY LIVER DISEASE: CLINICAL

1313 POPULATION BASED STUDY OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) SHOWS DISEASE IS MORE COMMON AMONG BANGLADESHIS IN ETHNICALLY DIVERSE BOROUGHS OF LONDON

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Background: Non-alcoholic fatty liver disease (NAFLD) is a menacing public health burden worldwide. NAFLD has recently been shown to be less common in Black populations and more common in Latinos compared to Caucasians, however NAFLD in other ethnicities has received scant attention. Barts Health NHS Trust serves one of the most ethnically diverse boroughs in the UK, with a large Bangladeshi population (approximately 30%). We sought to determine the frequency of NAFLD among different ethnic groups in East London.

Methods: We conducted a study in our liver clinic and a community-based study in Tower Hamlets and Newham using EMISWeb, the clinical data system used by all general practices in these two boroughs.

Results: In the community study 793 patients from Tower Hamlets and 334 patients from Newham had been diagnosed with NAFLD. The proportion of patients with NAFLD of Bangladeshi ethnicity was higher than expected: 30% of the Tower Hamlets population originate from Bangladesh but 56% of the patients with NAFLD were Bangladeshi (p < 0.0001). Similarly, in Newham 10% of the general population, but 36% of the NAFLD patients were of Bangladeshi ethnicity (p < 0.0001). In the General Hepatology out-patient clinic, 58 of 445 new patients (January-June 2011) were diagnosed with NAFLD. The proportion of patients with NAFLD of Bangladeshi ethnicity was greater than the Bangladeshi proportion of new referrals (41% vs 17%, p < 0.001). NAFLD was not more frequent in patients of other South Asian ethnicities in any cohort.

In all cohorts, the mean ages of Bangladeshi NAFLD patients were significantly lower than Caucasians (hospital: 43 vs 57 years, p = 0.0032; Tower Hamlets: 46 vs 55 years, p = 0.0001; Newham: 43 vs 52 years, p = 0.001). There were no differences in bodymass index or proportion of obese patients that could account for these differences, although the incidence of diabetes in the general practice NAFLD cohorts was higher in Bangladeshi compared to patients of other ethnicities.

Discussion: Ethnicity is likely to be a significant determinant of NAFLD development. NAFLD is more common among Bangladeshi than other (including South Asian) ethnicities. Bangladeshi with NAFLD are approximately a decade younger than Caucasians.

1314 FATTY LIVER ACCUMULATION CONTRIBUTES MORE TO THE RISK OF CORONARY ARTERY DISEASE THAN VISCERAL FAT ACCUMULATION

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Background: Liver and visceral fat accumulation are increasingly associated with metabolic syndrome, a condition carrying a high risk of coronary artery disease.

Aim: To evaluate the individual contribution of liver and visceral fat accumulation to the atherogenic risk profile.

Methods: 30 patients (age 53 ± 7) with excess of visceral fat (visceral fat area >330 ± 99 cm²), and 70 patients with NAFLD (aged 50 ± 9) and 30 sex, age matched healthy individuals were recruited. Coronary artery disease (CAD) was defined as a stenosis of >50% in at least one major coronary artery by cardiac CT. Fatty Liver was defined by CT liver minus spleen density > 10 (HU), Atherosclerosis by Intimal-Media thickness (IMT) of carotid artery by Doppler ultrasound, Visceral fat area was defined by abdominal CT (excessive if >330 ± 99 cm²). Biomarkers of insulin resistance (HOMA), inflammation (CRP) and oxidant-antioxidants status (MDA-Paraoxonase) were measured.

Results: Both patients with NAFLD and patients with high visceral adiposity showed higher prevalence of coronary soft plaques (50% vs. 25%, p < 0.001, and 38% Vs 25%, p < 0.01), higher prevalence of coronary stenosis (30% vs. 11%, p < 0.001, and 22% Vs 11%, p < 0.01), Higher IMT (0.98 ± 0.3 mm Vs 0.83 ± 0.1 mm,
P < 0.01, and 0.86±0.1 mm Vs 0.83±0.1 mm, P < 0.01), higher HOMA (4.0±3.0 vs 1.5±1.2, P < 0.005, and 3.0±1.0 Vs 1.5±1.2, P < 0.001) and higher triglyceride levels mg/dL (196.8±103 vs. 145±60, P < 0.005, and 182.6±90.87 Vs 145±60, P < 0.005) respectively than healthy controls. Multiple regression showed that fatty liver predicts coronary artery disease (OR 2.7), 95% CI 2.3–4.9, P < 0.001 independently by visceral fat accumulation (OR 2.01, 95% CI 1.2–2.8, P < 0.001).

Conclusion: Liver fat accumulation is an independent risk factor for coronary artery disease and early carotid atherosclerosis. NAFLD can contributes to a more atherogenic risk profile over and above the contribution of visceral adiposity.

1315 THE EFFECT OF VITAMIN D AND SITAGLIBTIN/METFORMIN COMBINATION ON HEPATIC FAT AND TRIGLYCERIDE CONTENT IN MO-1 MICE WITH FATTY LIVER DISEASE
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Background: Currently there are no established treatment options for fatty liver disease. Vitamin D and Sitaglibtin/Metformin have shown anti inflammatory and potential anti oxidative effects.

Aim: Evaluate the effects of Vitamin D with Sitaglibtin/Metformin Combination on the hepatic fat, Triglyceride content, and oxidative parameters in mice with NAFLD (MO-1 mice, C57BL/6J Background).

Methods: A total of 33 mice were randomly divided into four groups. The mice in the control group were on chow diet alone (n=10), mice treated with vitamin D (4000 IU/kg, n=6), mice treated with (Sitaglibtin/Metformin, 200 mg/kg, n=8), and mice treated with combination of vitamin D and Sitaglibtin/Metformin (n=9). After 12 weeks, blood and liver sections were examined for FAT, lipids composition, inflammation and oxidative stress parameters. Hepatic protein expression of (MTP, PPAR-alpha, TNF-alpha) protein were performed.

Results: The amount of liver fat (%) decreased to 50% in the mice treated with Vitamin D, to 18% in mice treated with Sitaglibtin/Metformin, and to (9%) in the mice treated with combination of Drugs, as compared to mice in the control group (70%, P < 0.001). The increase in hepatic TG (mg/gr liver) levels in mice with NAFLD was blunted by 58% in mice treated with vitamin D (2.5±1.7 mg/gr liver), blunted by 47% in mice treated with Sitaglibtin/Metformin (2.04±0.4, P < 0.001), and blunted by 62% in mice treated with combination of Drugs (2.7±1.4) as compared with mice in the control group (4.36±4.0, P < 0.001). In comparison to controls group (50.5±50.0), Hepatic Malonaldehyde content (MDA (mmole/L) in mice treated with vitamin D (42.2±25), mice treated with Sitaglibtin/Metformin (34.2±8.8), and mice treated with combination of Drugs (29.7±4.3) was decreased by 17%, 33% and 41%, respectively (P < 0.001). Hepatic microsomal triglyceride transfer protein (MTP), and PPAR-alpha proteins expressions were up regulated while hepatic paraoxonase and TNF-alpha protein expressions were down regulated significantly in all treatment groups (P < 0.001). Hepatic cholesterol levels remained unchanged.

Conclusion: Combination of vitamin D and Sitaglibtin/Metformin drugs improved hepatic steatosis with significant decreases in hepatic fat, TG, and MDA levels.

1316 GLUCOSE-INDUCED GLUCAGON-LIKE PEPTIDE 1 SECRETION IS DEFICIENT IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE
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Background and Aims: Glucagon-like peptide 1 (GLP-1) is an antihyperglycemic hormone inducing glucose-dependent insulin secretion from pancreatic β-cells. GLP-1 receptor agonism is a treatment concept in type 2 diabetes mellitus. Insulin resistance is the pathophysiologic hallmark also of non-alcoholic fatty liver disease (NAFLD). Recent experimental in vitro and in vivo data suggest a link between GLP-1 and hepatic steatogenesis, possibly through a mechanism involving hepatic GLP-1 receptors. GLP-1 agonism improved hepatic steatosis in different mouse models. Studies testing GLP-1 analogues for the treatment of NAFLD are ongoing. We analyzed for the first time glucose-induced GLP-1 secretion in patients with NAFLD compared to healthy controls.

Methods: Glucose induced GLP-1 secretion was measured in a cohort of non-diabetic patients with NAFLD (n=52), within n=16 with simple steatosis and n=36 with non-alcoholic steatohepatitis (NASH), and healthy controls (n=50). Standardized oral glucose tolerance test was performed. Glucose, Insulin and GLP-1 plasma levels were measured sequentially for 120 minutes after glucose administration.

Results: Glucose induced GLP-1 secretion was decreased in NAFLD compared to controls (cmax 8.8 pmol/l vs. 11.7 pmol/l, p < 0.001; area under the curve (AUC) 879 vs. 969, p < 0.001). There was no difference in GLP-1 secretion between NAFLD subgroups with simple steatosis and NASH (p=0.976). NAFLD patients were insulin resistant (HOMA2-IR 4.7 vs. 1.9, p <0.0001). HOMA2-IR was higher in NASH compared to simple steatosis (5.2 vs. 3.5, p=0.0054). Glucose-induced insulin secretion was higher in NAFLD compared to controls (AUC 10579 vs. 5981, p <0.001) while the glucose lowering effect was diminished. Highest insulin secretion and lowest glucose lowering effect was observed in NASH.

Conclusions: Our study provides first evidence for the deficiency of glucose-induced GLP-1 secretion in NAFLD. Impaired GLP-1 secretion is observed equally in simple steatosis and NASH, thus independently of hepatic inflammation. Insulin sensitivity is however lower in NASH compared to simple steatosis, linking GLP-1 deficiency to steatosis rather than insulin resistance. Our data hereby support a novel therapeutic approach, the use of GLP-1 agonists in the treatment of NAFLD. Further studies are required to assess the full potential of GLP-1 agonistic treatment in these patients.

1317 Withdrawn

1318 ALIMENTARY BEHAVIOUR AND NOT ESTROGEN DEPRIVATION IS RELATED WITH NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN WOMEN
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Background and Aim: The prevalence of NAFLD in women of reproductive age is lower than that in men of similar age. NAFLD increases in older women, in coincidence with menopausal onset. The protective role of estrogens on steatosis and fibrosis progression, however, is debated because aging is a main confounding factor.
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Our aim was to define the role of estrogens deprivation in women with NAFLD, comparing women in reproductive age vs. those with surgical and spontaneous menopause.

Methods: All women consecutively referred to our NAFLD/NASH Clinic were eligible for this single-center cross-sectional study. Inclusion criteria: evidence of liver steatosis at ultrasonography (US) (PLI criteria). Exclusion criteria: EtOH>14units/week, any other liver disease, celiac disease. Medical history, biometric parameters, physical examination, blood tests, US were prospectively recorded. Differences between groups were evaluated using t-test or chi-square test. Logistic regression identified variables related to steatosis.

Results: From November 2011 to October 2012, 108 women were studied. 22 patients were in childbearing age (age 41.4 ± 6.4y, BMI 29.5 ± 5.6); 63 spontaneous (age 62.3 ± 7.3y, BMI 29.8 ± 5.6) and 23 surgical (age 63 ± 6.2y, BMI 32.7 ± 11.5). The latter had longer estrogen deprivation (16.9 ± 9.1y vs. 10.2 ± 6.8y, p = 0.001). Post-menopausal women showed higher rate of hypertension (13.6 vs. 57%, p = 0.000) and metabolic syndrome (13.6 vs. 38.4%, p = 0.028) compared to fertile, ones; no differences were present between surgical and spontaneous menopause. At univariate analysis hyperglycemia (OR 2.53, 95%CI 1.076–5.989, P = 0.033), central obesity (OR 2.48, 95%CI 1.006–6.125, P = 0.048), metabolic syndrome (OR 2.17, 95%CI 0.903–5.213, P = 0.083), BMI (OR 1147, 95%CI 1.046–1.258, P = 0.003) waist circumference (OR 1.061, 95%CI 1.016–1.108, P = 0.008) and triglycerides (OR 1.014, 95%CI 1.003–1.025, P = 0.015) were related to steatosis. Neither menopause nor its length were related to steatosis. Multivariate analysis indicated triglycerides (OR 1.015, 95%CI 1.003–1.028, P = 0.024) and BMI (OR 1.165, 95%CI 1.018–1.334, P = 0.027) as independently associated with steatosis.

Conclusion: Estrogen deprivation, both spontaneous or surgically-induced, has a clear impact on metabolic syndrome and blood pressure but not on liver steatosis. Triglycerides and BMI were independently related to steatosis underlying the importance of alimentary behavior in the pathogenesis of NAFLD in all reproductive-age groups.

1319 SIMTUZUMAB, AN ANTIFIBROTIC MONOClonAL ANTIBOdy AGAINST LYSYL OXIDASE-LIKE 2 (LOXL2) ENZYME, APPEARS SAFE AND WELL TOLERATED IN PATIENTS WITH LIVER DISEASE OF DIVERSE ETIOLOGY

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Background and Aims: LOXL2, which promotes the cross-linking of type I collagen, is a key component in the core regulatory pathway of fibrogenesis, regardless of the etiology of the fibrosis or the organ affected. Liver fibrosis results from a wide range of liver diseases, many of which are poorly responsive to current therapeutic options. Simtuzumab (formerly GS-6624), a humanized monoclonal antibody with an immunoglobulin IgG4, isotype directed against human LOXL2, has been studied in pulmonary fibrosis and other fibrosing diseases including cancer. Here, we report on the safety of the first use of this compound in patients with liver fibrosis.

Methods: Twenty patients with liver fibrosis of diverse etiologies were included in two cohorts treated with two separate doses of simtuzumab (10mg/kg [cohort 1] or 3mg/kg [cohort 2] infused over 1 hour) every 2 weeks for a total of 3 infusions. Patients were aged 18–65 years, BMI <36kg/m². Metavir fibrosis stage of 1–3, and in general good health. Subjects underwent liver biopsy within a year of screening and liver fine needle aspirations immediately prior to the first infusion and at the end of the treatment period.

Results: All 10 patients have completed Cohort 1. In Cohort 2, all 10 patients have begun, and 3 have completed dosing. A total of 37 adverse events were reported from 12 subjects. The most frequently reported adverse events were abdominal pain (7 subjects), fatigue (3 subjects), musculoskeletal pain and headache (2 subjects each). Two subjects developed serious adverse events (psychiatric disorder and hypoaesthesia), but neither was related to study drug. No subject experienced a worsening in liver enzymes on therapy. Seven subjects had aminotransferase elevation prior to treatment and 6 experienced a decline in aminotransferases after the second infusion.

Conclusions: In this first experience in patients with liver disease, simtuzumab appeared to be well tolerated at doses up to 10mg/kg; lower doses are likely to be used in clinical practice. While simtuzumab is being developed as an antifibrotic agent, an acute reduction in transaminases was observed in response to treatment suggesting a potential anti-inflammatory effect in addition to the antifibrotic effect.

1320 PRE-DIABETES AND CHRONIC HEPATITIS C VIRUS (HCV) INFECTION: ASSESSMENT OF HOST AND VIRAL FACTORS IN AT-RISK LATINOS

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Background and Aims: The majority of patients with pre-diabetes develop overt diabetes and HCV infection is also associated with diabetes. We aimed to evaluate factors associated with pre-diabetes states among at-risk Latinos with and without HCV infection, including direct measurement of insulin action.

Methods: One hundred twenty-three non-cirrhotic, non-diabetic, Latino subjects (52 with HCV infection) were enrolled. All subjects underwent clinical and metabolic evaluation, including oral glucose tolerance testing and measurement of insulin-mediated glucose uptake by steady-state plasma glucose (SSPG) concentration during the insulin suppression test.

Results: Patient characteristics were similar among subjects with and without HCV, except HCV-infected subjects had higher mean age (47 vs. 39 years, P < 0.001), rate of U.S. birth (48% vs. 23%, P = 0.004), tobacco use (12 vs. 3 pack-years, P < 0.001), duration of alcohol use (25 vs. 16 years, P = 0.0027), fasting plasma glucose (100 vs. 95mg/dL, P = 0.0086), ALT (68 vs. 25 units/L, P < 0.001), and ferritin (119 vs. 73mg/mL, P = 0.010), but lower LDL levels (90 vs. 113mg/dL, P < 0.001). Overall, HCV-infected subjects had higher rates of pre-diabetes compared to non-HCV subjects (61% vs. 33%, P = 0.003), including higher rates of impaired fasting glucose (IFG, 37% vs 17%, P = 0.020), impaired glucose tolerance (IGT, 25% vs. 16%, P = 0.24) and combined IFG/IGT (17% vs. 9%, P = 0.25). On univariate analysis, pre-diabetes was positively associated with HCV (OR 3.3, P = 0.002), age (OR 2.2 per 10 years, P < 0.001), waist circumference (OR 1.03, P = 0.049), duration of alcohol consumption (OR 1.04, P = 0.031), ALT (OR 1.2 per 10 units/L, P = 0.003), ferritin (OR 1.01, P = 0.002), and insulin resistance (SSPG >180mg/dL, OR 3.05, P = 0.005). Adjusting for sex and family history of diabetes on multivariable analysis, increasing age (OR 2.5 per 10 years, P = 0.001) and SSPG levels (OR 1.09 per 10 mg/dL, P = 0.016) predicted pre-diabetes.

Conclusions: Pre-diabetes is highly prevalent among Latinos with HCV, particularly impaired fasting glucose. However, while host factors including age and insulin resistance were associated with pre-diabetes, HCV infection per se was not predictive of pre-diabetes in this at-risk population.

This work was supported by National Institute of Health, R01 DK074673 (M.K.), American Diabetes Foundation 1–08-CR-30 (M.K.), and UL1 RR024131 (NIH/NCRR UCSF-CTSI).
1321 PREDICTORS OF DIRECTLY MEASURED INSULIN RESISTANCE IN AT-RISK LATINOS WITH AND WITHOUT HEPATITIS C (HCV) INFECTION

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Background and Aims: HCV is associated with insulin resistance and disproportionately affects Latinos, who in turn have higher rates of diabetes. Factors predictive of insulin resistance in HCV-infected Latinos are not well understood. We aim to compare insulin sensitivity using direct measurements, and assess host and viral factors associated with insulin resistance in Latinos.

Methods: 123 non-cirrhotic, non-diabetic Latinos (52 with HCV) were enrolled. All subjects underwent clinical and metabolic evaluation including oral glucose tolerance test and insulin sensitivity by steady-state plasma glucose (SSPG) concentration during the insulin suppression test. Patients in the lowest tertile of SSPG were defined as insulin sensitive (IS) and in the highest tertile as insulin resistant (IR).

Results: Overall patient characteristics were: mean age 43±10 years, 64% male, and BMI 27±5 Kg/m². Among HCV-positive patients, 67% were genotype 1, mean log10 HCV viral load was 5.83 IU/mL, and mean ALT 89±73 IU/mL. The mean SSPG in IS (N=40) was of 65±14 mg/dL and in IR (N=41) was 239±35 mg/dL. On univariable analysis, IS and IR patients were similar with respect to age, family history of diabetes, and tobacco use. However, IR was positively associated with HCV status (OR 1.83, 95% CI 0.7–4.2), female gender (OR 2.38, 95% CI 0.9–5.8), waist circumference (OR 1.21, 95% CI 1.1–1.3), serum LDL (OR 1.02, 95% CI 1.00–1.03), and triglycerides (OR 1.02, 95% CI 1.01–1.04); and negatively associated with HDL (OR 0.93, 95% CI 0.89–0.97), and current alcohol use (OR 0.43, 95% CI 0.37–1.04). On multivariable analysis, controlling for age and family history of diabetes, female gender (OR 13.74, 95% CI 1.99–94.9, p=0.008), HCV infection (OR 9.13, 95% CI 1.2–70.1, p=0.03), waist circumference (OR 1.14, 95% CI 1.04–1.24, p=0.006), and triglycerides (OR 1.02, 95% CI 1.00–1.04, p=0.04) were significantly associated with IR.

Conclusions: Along with host factors, HCV infection was associated with nine-fold higher odds of directly measured insulin resistance in Latinos. Although male gender is associated with higher diabetic rates, female gender was a strong predictor of insulin resistance among Latinos. The role of other gender-specific factors influencing insulin resistance in this population requires further investigation.


1322 RELATION BETWEEN THE ADIPOCYTE HYPERTROPHY DEGREE AND ADIPOSE TISSUE MACROPHAGE INFLTRATION WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) PROGRESSION IN MORBID OBESITY

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Background and Aims: Two mechanisms are implicated in the enlargement of white adipose tissue: hypertrophy and hyperplasia. Adipocyte hypertrophy takes place after chronic overfeeding and it is associated with metabolic syndrome. The ability to recruit new adipocytes decreases after weight gaining and after puberty. The aim of this contribution deals with determining the correlation between the presence and progression of NAFLD with the three following parameters: adipocyte hypertrophy degree, number of adipocytes and quantity of macrophages, in adipose tissues derived from morbidly obese patients.

Methods: 153 morbidly obese subjects who underwent bariatric surgery were analyzed. Liver and adipose tissues (visceral and subcutaneous) biopsies were obtained at the moment of surgery. Liver biopsies were evaluated by a single experienced pathologist using the Kleiner scoring. Digital micrographs from adipose tissue biopsies were analysed using Image J software to obtain both, the number of adipocytes (cells/mm²) and size (large diameter-μm). The tissue macrophage infiltration was estimated measuring CD68 expression by real time PCR (data were normalized by three housekeeping genes).

Results: Patients were classified in four groups: i. 37 patients without NAFLD; ii. 41 patients with liver steatosis (Kleiner score <3); iii. 52 patients with probable steatohapatitis (Kleiner score 3–4); iv. 23 patients with steatohepatitis (Kleiner score ≥5). It was found that number of adipocytes was lower in subcutaneous adipose tissues compared with visceral tissues (99.1 vs. 124.8, P<0.001). The hypertrophy degree was higher in subcutaneous adipose tissues compared with visceral tissues (113 μm vs. 98, P<0.001). Hypertrophy degree increased with NAFLD progression only in visceral tissues (from 91 μm in group 1 to 115 μm in group 4, P<0.001). CD68 expression was larger in subcutaneous tissues compared with visceral tissues (9.2 vs. 6.5, P<0.05). Moreover, the expression of CD68 increased with NAFLD progression specially in visceral tissues (from 3.6 in group 1 to 9.3 in group 4, P<0.001).

Conclusion: This contribution confirms that there is a correlation between adipose tissue morphometric data with NAFLD. These results offer new perspectives about the influence of adipose depots in the origin and development of NAFLD in morbid obesity.

1323 CONTROLLED ATTENUATION PARAMETER (CAP) FOR NON-INVASIVE ASSESSMENT OF LIVER STEATOSIS IN THE GENERAL POPULATION: CORRELATION WITH ULTRASOUND (US) AND FATTY LIVER INDEX (FLI)

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Background: Liver steatosis measurement by controlled attenuation (CAP) for non-invasive assessment of liver steatosis in the general population. Steatosis was evaluated using CAP, ultrasound (US) and Fatty Liver Index (FLI).

Purpose: To compare CAP with US and FLI for the diagnosis of liver steatosis in the general population.

Methods: 153 morbidly obese subjects who underwent bariatric surgery were analyzed. Liver and adipose tissues (visceral and subcutaneous) biopsies were obtained at the moment of surgery. Liver biopsies were evaluated by a single experienced pathologist using the Kleiner scoring. Digital micrographs from adipose tissue biopsies were analysed using Image J software to obtain both, the number of adipocytes (cells/mm²) and size (large diameter-μm). The tissue macrophage infiltration was estimated measuring CD68 expression by real time PCR (data were normalized by three housekeeping genes).

Results: Patients were classified in four groups: i. 37 patients without NAFLD; ii. 41 patients with liver steatosis (Kleiner score <3); iii. 52 patients with probable steatohapatitis (Kleiner score 3–4); iv. 23 patients with steatohepatitis (Kleiner score ≥5). It was found that number of adipocytes was lower in subcutaneous adipose tissues compared with visceral tissues (99.1 vs. 124.8, P<0.001). The hypertrophy degree was higher in subcutaneous adipose tissues compared with visceral tissues (113 μm vs. 98, P<0.001). Hypertrophy degree increased with NAFLD progression only in visceral tissues (from 91 μm in group 1 to 115 μm in group 4, P<0.001). CD68 expression was larger in subcutaneous tissues compared with visceral tissues (9.2 vs. 6.5, P<0.05). Moreover, the expression of CD68 increased with NAFLD progression specially in visceral tissues (from 3.6 in group 1 to 9.3 in group 4, P<0.001).

Conclusion: This contribution confirms that there is a correlation between adipose tissue morphometric data with NAFLD. These results offer new perspectives about the influence of adipose depots in the origin and development of NAFLD in morbid obesity.
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From 206 studied, age: 51.3±17.4 years, male 57.1%, body mass index (BMI) 27.2±4.9 kg/m² and waist circumference (WC): 91.4±13.9 cm. Mean TE was 5.37±1.8 kPa and mean CAP was 239.1±69.2 dB·m⁻¹. Prevalence of steatosis (US score) was: S<2 61.6%, S≥2 38.4% and S≥4 12.1%.

Cap significantly correlated with presence of steatosis (p=0.73, p<0.0001), steatosis grade (p=0.76; p=0.0001), FLI (p=0.69), WC (p=0.62), BMI (p=0.55), triglyceride (p=0.49), homeostatic model assessment (HOMA-IR) (p=0.26) and total cholesterol (p=0.19), but not with fibrosis (TE). Using CAP and FLI, (AUROC)s were 0.94 (95%CI 0.91–0.97, p<0.001) and 0.91 for S≥2; 0.95 (95%CI 0.90–0.99, p<0.001) and 0.93 for S≥4, respectively. The optimal cut-off value of CAP and FLI (maximum Youden index) were 243 dB·m⁻¹ [93% sensitive (Se), 79% specific (Sp), 71% positive predictive value (PPV) and 95% negative predictive value (NPV)] and 48 for S≥2; 303.5 dB·m⁻¹ (88% Se, 92% Sp, 61% PPV and 98% NPV) and 62 for S≥4, respectively.

Conclusion: Given the good correlation of CAP with US, it seems a promising tool for screening and quantification of steatosis in general population. Larger studies should be performed to validate our results.

1324 QUANTIFICATION OF LIVER FAT USING NON-CONTRAST CT: COMPARISON OF LIVER/SPLEEN RATIO (CTL₁₅), LIVER-SPLEEN DIFFERENCE (CTL₁₅₋₁₇) WITH LIVER FAT PERCENTAGE (CTLFP)

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Objective: Non-contrast CT scans have been used to quantify the degree of hepatic steatosis using the metrics of liver spleen ratio (CTL₁₅), and liver spleen difference (CTL₁₅₋₁₇). The objective of this study was to compare the quantity of liver fat using CTL₁₅, CTL₁₅₋₁₇ with the method of CT liver fat percentage (CTLFP) in a cohort of three body mass index (BMI) types.

Methods: This IRB approved, prospective study included 89 patients stratified according to BMI into overweight (25–29.9, N=28); obese (31–34.9, N=31) and severely obese (≥35, N=30). Scanning was performed on 320-row detector CT at 135 kVp, 350 mA, gantry rotation 350 ms, voxel 0.42–0.60mm³, slice thickness 3.2mm with pitch factor 1.484. Cross sectional images with liver and spleen visible were selected and 6 separate ROIs (205–285mm²) in the liver and 3 in the spleen were averaged for CT L/S, CTL₁₅₋₁₇ for evaluating the concentration of liver fat especially in the clinical setting when liver biopsy is not indicated. The formula appears to provide an accurate and expedient tool to evaluate the degree hepatic steatosis without the need for complicated statistical calculation.

Conclusion: The CTLFP is a convenient and accurate alternative to CTL₁₅ and CTL₁₅₋₁₇ for evaluating the concentration of liver fat especially in the clinical setting when liver biopsy is not indicated. The formula appears to provide an accurate and expedient tool to evaluate the degree hepatic steatosis without the need for complicated statistical calculation.

1325 RIFAXIMIN IN NON-ALCOHOLIC STEATOHEPATITIS: AN OPEN-LABEL PILOT STUDY

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Background and Aims: Mounting evidence implicates gut microbial dysbiosis in the pathogenesis of non-alcoholic steatohepatitis (NASH) by mechanisms including caloric salvage, lipopolysaccharide production, upregulation of proinflammatory cytokines, increased insulin resistance and consequent increases in body mass and hepatic steatosis. Rifaximin is a minimally-absorbed, gut-selective antibiotic with bactericidal activity against a broad spectrum of gut microbes, making it an attractive candidate therapy. We aimed to study the effect of Rifaximin on markers

<table>
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<th>Table 1.</th>
<th>Overweight</th>
<th>Obese</th>
<th>Severely obese</th>
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<tr>
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<td>54.88±6.58</td>
<td>53.05±7.94</td>
<td>46.07±13.89</td>
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</tr>
<tr>
<td>Liver/spleen ratio</td>
<td>1.13±0.14</td>
<td>1.1±0.16</td>
<td>0.92±0.27</td>
<td>0.0003</td>
</tr>
<tr>
<td>Liver–spleen difference</td>
<td>6.18±7.09</td>
<td>4.7±7.79</td>
<td>−3.82±13.00</td>
<td>0.0002</td>
</tr>
<tr>
<td>Liver fat% (CTLFP)</td>
<td>3.2±4.11</td>
<td>4.3±4.96</td>
<td>8.7±8.68</td>
<td>0.0027</td>
</tr>
</tbody>
</table>
of hepatic inflammation, hepatic steatosis, hepatic and peripheral insulin sensitivity.

**Methods:** Patients with biopsy-proven NASH, elevated aminotransferase values and no hepatic comorbidities were included in this open-label, randomised, cross-over study, all receiving 6 weeks of Rifaximin 400 mg twice daily, before or after one of two 6 week observation periods on standard therapy. The primary endpoint was change in alanine aminotransferase (ALT) values after Rifaximin therapy. Secondary endpoints were change in percentage hepatic lipid assessed by hepatic proton magnetic resonance spectroscopy and change in hepatic and peripheral insulin sensitivity assessed by the hyperinsulinemic euglycemic clamp. Patients also had anthropometrics, serum biochemistry and cytokine profiling at each timepoint. Stool and urine were collected for subsequent analysis.

**Results:** 15 patients, 13 male, 2 female, mean (SD) age 48 (9.5) years were included. 7 had diabetes on oral hypoglycaemic medications and 8 did not have diabetes. After 6 weeks of therapy, there was no difference in ALT before, 69 (40) IU/L and after, 71 (43) IU/L, treatment, p = 0.7. Hepatic lipid content was 23.3 (12.8) % before and 26.5 (15.9) % after Rifaximin, p = 0.16. Peripheral insulin sensitivity (Rd) was unchanged, 29.5 (6.5) to 29.4 (10.0) nmol/kg min, p = 0.91, hepatic insulin sensitivity (% suppression of endogenous glucose production) was unchanged (35.2 (10.1) % to 31.2 (13.2) %, p = 0.35). There were no significant differences in body mass index, waist and hip circumference, IL 1b, IL6, IL10, IL18, CD14, TNFα, Leptin, Resistin and Adiponectin values with treatment.

**Conclusion:** Treatment with Rifaximin was not associated with changes in markers of hepatocellular damage, hepatic lipid content, cytokine profile or insulin sensitivity in patients with NASH.

**1326 IMPROVEMENT OF PLASMA PARAMETERS LINKED TO NONALCOHOLIC FATTY LIVER DISEASE AFTER WEIGHT LOSS INDUCED BY PROXIMAL SMALL INTESTINAL EXCLUSION BY DUODENAL-JEJUNAL BYPASS LINER**

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The rising prevalence of obesity is accompanied with an increase in people suffering from obesity-related comorbidities, such as type 2 diabetes (T2DM) and nonalcoholic fatty liver disease (NAFLD). While the early stage of NAFLD, steatohepatitis, is considered benign, progression to nonalcoholic steatohepatitis (NASH) and liver-related complications often occurs. Bariatric surgery improves obesity, T2DM, and NAFLD. However, traditional bariatric techniques are invasive. Recently, a non-surgical bariatric device, the duodenal-jejunal bypass liner (DJBL), has been developed to mimic the bypass component of the Roux-en-Y gastric bypass in a minimal invasive way. Previous studies with this device have revealed positive effects on obesity and T2DM. We investigated the effect of DJBL treatment on plasma parameters that reflect NAFLD.

Seventeen subjects with obesity and T2DM received the DJBL for 24 weeks. At baseline and at three and six months post-implantation of the DJBL, plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (γ-GT), albumin, caspase-cleaved cytokeratin-18 (caspase-cleaved CK-18), and liver fatty acid-binding protein (L-FABP) were determined.

At baseline, patients had a BMI of 37.0 ± 1.3 kg/m2. Mean levels of AST, ALT, and γ-GT were elevated (AST: 35.1 ± 4IU/L, ALT: 54.5 ± 9IU/L, and γ-GT: 66.5 ± 14IU/L). Caspase-cleaved CK-18 and L-FABP concentrations were 214.4 ± 35.6U/L and 29.3 ± 2.6 ng/mL respectively. Three months after DJBL placement, BMI had decreased to 33.6 ± 1.2 kg/m2 (p < 0.05). Plasma levels of AST, ALT, γ-GT, caspase-cleaved CK-18, and L-FABP had also decreased (AST: 28.3 ± 3IU/L, ALT: 32.2 ± 2IU/L, γ-GT: 44.6 ± 7IU/L, caspase-cleaved CK-18: 140.6 ± 16.3IU/L, and L-FABP: 18.2 ± 1.5 ng/mL, all p < 0.05). A further decrease of AST, ALT, and γ-GT was observed at six months post-implantation (AST: 23.2 ± 2IU/L, ALT: 28.2 ± 1IU/L, and γ-GT: 35.5 ± 5IU/L, all p < 0.05). At that time, mean BMI had decreased to 32.9 ± 1.2 kg/m2 (p < 0.05). No further change in caspase-cleaved CK-18 and L-FABP was observed (caspase-cleaved CK-18: 149.2 ± 23.1IU/L, L-FABP: 20.2 ± 1.6 ng/mL, both p=ns). Albumin levels were within the normal range at all time points.

DJBL treatment improves established clinical plasma liver parameters. In addition, a decrease in L-FABP, reflecting decreased liver injury, was observed. Moreover, levels of the recently identified more specific NAFLD-marker, caspase-cleaved CK-18, reduced. These data suggest that proximal small intestinal exclusion by DJBL positively affects NAFLD in obese patients with T2DM.

**1327 STEPSIZE COMBINATION OF SIMPLE NON-INVASIVE FIBROSIS SCORING SYSTEMS INCREASES DIAGNOSTIC ACCURACY IN NON-ALCOHOLIC FATTY LIVER DISEASE**

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Objective: Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease ranging from simple fatty liver (NLAF) to steatohepatitis (NASH), fibrosis, and cirrhosis. We aimed to analyze the diagnostic performance and clinical utility of simple non-invasive tests alone or in combination for the detection of advanced fibrosis in patients with NAFLD.

**Design and Subjects:** Data from 323 patients with biopsy-proven NAFLD/NASH who presented to the Clinic for Gastroenterology and Hepatology, University Hospital of Cologne between July 1998 and November 2009, were analyzed retrospectively. Sensitivity, specificity, positive and negative predictive values were determined along with the area under receiver operating characteristic curves (AUROC) using published formulas for NAFLD, FIB-4, and BARDFibrosis scores.

**Results:** The AUROC were as follows: NAFLD fibrosis score 0.96 (95% CI 0.92–0.99), FIB-4 0.95 (95% CI 0.91–1.00), BARD 0.82 (95% CI 0.71–0.92) with negative predictive values for advanced fibrosis of 96%, 98% and 96%, respectively. When applying the NAFLD, FIB-4 or BARD scoring systems, combining 25%, 15% or 26% of cases with advanced fibrosis would have been missed. Combining FIB-4 and BARD in a stepwise fashion, patients would have been correctly classified without biopsy in 67% of cases without missing a single case of advanced fibrosis.

**Conclusions:** The FIB-4 and NAFLD fibrosis scores perform better than the BARD scoring system. Liver biopsy can securely be replaced only with a stepwise combination of simple non-invasive tests, otherwise the assessment of risk due to advanced fibrosis may be misleading in a clinically meaningful proportion of patients.

**1328 NON-ALCOHOLIC FATTY LIVER DISEASE IN LEAN PATIENTS: IDENTIFYING HIDDEN CULPRITS**


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**Introduction:** Nonalcoholic fatty liver disease (NAFLD) prevalence is increasing worldwide. Little research has been conducted in lean patients suffering from this condition.
OBJECTIVES: To describe the prevalence of and factors associated with hepatic steatosis among lean population. 

METHODS: The 2012 liver disease detection survey conducted at the Muniz Hospital in Buenos Aires was a population based cross-sectional study of non institutionalized residents aged 15 years or older. Anthropometric features were analyzed. Hepatic steatosis was detected by ultrasonography (US); measurements of prehepatic (in a longitudinal view of the caudal lobe, measured in the anterior surface of the liver, 1cm distal to the diaphragm) and subcutaneous fat were performed. Fasting blood glucose, ALT, AST were measured. Liver stiffness was evaluated by transient elastography. We conducted logistic regression analysis to identify factors associated with liver steatosis in a subgroup of lean patients defined as BMI <25 and waist circumference lower than 88cm in women/102 cm in men. Patients with viral hepatitis or alcohol consumption (more than 140gr/females and 210gr/males) were excluded.

RESULTS: 966 patients were surveyed from July to August 2012; of them 165 were excluded for further analysis. Of this population, 183 (22.8%) patients fitted our lean patient definition; n=126 (68.8%) were female. The median age was 41±16 years old. NAFLD was present in n=13 (7.1%) patients. Lean patients with NAFLD were older (49±21 vs 41±16 years p=0.20), had similar fasting blood glucose (94±10 vs 92±20 mg/dl p=0.20), TGO values (25±8 vs 25±20 UI p=0.27), transient elastography (4.9±0.8 vs 4.9±2.2 Kpa p=0.25) and subcutaneous fat (8.3±4 vs 6.6±4 mm p=0.08). However, NAFLD lean patients had higher TGP values, 24 (18–38) vs 17 (14–22) UI/dl p=0.006; and visceral fat (10.4±4 vs 6.3±3 mm p=0.001). Three patients had high blood pressure, none were diabetic. The only independent predictors identified for NAFLD in lean patients was visceral fat above 10.4mm OR= 10 (IC95%: 2.99–33.89) p=0.001.

CONCLUSIONS: The presence of NAFLD in lean patients positively correlates with prehepatic fat. Having more than 10mm of prehepatic fat increases tenfold the risk for NAFLD. A specific role of visceral fat in the pathogenesis of NAFLD should be further analyzed.

1329 DIAGNOSTIC ACCURACY OF A NEW ELASTOGRAPHIC METHOD (SHEAR WAVE™ ELASTOGRAPHY IMAGING) IN THE NONINVASIVE ASSESSMENT OF NON-ALCOHOLIC STEATOSIS IN PATIENTS WITH TYPE 2 DIABETES

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BACKGROUND: Hepatic steatosis is often associated with diabetes and obesity and may be secondary to alcohol and drug use, toxins and metabolic diseases. Detection and quantification of liver fat have many clinical applications, and early recognition is crucial to institute appropriate management and prevent progression.

AIMS: Evaluate the performance of a new method sonographic SHEAR WAVE™ Elastography (SWE) in noninvasive steatosis assessment in patients with NAFLD and type 2 diabetes (T2D).

MATERIALS AND METHODS: 130 patients with T2D who underwent liver ultrasonography coupled with SWE were enrolled. Patients depending on the degree of steatosis divided into groups. The amount of liver steatosis was classified as follows: none (S0) – ≤5%, mild (S1) – ≤10% and <30%, moderate (S2) – ≥30% and <60%, and severe (S3) ≤60%. We performed 10 valid measurements of liver stiffness (LS) in every patient, and a median value was calculated, the result being measured in kPa. Significance was tested using analysis of variance (ANOVA). Evaluation of diagnostic accuracy of SWE performed using ROC-analysis.

RESULTS: A valid LS determination (success rate of at least 60%) was observed in 125/130 (93.5%). We noted statistically significant difference between median values of LS in our group (p<0.001). Median values, 25th and 75th percentiles of LS were as follows: control group (S0) – 5.4kPa (4.9–6.1); S1 – 6.4kPa (6.2–6.7); S2 – 7.4kPa (7.0–7.8) and S3 – 8.4kPa (8.0–8.7). The AUROC of SWE for S0 vs S (1–3) and S0 vs S (2–3) was 0.947 (95%CI 0.908–0.985) and 0.993 (95%CI 0.954–0.999) respectively. The optimal LS cutoff point for the prediction of steatosis >5% (S1–3) was 6.35, with sensitivity, specificity, positive (PPV) and negative predictive value (NPV) respectively 89.6%, 84%, 95.5% and 65.6%. The optimal LS cutoff point for the prediction of advanced steatosis >30 (S2–3) was 6.6 with sensitivity, specificity, PPV and NPV respectively 98.0%, 92.0%, 97.4% and 95.8%. LS values were strongly correlated with degree of steatosis (r=0.883, p<0.001).

CONCLUSION: SHEAR WAVE™ Elastography can be used as noninvasive method for the detection of non-alcoholic steatosis.

1330 NON-INVASIVE MEASUREMENT OF LIVER STEATOSIS BY CONTROLLED ATTENUATION PARAMETER (CAP) USING Fibroscan® IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)


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BACKGROUND AND AIM: The aim of this study was to evaluate the performance of controlled attenuation parameter (CAP) for the assessment of steatosis grade in patients with nonalcoholic fatty liver disease (NAFLD).

METHODS: We enrolled patients with chronic liver injury who met the following criteria: (1) those who visited the authors’ hospital from March 2012 to November 2012, (2) diagnosed as having NAFLD by liver biopsy with a history of alcohol consumption <20 g/day, (3) underwent 10 valid liver stiffness measurements by Fibroscan. Blood sample, liver stiffness measurement, CAP assessment and liver biopsy were performed on each patient. The following parameters were also determined at the time of liver biopsy: weight, height, waist circumference, and alcohol consumption. Steatosis was categorized according to the NAFLD Activity Score (NAS) (0, <5%; S1, 5–33%; S2, 34–66%; and S3, >66%) and assessed as the percentage of hepatocytes containing lipid droplets. The performance of CAP for diagnosing steatosis as compared with biopsy was assessed using areas under receiver operating characteristic curves (AUROC) and receiver operating characteristic curve (AUROC).

RESULTS: A total of 62 patients fulfilled the inclusion criteria. The majority (75.8%) was male and the median age was 60.9 years (IQR 43.5–69.5). The median BMI was 27.7 kg/m2 (IQR 26.1–29.3). CAP was significantly correlated with sex, age, BMI, Platelet count, ALT, HDL cholesterol, triglycerides level, but not with GGT and AST levels. The median CAP values of patients with S0, S1, S2 and S3 were 226 (IQR 207–257.5), 285.0 (273–324), 335 (285–356.5), and 334 (316.2–340.5) dB/m respectively. Although CAP was not significantly different between patients with S2 and S3 steatosis (P=0.60), differences between the remaining steatosis categories were significant (P<0.05). The AUROCs of the CAP for ≥5%, >33% and >66% steatosis were 0.87, 0.81 and 0.75, respectively. With the optimal cut-off value of 270 dB/m for detecting ≥5% steatosis, CAP had 84.3% sensitivity, 81.8% specificity, and 95.6% positive and 52.9% negative predictive values.
Conclusions: CAP is a useful tool to assess steatosis of NAFLD patients non-invasively. Especially, CAP can detect steatosis at a level of ≥5% very efficiently.

1331
A BIOMARKER PANEL WITH HIGH SENSITIVITY AND SPECIFICITY FOR NONALCOHOLIC STEATOHEPATITIS IDENTIFIED BY SUPPORT VECTOR MACHINE
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Background and Aims: Multivariate data analysis of multiple biomarkers can be performed by means of innovative bioinformatic tools like support vector machines (SVMs), a classification method based on the statistical learning theory. In this study, we used a SVM algorithm to discriminate with high accuracy at the individual level between obese subjects with NASH and those without using a blood-based biomarker panel. First, we measured serum levels of 17 targeted biomarkers of NASH covering a broad range of pathophysiological mechanisms in chronic hepatic inflammation and metabolic derangements. We then applied a SVM algorithm to obtain a non-linear combination of the potential candidate biomarkers.

Methods: A total of 17 biomarkers were measured by commercially available enzyme-linked immunosorbent assays in 136 serum samples from patients with biopsy-proven NASH (n = 60) and subjects with normal ALT and no evidence of fatty liver on ultrasound (n = 76). The database was randomly divided (1:1 fashion) into a discovery set for classification training and in a validation set of the chosen biomarkers in blinded samples. Multivariate analysis was performed by means of SVM.

Results: After the identification of a group of three most discriminative biomarkers (osteoprotegerin, fibroblast growth factor 21, and M30) in the discovery set, the application of SVM to the validation test resulted in a 92.5% sensitivity and 84.1% specificity for distinguishing subjects with NASH from controls.

Conclusions: We identified a highly selected blood-based biomarker panel consisting of osteoprotegerin, fibroblast growth factor 21, and M30 which allowed a SVM-based discrimination of NASH patients from controls with clinically relevant accuracy and validity. The present results demonstrate the potential clinical usefulness of highly focused serum marker profiling in conjunction with multivariate pattern recognition as a supportive diagnostic tool in NASH. Blood-based biomarkers might have utility in NASH diagnostics before further classification with liver biopsy.

1332
HEPATIC EXPRESSION AND SERUM LEVELS OF SYNDECAN 1 (CD138) IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE
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Background and Aims: Syndecan-1 (CD138) is a transmembrane heparan sulfate proteoglycan expressed in the liver which may exert metabolic effects by mediating the hepatic clearance of triglyceride-rich lipoproteins. In the present study, we assayed serum levels and the hepatic expression of syndecan-1 and examined their association with clinical, biochemical, and histologic phenotypes in patients with histology-proven nonalcoholic fatty liver disease (NAFLD).

Methods: A total of 59 patients with biopsy-proven NAFLD and 54 matched controls were enrolled. The analysis of syndecan-1 expression in liver biopsies was performed by immunohistochemistry on formalin-fixed, paraffin-embedded samples. Serum syndecan-1 levels were measured by ELISA.

Results: NAFLD patients had significantly higher serum syndecan-1 levels [median: 61 ng/mL (interquartile range: 36–97 ng/mL)] than controls [median: 37 ng/mL (interquartile range: 25–59 ng/mL), Mann–Whitney U test, P < 0.001]. However, we did not find any significant association between serum syndecan-1 and the mean syndecan-1 immunohistochemical score (r = 0.064, P = 0.63). Interestingly, the syndecan-1 immunohistochemical score was an independent predictor of HDL cholesterol in NAFLD patients (β = 0.27, t = 1.99, P < 0.05).

Conclusions: Our data suggest that serum syndecan-1 levels are raised in patients with NAFLD. Moreover, the syndecan-1 immunohistochemical score in the liver is independently associated with HDL cholesterol in this group of patients. These pilot results support further investigation of this molecule in metabolic liver diseases.
POSTERS

1334
CORRELATION OF HUMAN LIVER PPAR GENE EXPRESSION WITH HISTOLOGICAL SEVERITY OF NAS AND ASSOCIATED METABOLIC DERRANGEMENTS: RATIONALE FOR TARGETED THERAPY
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Introduction: Peroxisome proliferator-activated receptors (PPARs) have been implicated in the pathogenesis of NASH, merely based on animal models. Data on gene expression in liver tissue of NASH patients are scarce.
Aims: To study PPARα, β/δ and γ expression in liver tissue of a large cohort of obese patients assessed for the presence of NAFLD.
Methods: Patients presenting to the obesity clinic underwent a thorough metabolic and hepatic work-up. If NAFLD was suspected, a liver biopsy was performed. Gene expression was studied by mRNA quantification (real time RT-PCR). Liver histology was scored using the Brunt definition and the NASH CRN Scoring System.
Results: 125 patients were consecutively included (mean age 45.0±12.4y, mean BMI 38.7±6.67kg/m2). Liver PPARα expression negatively correlated with the presence of NASH according to Brunt et al (p<0.001) and with the severity of steatosis (p=0.003), inflammation (p=0.001), the NASH activity score (p=0.008), and fibrosis (p=0.003). PPAR β/δ and PPARγ expressions did not correlate with any of the histological features. PPARα expression was positively correlated to adiponectin (R²=0.345, p=0.010) and inversely correlated to visceral fat (R²=-0.343, p<0.001), HOMA IR (R²=-0.411, p<0.001) and CK18 (R²=-0.233, p=0.012) but not to PNPLA3 polymorphism. Liver PPARγ expression did not correlate with parameters of glucose metabolism or serum lipids.
Conclusion: Human liver PPARα gene expression negatively correlates with NASH severity, visceral adiposity and insulin resistance and positively with adiponectin, suggesting that PPARα is a potential therapeutic target for NASH treatment.

1335
HIGH PREVALENCE OF NON-ALCOHOLIC FATTY LIVER DISEASE IN THE CHINESE – RESULTS FROM THE HONG KONG LIVER HEALTH CENSUS
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Background and Aims: To determine the prevalence and risk factors of non-alcoholic fatty liver disease (NAFLD) in a healthy Chinese population.
Methods: Participants of the Liver Health Census were recruited from two sources – blood donors from the Hong Kong Red Cross Blood Transfusion Service and volunteers from the general population. All participants included in the census were screened negative for hepatitis B surface antigen, antibody to hepatitis C virus, and antibody to human immunodeficiency virus. All subjects underwent weight, height, and hip and waist circumference measurements. Blood pressure was also recorded. A detailed questionnaire of medical history, alcohol, medicine, and herbal intake was undertaken. Laboratory blood testing, ultrasonography and transient elastography were performed on the same day.
Results: A total of 2,493 subjects were recruited into the census. The prevalence of NAFLD was 42% (1,054 patients). Univariate analysis identified male gender, increasing age, weight, height, body mass index, waist circumference, hip circumference, waist-hip ratio, systolic and diastolic blood pressure, fasting cholesterol and glucose levels to be significant factors associated with NAFLD. Using multivariiate analysis, gender, age, waist circumference, systolic blood pressure, fasting cholesterol and glucose levels remained significant factors. The relative risk of NAFLD in those with high waist circumference (current recommendation: male 90cm, female 80cm), diabetes, hypertension, and hypercholesterolemia was 2.99, 2.01, 1.79, and 1.54 respectively. The degree of steatosis increased with levels of fasting glucose, cholesterol, systolic blood pressure and waist circumference (all p<0.001). The optimal waist circumference was found to be 84 and 74cm for male and females respectively, with a relative risk of 5.16 for those above this limit. 1.2% and 0.002% of subjects with NAFLD had advanced liver fibrosis and cirrhosis respectively.
Conclusions: NAFLD was found to be highly prevalent in the Chinese population. Despite this, the prevalence of severe liver disease was low. Increasing levels of risk factors were associated with increasing severity of NAFLD. Lower cut-off levels of waist circumference to predict NAFLD should be adopted for Chinese population.

1336
NON-ALCOHOLIC STEATOHEPATITIS INCREASES THE PRODUCTION OF ANTIBODIES AGAINST OXIDIZED LDL, ATHEROSCLEROSIS AND CARDIOVASCULAR RISK
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Background and Aims: Recent studies suggest that non-alcoholic steatohepatitis (NASH) could promote an increase cardiovascular morbi-mortality (Ampuero et al, Hepatology 2012; vol 56, 4 (suppl); 889A). On the other hand, oxidized LDL (oxLDL) is proatherogenic and immunogenic, so generates antibodies against oxidized LDL (anti oxLDL). The aim was to analyze the impact of steatohepatitis on cardiovascular disease (CVD) in a cross-sectional study.
Methods: Patients with NAFLD diagnosed by percutaneous liver biopsy assessed by a single pathologist were included. Macrovesicular steatosis, lobular and portal inflammation, ballooning, perisinusoidal fibrosis, Kleiner stage and NAS score were determined. In 83 patients metabolic, hepatic and kidney profiles were analyzed in serum samples that were obtained and stored in aliquots at −80°C until measurement of circulating oxLDL antibodies anti-cholesterol antibodies by ELISA. Seventeen patients with suspected CVD underwent ergometry assessed by minutes and Metabolic Equivalent of Task (METs).
Results: Mean age of overall cohort was 47±12 years old; 48/83 (58%) males; fibrosis stage: F0–F1: 50/83 (60%), F2: 18/83 (22%) and F3–F4: 15/83 (18%); steatohepatitis in 54% (45/83) and ballooning 16/83 (19%). Anti oxLDL levels were higher in patients with ballooning in liver biopsy (2.212 EU/mL±946 EU/mL vs 1.617 EU/mL±1.017 EU/mL; p=0.036), with severe fibrosis (2.089 EU/mL±1.704 EU/mL vs 1.493 EU/mL±0.905 EU/mL; p=0.009) and in patients with steatohepatitis (2.095 EU/mL±1.139 EU/mL vs 1.575 EU/mL±0.940 EU/mL; p=0.033), besides anti oxLDL correlated with NAS score (r=0.33; p=0.002; n=83). However, there was no association with simple steatosis or steatosis degree. Patients with severe fibrosis (n=7) endured less ergometry (1.37±2.53 min vs 8.58±2.11 min;
1337 CARDIAC FUNCTION IMPROVES FOLLOWING HIGH INTENSITY INTERMITTENT EXERCISE THERAPY IN NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is associated with a twofold greater risk of developing cardiovascular disease than those without. Recent research has shown that there are alterations in cardiac structure and function in NAFLD in the absence of overt cardiac disease. This study aimed to investigate exercise as a therapeutic tool to decrease cardiac risk in NAFLD.

Patients and Methods: Twenty adults with NAFLD were randomised to 12 weeks of high intensity intermittent training or continue standard care. Cardiac structure and function were assessed using high resolution cardiac magnetic resonance imaging and tagging at 3.0T. High energy phosphate metabolism was assessed using 31P-spectroscopy to measure PCr/ATP ratio. People with a history of discrete cardiac events or potential cardiac change. To date, no studies have reported the relationship between liver fat and metabolic control in people with NAFLD. This study determined the level of objectively measured sedentary behaviour and physical activity in people with NAFLD, and investigated links between physical activity, liver fat, glucose control and body composition.

Methods: Sedentary behaviour, physical activity and energy expenditure were assessed in 37 adults with NAFLD using a multi-sensor array (SenseWear Pro3, Bodymedia Inc, PA, USA) over 7 days. Liver fat, glucose control, and body composition were assessed respectively by 1H-MRS, fasting blood samples, and air displacement plethesmography. Patterns of sedentary behaviour were assessed by power law analyses of the lengths of sedentary bouts fitted from raw sedentary data. An age and sex-matched healthy control group wore the activity monitor for the same time period.

Results: People with NAFLD spent approximately half an hour extra a day being sedentary (1318±68 vs. 1289±60min; p<0.05) and the number of steps walked was reduced by 18% (8483±2926 vs. 10377±3529; p<0.01). As a consequence, active energy expenditure was reduced by 40% (432±258 vs. 732±345kcal; p<0.01) and total energy expenditure was lower in NAFLD (2690±440 vs. 2901±511kcal/day; p<0.01). Power law analyses of the lengths of sedentary bouts demonstrated that patients with NAFLD also have a lower number of transitions from being sedentary to active compared with matched controls (15±0.03 vs. 15±0.03%; p<0.05). Liver fat and fasting glucose were not associated with any of the physical activity parameters. Body fat percentage was negatively associated with steps (r=-0.542; p<0.01), total energy expenditure (r=-0.514; p<0.01) and physical activity duration (r=-0.581; p<0.01). There was also a positive association between body fat percentage and sedentary time (r=0.336; p<0.05).

Conclusions: People with NAFLD spend more time sedentary and undertake less physical activity on a daily basis than healthy controls. Low levels of physical activity represent a therapeutic target which may prevent progression of metabolic conditions in people with NAFLD and should be considered by clinical care teams.

1339 DIAGNOSTIC VALUES OF UBIQUITIN-POSITIVE HEPATOCELLULAR INCLUSIONS IN PATHOLOGIC DIAGNOSIS OF NONALCOHOLIC STEATOHEPATITIS

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Background and Aims: Nonalcoholic steatohepatitis (NASH), an aggressive form of nonalcoholic fatty liver disease (NAFLD), is recognized to progress to cirrhosis. Hence, pathologic differentiation between NASH and simple steatosis is important in managing NAFLD patients. Pathologic diagnosis of NASH is based solely on presence of ballooned hepatocytes in inflamed fatty livers. However, hepatocyte ballooning (≥1.5-fold cellular enlargement with clear cytoplasm) is often difficult to be recognized even by expert pathologists. Mallory-Denk body (MDB) is one of the other histopathological features of NASH, and is clearly demonstrated by immunostaining with anti-ubiquitin. In this study, we tested the diagnostic value of ubiquitin immunohistochemistry in histopathological examination of NAFLD.

Methods: Thirty liver biopsies obtained from NAFLD patients (22 women and 8 men, 60±12 yr) were examined morphologically and immunohistochemically using anti-ubiquitin antibody (Dako Japan Inc., Tokyo, Japan). The histological severity of the liver disease is evaluated with Matteoni’s classification and NAFLD activity score (NAS). Ubiquitin-positive hepatocellular inclusions were semi-quantified as absent (0), few (1), and many (2). Nonparametric
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THE INFLUENCE OF WAIST CIRCUMFERENCE ON INSULIN RESISTANCE AND NONALCOHOLIC FATTY LIVER DISEASE IN APPARENTLY HEALTHY KOREAN SUBJECTS
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Background and Aims: Waist circumference (WC) is a valuable component of metabolic syndrome. Waist circumference is related to insulin resistance (IR) and nonalcoholic fatty liver disease (NAFLD). The purpose of this study was to examine the association between waist circumference and IR, NAFLD in apparently healthy Korean subjects.

Methods: After exclusion criteria, 9159 (5052 men, 4107 women) subjects who participated in comprehensive health checkup program enrolled this study. Cross-sectional study was performed. IR was evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR) and IR was considered with HOMA-IR>2. NAFLD was presented by ultrasonographic finding. We considered alanine aminotransferase (ALT) elevated if above the upper normal limit (>40IU/L in men, >35IU/L in women). Logistic regression analysis was performed to evaluate the odds ratio in NAFLD, IR and ALT according to the categorized levels of the waist circumference.

Results: NAFLD was found in 27.9% (41.7% in men, 10.8% in women) of the subjects. IR was 17.2% (21.3% in men, 12.2% in women), elevated ALT was 10% (15.0% in men, 3.8% in women) of subject. After adjusted for confounding factor, the prevalence of NAFLD, IR and elevated ALT was significant associated with increasing quartile of waist circumference. (highest quartile in NAFLD; OR 15.539 [95% CI 12.687–19.033] in men, 23.918 [95% CI 23.918–99.288] in women, P<0.001), (highest quartile in IR; OR 17.576 [95%CI 13.283–23.255] in men, 11.078 [95% CI 7.813–15.708] in women, P<0.001) (highest quartile in elevated ALT; OR 7.952 [95% CI 6.046–10.459] in men, 8.487 [95% CI 4.679–15.395] in women P<0.001).

Conclusion: Waist circumference is contribute to IR and NAFLD in apparently healthy Korean subjects. So waist circumference may be a most important factor of developing IR and NAFLD.

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ARE CURRENT NORMAL RANGES SET TOO HIGH? PREVALENCE OF ABNORMAL LIVER TRANSAMINASES IN A LARGE POPULATION-BASED COHORT OF THE RUHR AREA
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Background and Aims: Chronic liver disease is a major cause of morbidity and mortality in the industrial nations. Abnormalities in serum content of liver transaminases (LT) often reflect significant liver disease. However, normal values can persist with severe liver damage. The prevalence of liver related diseases in Germany is estimated at roughly 5 Mio. people (6.25%). Though, the prevalence of elevated aminotransferases in asymptomatic subjects in Germany is unknown.

Methods: We analyzed data of participants (n=4789, aged 45 to 75 years) from the Heinz-Nixdorf-Recall study, a population-based cohort study. LT (alanine aminotransferase [ALT], aspartate aminotransferase [AST]) were considered abnormal if they were >50U/l for male, >35 U/l for female.

Tests (Mann–Whitney test and Spearman’s correlation coefficient) were used for statistical analyses.

Results: Of 30 cases, 25 were NASH of Matteoni type 4 (including four suspicious cases), four were simple steatosis of Matteoni type 2, and one was cirrhosis of probably burnt-out NASH. The mean NAS (±SD) was 4.7 (±1.8) and the mean fibrosis stage was 2.3 (±1.1).

Morphologically detectable MDBs were seen only in six of 30 samples (Figure 1: arrow). In contrast, ubiquitin-positive inclusions were seen more frequently in 22 samples, principally in ballooned hepatocytes (Figure 2: arrows). The presence of the inclusions was closely related to the degree of hepatocyte ballooning (Rs=0.57, P=0.002). In the four suspicious and one burnt-out NASH cases, the finding of ubiquitin-positive inclusions made the diagnoses more definitive.

Conclusions: Detection of ubiquitin-positive inclusions was easy and provided worthy information for pathologic diagnosis of NASH. In the light of difficulty in recognizing hepatocyte ballooning morphologically, ubiquitin immunohistochemistry may be recommended as a routine examination in cases of NAFLD.
Results: Mean ALT was at 16±8.8U/l and well below the normal threshold for normal values. The same result was obtained for AST, with a mean of 13±4.6U/l. When stratified by gender, ALT was significantly higher in male subjects than in females (19±9.7U/l vs. 14±7.1U/l; p<0.0001), which was also observable for AST (14±4.8U/l vs. 12±4.2 U/l; p<0.0001), respectively. However, the percentage of female subjects with elevated AST was significantly higher compared to male subjects [15/2405 (0.6%) vs. 5/2383 (0.2%); p=0.0045]. A similar proportion was found for ALT, with 1.8% (43/2407) females above normal range compared to 1.5% (36/2384) males (p=0.0075).

Conclusion: A large discrepancy was observed between estimated numbers of liver disease (6.25% of the population) and the number of subjects with elevated LT (below 2%) in the analyzed study cohort. Being the main alarm signal for liver diseases or injury before enrolling further diagnostics, current LT thresholds might miss a significant part of liver pathologies. Current normal range limits should be re-assessed, to provide a more focused approach concerning chronic liver disease.

1342 CHANGE IN SOLUBLE CD163, A MARKER OF ACTIVATED MACROPHAGES, IS ASSOCIATED WITH IMPROVEMENT IN LIVER ENZYMES AND METABOLIC PROFILE IN OBESE CHILDREN DURING LIFESTYLE INTERVENTION

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Background and Aims: Macrophages are involved in inflammation and fibrosis in liver diseases. When activated the macrophages shed the haemoglobin-haptoglobin scavenger receptor CD163 into the circulation as soluble CD163 (sCD163), which thus acts as a marker of macrophage activation. We aimed to associate sCD163 with clinical and biochemical parameters of pediatric obesity and fatty liver disease, as well as changes in these parameters during a weight loss camp.

Methods: We studied 117 obese children enlisted into a 10-week lifestyle intervention programme consisting of a healthy diet and moderate physical activity. Seventy-one children completed the 12-month follow up. Clinical, biochemical and metabolic parameters were recorded at each visit, and liver ultrasonography was performed. Soluble CD163 was measured by ELISA.

Results: The children lost weight during camp, and 24% sustained or further decreased their standardized BMI (BMI-SDS) after the 12-month follow up. Soluble CD163 was not associated with BMI-SDS or changes in weight and BMI-SDS. Baseline sCD163 was higher in patients with increased ALAT (2.27±0.7 vs. 2.0±0.6 mg/L; p=0.03) and ultrasonographic steatosis (2.33±0.72 vs. 2.02±0.58 mg/L, p=0.01). At baseline, sCD163 was independently associated with ALAT, total cholesterol and high sensitivity C-reactive protein (hs-CRP) in a multiple regression model. In an equivalent model, there were independent associations between the change in sCD163 during the 10-week lifestyle intervention and corresponding changes in ALAT, HOMA-IR, hs-CRP and total and HDL-cholesterol.

Conclusion: sCD163 was associated with the parameters of fatty liver disease and the metabolic syndrome in obese children, as well as changes in these parameters during lifestyle intervention. sCD163 may serve as a biochemical marker for disease and treatment in NAFLD.

1343 THE EFFECT OF METFORMIN TREATMENT ON CARDIOVASCULAR RISK IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: There is no data regarding the effect of insulin sensitizers on cardiovascular risk in NAFLD patients. The aim of this study was to investigate the effect of metformin on cardiovascular risk in patients with NAFLD.

Materials and Methods: A total of 76 consecutive biopsy-proven NAFLD patients with insulin resistance (IR) (M/F: 41/35, mean age: 49.5±8.0 years) were included to study. All subjects were divided basically into two distinct groups as follows: group 1 (n = 34) received a conventional diet of 25 kcal/kg x ideal body weight and an exercise program. Group 2 (n = 42) received the diet and exercise program plus metformin at a dose of 850 mg b.d. All subjects were seen at the fourth week and at 3-month intervals. Clinical and laboratory examinations were performed. Clinical and laboratory examinations were performed. Carotis intima media thickness (CIMT) was measured, and cardiovascular risk score was calculated by using “Heart Score Risk Calculator” program of European Society of Cardiology (ESC) at the beginning of study and thereafter annually.

Results: With respect to baseline parameters, no significant differences in terms of the metabolic, biochemical parameters, CIMT and CVR score were observed between the two groups (p > 0.05). When compared to baseline, IR had significantly decreased in the two groups at the end of 12 months (HOMA score, p<0.002, p<0.001, respectively) However, there was no significant improvement in terms of CIMK and CVR scores observed in both groups (p > 0.05).

Conclusion: Metformin can lead to improvement in insulin resistance, but not improve on cardiovascular risk in patients with NAFLD.

1344 VISCERAL OBESITY IS ASSOCIATED WITH SIGNIFICANT FIBROSIS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is related to dyslipidemia, diabetes, and metabolic syndrome, which are closely linked with visceral obesity. However, association between visceral adipose tissue area and fibrosis in patients with NAFLD has not been completely established. The aim of this study was to determine the relationship between by computed
ASSOCIATION OF PSORIASIS WITH NONALCOHOLIC FATTY LIVER DISEASE: RESULTS FROM A POPULATION-BASED STUDY

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Background: Recent small studies have found an increased prevalence of nonalcoholic fatty liver disease (NAFLD) in psoriasis patients. The exact mechanism explaining this association is unknown, but may be related to metabolic conditions that are highly prevalent in both diseases. In addition, a pathophysiological circle, in which the release of inflammatory cytokines (e.g. TNF-α, IL-6) as a result of both conditions plays a key role, is suggested to link psoriasis with NAFLD.

Aim: To investigate the association of psoriasis with NAFLD in a large population-based cohort study of older adults.

Methods: This cross-sectional study was based on participants (aged ≥65 years) of a population-based study. Each participant was interviewed and had a clinical examination, including a fasting blood collection, and liver ultrasonography. NAFLD was defined as fatty liver on ultrasonography (Hamaguchi criteria), in the absence of alcohol consumption (>14 drinks/week), positive HBsAg or anti-HCV, or use of drugs associated with fatty liver.

Psoriasis was either diagnosed by trained physicians in Dermatology at the research center or by records of general practitioners, which were checked if participants used drugs associated with psoriasis (98% of participants were registered at an automated pharmacy). We used logistic regression analysis to analyze the association of psoriasis with NAFLD.

Results: Data on psoriasis was available for 2617 of 3205 participants that had liver ultrasonography. In total, 325 participants were excluded for presence of secondary causes of fatty liver, leaving 2292 participants for analyses (mean age 76.2±6.0 years; 58.7% female; mean BMI 27.4±4.2kg/m²). In this study population, the prevalence of NAFLD was 33.9% and 118 elderly participants (5.1%) had psoriasis. Psoriasis was present in 7.0% of participants with NAFLD, and in 4.2% of participants without NAFLD (p=0.007). In logistic regression analysis, psoriasis was significantly associated with NAFLD after adjustment for age and gender (OR1.7, 95%CI 1.2–2.5; p=0.006). After additional adjustment for alcohol consumption, pack years of smoking, smoking status, ALT, and presence of the metabolic syndrome, psoriasis remained significantly associated with NAFLD (OR1.7, 95%CI 1.1–2.5; p=0.01).

Conclusion: This is the first cross-sectional population-based study, to demonstrate an association of psoriasis with NAFLD.

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) IS ASSOCIATED WITH IMPAIRED LEFT VENTRICULAR DIASTOLIC DYSFUNCTION AND INCREASED MEDIASTINAL FAT

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Background: NAFLD is considered the liver component of the metabolic syndrome. Data on cardiac function in patients with NAFLD are limited and conflicting.

Aim: We assessed whether NAFLD is associated with abnormalities in cardiac function.

Methods: We studied 43 consecutive NAFLD individuals without a history of ischemic heart disease, hepatic diseases, or excessive smoking.

Conclusions: Fatty liver is known to be associated with metabolic syndrome and increased coronary heart disease (CHD) risk. But there is no validated tool to discriminate these patients. The aim of the current study is to evaluate the validity of fatty liver panels in predicting subjects of fatty liver with metabolic syndrome (MS) and with increased CHD risk.

Methods: We retrospectively reviewed the medical records of subjects who visited KonkukUniversity hospital between January and December 2010, for health check up and collected their anthropometric and clinical data. Diagnosis of fatty liver was made with liver ultrasonography. MS was defined according to NCEP-ATPIII criteria adjusted for Asians. CHD risk was calculated based on revised Framingham risk scoring system at 2002. Fatty liver index (FLI), visceral adiposity index (VAI), liver fat score (LFS) and liver fat percent (LFP) were calculated as described previously.

Results: Fatty liver was diagnosed at 4,122 among total 10,663 subjects. Fatty liver group had higher prevalence of males, smoker, and older age than non fatty liver group (71.3 vs 52.1%, P<0.001, 40.4vs 59.3, P<0.001, 46.0 vs 43.6, P<0.001, respectively). All other anthropometric and biochemical values were different between the two groups, showing higher tendency for increased metabolic riskin fatty liver group. Liver fat score and fat percent showed highest AUCROC values (0.925, 0.920) for the prediction of fatty liver with MS. For the prediction of fatty liver with CHD over 10%, FLI andLFS showed higher AUCROC values (0.797, 0.769), and for those with over 20%, both FLI and LFS showed highest AUCROC value (0.847, 0.847).

Conclusion: FLI and LFS were useful indexes in predicting presence of fatty liver with MS. For the presence of fatty liver with increased CHD risk, both FLI with LFS and LFP were useful indexes.
alcohol consumption, in whom NAFLD was diagnosed by non enhanced CT and were compared to 33 healthy controls matched for age, and BMI. Tissue Doppler echocardiography (TDI) was performed in all patients. 

**Results:** Patients with NAFLD were male (95% VS 72%, P < 0.01) and had increased mean systolic blood pressure (140±14 VS 132±14, P < 0.02) than controls. NAFLD patients had lower early diastolic tissue velocity (e' on TDI, 10.2±2.9 cm/sec vs 12.2±3.1 cm/sec, P < 0.011), lower ratio of early diastolic to late diastolic mitral inflow (E/A ratio 1.12±0.4 vs 1.42±0.35, P < 0.28), higher atrial reversal velocity of mitral inflow (Ar velocity 28±6 vs 25±4.4, p < 0.03), higher left ventricle mass (156±30 VS 137±31, p < 0.013), higher deceleration time (DT 208.2±40 ms vs 184.2±26, P < 0.004), and higher short axis mediastinal fat (SAX 6.7±2.9 mm vs 5.1±2.6, P < 0.07) than healthy controls. All of these differences remained significant after adjustment for hypertension and other cardio metabolic risk factors. Pericardial fat thickness, left ventricular volumes, ejection fraction, and relation time (IVRT) were not different.

**Conclusions:** Our data show that patients with NAFLD have early features of LV diastolic dysfunction.

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**PREDICTIVE VALUE OF EPICARDIAL FAT AS NEW MARKER OF METABOLIC SYNDROME AND EARLY VASCULAR DAMAGE IN PATIENT WITH NAFLD**

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**Background:** Increased visceral adiposity is considered the hallmark of the metabolic syndrome (MS) and Non-alcoholic fatty liver disease (NAFLD). Epicardial adipose tissue has been implicated in the pathogenesis of coronary atherosclerosis.

**Aim:** To evaluate in patients with MS and NAFLD the threshold values of echocardiographic epicardial fat thickness and their association with a) metabolic and clinical parameters, b) early atherosclerotic vascular damage, by carotid intima media thickness (IMT).

**Methods:** 41 patients with MS and with clinic, laboratory, ultrasound, histology proven NAFLD were enrolled in the study (20 men, age 48±13 years, BMI 33±5 kg/m2, waist circumference (men) 114±11 cm, (women) 109±10 cm) and 20 controls without MS and without NAFLD (11 men, age 47±9 years BMI 23±1 kg/m2). Physical examination, blood tests, carotid ultrasound were performed. Epicardial fat thickness was evaluated by transthoracic echocardiogram.

**Results:** Patients with MS and NAFLD had significantly higher epicardial fat thickness than controls (4.95±2.6 and 2.69±1.8 mm, p = 0.01). Considering as increased values higher than 2.7 mm (median of controls) we evaluated variables associated with increased epicardial fat. Age, BMI, waist circumference, fasting glucose, HOMA-IR, IMT were significantly higher in subjects with increased epicardial fat than without, while the echocardiographic diastolic function index early/atrial peak flow (E/A) was significantly lower. At multivariate analysis HOMA-IR remained the independent variable associated with epicardial fat (p = 0.04, OR1.8, 95% CI1.037–3.58).

**Conclusion:** In conclusion patients with MS and NAFLD had higher value of epicardial fat thickness than controls, the increased epicardial fat values were associated with insulin-resistant and with early vascular damage. Epicardial fat measurement, an easy diagnostic tool to define visceral and cardiac adiposity could be proposed to better predict the cardiovascular risk and connection with NAFLD.
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Methods: In 22 non-diabetic, non-dyslipidaemic patients with biopsy-proven NAFLD we measured whole abdomen visceral fat (VF) by MRL, fasting endogenous glucose production (EGP) and lipolysis by tracer infusion, peripheral insulin resistance (IR) as HOMA, Hepatic-IR as EGP x fasting insulin, and adipose tissue (Adipo)-IR as basal FFA levels x fasting insulin. Hepatic histology was scored according to Kleiner, liver fat was also reported as percentage (LF%).

Results: In our study cohort, VF was increased in proportion to BMI (r=0.54, p<0.004) and to LF%, (r=0.43, p<0.03), whereas LF% was not correlated with BMI. Only LF%, but not VF, was associated with circulating FFA levels (r=0.51, p<0.01), and Adipo-IR (r=0.44, p<0.05), while no correlation was found between either LF% or VF with Hepatic-IR or HOMA. Patients with NAS score ≥4 (compared to those with NAS 0–3) had more VF (3.9±0.7 vs 2.8±0.4 kg), increased FFA concentrations (525±52 vs 804±98 mmol/l), peripheral IR (HOMA: 3.7±0.5 vs 2.8±0.8), hepatic-IR (169±23 vs 124±32), and adipose-IR (13.2±2.5 vs 6.3±1.8) (all p<0.05). When taking into account only ballooning and lobular inflammation in the NAS score, we found that subjects with a composite score ≥2 had an impaired suppression of lipolysis. Compared to patients without fibrosis, those with fibrosis had more VF (3.9±0.6 vs 2.7±0.7 kg, p<0.03), but not LF%, and no differences were found in the indexes of IR.

Conclusions: In NAFLD subjects, liver fat is associated with metabolic derangements and insulin resistance, but insulin resistance of the adipose tissue and VF accumulation appear to provide a major contribution to liver damage.

Funding from the FP7/2007–2013 under grant agreement no. Health-F2–2009–241762, for the FLIP project.

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INTEROBSERVER CONCORDANCE IN CONTROLLED ATTENUATION PARAMETER (CAP) MEASUREMENT, A NOVEL TOOL FOR THE ASSESSMENT OF HEPATIC STEATOSIS BASED ON TRANSIENT ELASTOGRAPHY

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Introduction: The combination of transient elastometry (TE) with controlled attenuation parameter (CAP) allows non-invasive measurements of hepatic steatosis (HS) simultaneously to liver stiffness. TE is characterized by a high reproducibility and low inter-observational variability in HCV-infected patients with or without HIV coinfection. Nevertheless, no data are available on interobserver differences in CAP values. This is a relevant point, since HS is a very common disorder among HIV-infected patients, and accurate non-invasive diagnosis is critical.

Objective: To assess the concordance of CAP measurements between two independent observers in patients infected with HIV and/or hepatitis virus.

Methods: In a cross-sectional, prospective study conducted from December 2011 to March 2012 in a university hospital in Spain, CAP-enabled TE acquisitions were performed by two independent observers in 118 consecutive patients with HIV and/or hepatitis virus infection. The interobserver concordance between the CAP value measurements was assessed using the intraclass correlation coefficient (ICC) and the concordance of the classification of patients regarding the grades of HS was characterized using the kappa index. Patients with CAP ≥238 dB/m were considered to bear significant HS (≥10% hepatocytes involved), as previously reported.

Results: 78% patients were male. Twenty (17%) patients were HIV monoinfected, 44 (37.3%) hepatitis C virus (HCV)-monoinfected and 52 (44%) showed HIV/HCV coinfection. The median (Q1-Q3) values of CAP obtained by the first and the second observer were 228 (205–265) and 227 (196–269) dB/m, respectively. The median (interquartile range) of the absolute difference of CAP values between the two observers was 20 (10–41) dB/m. The overall ICC was 0.84 (95% confidence interval: 0.77–0.88). The kappa index for the concordance of classification for the presence of significant HS was 0.55. No factor was associated with a greater concordance between observers.

Conclusions: The concordance of CAP values obtained by two observers is good. Therefore, the determination of HS by means of CAP in HIV and/or hepatitis virus infection represents an observer-independent and easily performable method. However, the concordance of the diagnosis of significant HS, defined by the cutoff of 238 dB/m, is suboptimal.

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EFFECTS OF omega-3 POLYUNSATURATED FATTY ACIDS (PUFA) FROM FISH AND FLAXSEED OILS ON NONALCOHOLIC STEATOHEPATITIS (NASH)

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Introduction: There are very few intervention strategies that have been proven in non-alcoholic fatty liver disease (NAFLD). Omega-3 polyunsaturated fatty acids (PUFA) seem to be efficacious on NAFLD treatment from experimental models, but few randomized trails have been realized. The aim of this study was to evaluate prospectively the efficacy of Omega-3 PUFA derived from fish and flaxseed in non-alcoholic steatohepatitis (NASH) patients.

Methods: Sixty patients with biopsy proven NASH were included in the randomized placebo controlled trial. The patients were randomized into two groups. Omega-3 group (n=30) received capsules containing 945mg of Omega-3 PUFA [alpha linolenic acid/64%, eicosapentaenoic acid (EPA)/16% and docosahexaenoic acid (DHA)/21%], in 3 capsules/day. Placebo Group (n=30), received 3 placebo capsules containing mineral oil. The intervention was carried out for 6 months, when patients were re-submitted for new liver biopsy. Primary endpoint was liver histology according to the NASH activity score (NAS) at baseline and 6 months. Second endpoints were evaluated by analysis of serum aminotransferases, fasting lipoprotein profile, serum glucose, anthropometric parameters and serum levels of cytokines at 0, 3 and 6 months.

Results: These 60 patients enrolled, 10 were not finished the study (5 in Omega-3 group and 5 in the Placebo group). Concerning the primary endpoint, the NAS activity improved by 57% in the placebo group and 67% in the omega-3 group, however, no significant difference was seen (p=0.33), the hepatocellular ballooning reduced 22% in the placebo group and 33% in the omega-3 group, also with no difference between groups (p=0.28). Omega-3 did not reduce steatosis, lobular inflammation and fibrosis. Sera aminotransferases, fasting lipoprotein profile, serum glucose, anthropometric parameters and serum levels of IL-6 and TNF-α were not altered with the treatment.

Conclusion: Our results indicate that Omega-3 PUFA from fish and flaxseed oil compound cannot improve, after 6 months, the liver histology, biochemical parameters and serum levels of IL-6 and TNF-α. The limitations of this study were the small number of patients enrolled and the composition of Omega-3 compound that was enriched with linolenic acid (64%) than EPA/DHA. Further study is needed to confirm these results.
1354 GLYCEMIC VARIABILITY IS AN INDEPENDENT PROGNOSTIC FACTOR FOR DEVELOPMENT OF HEPATIC FIBROSIS IN NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Patients with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) often have metabolic disorders including insulin resistance and type 2 diabetes mellitus (T2DM). We clarified the predictive factors in glucose metabolism for progression of hepatic fibrosis in patients with NAFLD by the 75-g oral glucose tolerance test (75gOGTT) and a continuous glucose monitoring system.

Methods: One hundred sixty-nine patients (68 female and 101 male patients) with biopsy-proven NAFLD with performance with 75gOGTT were enrolled and divided into four groups according to the stage of hepatic fibrosis (F0–3).

Results: The proportion of patients with T2DM significantly gradually increased with the progression of fibrosis. In addition, HbA1c and the homeostasis model assessment of insulin resistance were significantly elevated and 1,5-anhydroglucitol (1,5-AG) was remarkably increased with the progression of fibrosis. In the 75gOGTT, areas under curve of glucose and insulin secretion were remarkably decreased with the progression of fibrosis. In the 75gOGTT, HbA1c was associated with CP (P=0.003) and C-IMT (P=0.001), prevalence of CP (63 vs. 50%, P=0.0001) and FRS (p=0.003). NAS score and liver steatosis were significantly correlated with CP (r=0.173, P=0.04 and r=0.198, P=0.02) and FRS (r=0.208, P=0.01 and r=0.164, P=0.05). Only ballooning was correlated with C-IMT (r=0.273, P=0.01). In multivariate logistic regression, NAS score was associated with CP (P=0.02), independent of age, LDL, diabetes, high blood pressure and tobacco.

Conclusion: In NAFLD histological severity is an independent predictor of early atherosclerotic lesions and 10-year CV risk and should prompt screening for early atherosclerosis.

Acknowledgements: Funding from the FP7/2007–2013 under grant agreement no. Health-F2-2009-241762, for the FLIP project.

1356 NAFLD IS SIGNIFICANTLY INCREASED IN PATIENTS AT HIGH RISK FOR CARDIOVASCULAR EVENTS AND IS CORRELATED WITH EARLY PREDICTORS OF ATHEROSCLEROSIS AND THE FRAMINGHAM SCORE

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Patients with NAFLD have an excess prevalence of cardiovascular (CV) events. It is unknown if this is mediated through a higher risk of early atherosclerotic lesions.

Aim: To evaluate the relationship between NAFLD, early predictors of atherosclerosis and the 10-year Framingham risk score (FRS) in patients at high cardiovascular risk.

Methods: Patients with ≥2 CV risk factors (high BP, diabetes or high fasting glucose, dyslipidemia, obesity, tobacco), without previous CV events, known liver disease and drinking <50g of alcohol/day underwent carotid ultrasonography with measurement of carotid intima-media thickness (C-IMT). The Fatty Liver Index (FLI), a surrogate marker of hepatic steatosis when >60, and the Framingham score (FRS) were calculated. Carotid plaques (CP) were defined as C-IMT>1.5 mm at carotid bifurcation.

Results: 5685 subjects were enrolled: 53% males, mean age 55 years (69% >50yrs), mean BMI 26.4 kg/m², 26% had ≥1 CP, mean C-IMT: 0.62 mm (s.d. 0.14), mean FRS 10.6 (s.d. 8.1), mean FLI 45 (s.d. 30.4). Compared to subjects with FLI <60, those with FLI>60 had higher BMI, ALT, AST and GGT (p<0.0001). They also had higher C-IMT (0.64±0.16 vs. 0.61±0.13, p<0.0001), and higher FRS (14.7±8.8 vs. 8.3±6.6, p<0.0001). The interaction between FLI and CP was age-dependent. In subjects ≥50, a high FRS (>60) was associated with CP (36 vs. 32%, p<0.01) while no difference existed in subjects <50yrs (10% vs. 12%). Moreover, FLI was significantly correlated with FRS (r=0.46, p<0.0001) and increased with each FRS quintile (p<0.0001). In multivariate analysis FLI measurement of carotid intima-media thickness (C-IMT). Patients with other chronic liver diseases or excess alcohol consumption (>50g/day) were excluded. NAFLD was categorized in steatosis (S), steatosis and non-specific inflammation (SNS) and steatohepatitis (NASH) and staged using Kleiner’s classification. Carotid plaques (CP) were defined as a C-IMT>1.5 mm at carotid bifurcation. 10-year Framingham risk score (FRS) was calculated. A control group of 100 age and sex-matched, asymptomatic patients with no NAFLD (i.e. normal ALT, and fatty liver index, FLI <60) was included.

Results: 135 patients (71% males) from France, Italy and Spain were enrolled; mean age was 49.6±11.4 years, mean BMI, 29.9±4.88. The interval between LB and CUS was 1.9±2.12 yrs. NAS was diagnosed in 61% and ≥2 fibrosis in 43%. Compared to controls, patients with NAFLD had higher BMI, ALT, AST, TG, fasting glucose (p<0.0001 for all), FRS (p=0.003), prevalence of CP (33 vs. 16%, p=0.003) and C-IMT (after excluding patients with CP), (0.72±0.22 vs. 0.56±0.12, p<0.0001). FRS was higher in patients with NASH vs. S or SNS (12.2±6.3 vs 9.4±6.2, p=0.05). Patients with ≥2 fibrosis had a higher prevalence of CP (46 vs. 21%, p<0.05). NAS score and liver fibrosis were significantly correlated with CP (r=0.173, P=0.04 and r=0.198, P=0.02) and FRS (r=0.208, P=0.01 and r=0.164, P=0.05). Only ballooning was correlated with C-IMT (r=0.273, P=0.01). In multivariate logistic regression, NAS score was associated with CP (p=0.02), independent of age, LDL, diabetes, high blood pressure and tobacco.

Conclusion: In NAFLD histological severity is an independent predictor of early atherosclerotic lesions and 10-year CV risk and should prompt screening for early atherosclerosis.

Acknowledgements: Funding from the FP7/2007–2013 under grant agreement no. Health-F2-2009-241762, for the FLIP project.

1355 HISTOLOGICAL SEVERITY INDEPENDENTLY PREDICTS EARLY ATHEROSCLEROTIC LESIONS AND 10 YEAR CARDIOVASCULAR RISK IN NAFLD PATIENTS

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Background: Patients with NAFLD are at higher risk of atherosclerosis but the relationship between early atherosclerotic lesions and histological severity of NAFLD is uncertain.

Aim: To evaluate the relationship between liver histology, early atherosclerotic lesions and the 10 year Framingham risk score in patients with histologically defined NAFLD.

Method: This multicentric study enrolled consecutive patients with available liver biopsy (LB) and carotid ultrasound (CUS) with
was independently associated with C-IMT (p < 0.0001) and CP (beta = 0.179, p = 0.01), independent of age, cholesterol level, presence of diabetes or high blood pressure.

Conclusions: NAFLD increases the risk of early atherosclerotic lesions independent of established CV risk factors. NAFLD is an independent predictor of 10-year CV risk.

Acknowledgements: Funding from the FP7/2007–2013 project under grant agreement no. Health-F2-2009-241762, FLIP project.

1357 MAY HEMOGLOBIN BE A MEDIATOR OF INCREASED CARDIOVASCULAR RISK IN NAFLD?

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Background: NAFLD is associated with a significantly increased risk of adverse vascular events; the exact mechanisms underlying these associations have been only partially elucidated. Hemoglobin (Hb) concentration could affect the cardiovascular system through oxygen supply and blood viscosity. Several recent epidemiological studies have found positive associations of Hb with incident cardiovascular events.

Aim: To investigate the association of Hb concentration with the 10-years risk of cardiovascular events in patients with NAFLD.

Methods: A total of 117 patients (mean age: 49.66 ± 9.4 years, 63/117 – 54% females) who had NAFLD were included in the study. The 10-years risk of cardiovascular events was calculated according to Framingham equation. The risk score were categorized using predefined cutoffs as intermediate (≤20%) and high (>20%). Hemoglobin concentration was divided into age- and sex-dependent quintiles to evaluate the association of Hemoglobin concentration with CVD risk factors and clinical covariates including age, sex, systolic and diastolic BP, LDL and HDL cholesterol, serum triglycerides, diabetes, body mass index (BMI), and smoking status. We used multiple-adjusted Cox proportional hazards regression models to assess the effect of hemoglobin concentration on CVD risk factors.

Results: In higher hemoglobin level quartiles (all patients had hemoglobin level more than 152g/L) were younger (p < 0.001), had elevated lipids (p < 0.05), and liver enzymes (ALT, ASAT) (p < 0.001), but were not more likely to have hypertension and diabetes mellitus or higher BMI (p > 0.05). After adjusting for known CVD risk factors, compared with the lowest quintiles (Hb less or equal than 128.5 g/L) with the highest quintile (Hb more than 152 g/L), odds ratios (95% confidence intervals) was 3.45 (1.12–10.7). Hemoglobin concentration was independently associated with C-IMT (p < 0.001), had elevated lipids (p < 0.05). After adjusting for known CVD risk factors, compared with the lowest quintiles (Hb less or equal than 128.5 g/L) with the highest quintile (Hb more than 152 g/L), odds ratios (95% confidence intervals) was 3.45 (1.12–10.7).

Conclusion: Elevated hemoglobin level (more than 152 g/L) influence cardiovascular risk profile in patient with NAFLD. Measurement of hemoglobin level may be useful in predicting cardiovascular risk in patient with NAFLD.

1358 SERUM HEPcidIN LEVELS IN PATIENTS WITH CHRONIC LIVER DISEASES

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Introduction: Chronic liver diseases, especially alcoholic fatty liver disease (AFLD), nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis C (CHC), are frequently associated with iron overload. Hepcidin is the iron-regulatory hormone, which is synthesized mainly in the liver and plays an important role in iron homeostasis.

Aim: The aim of this study was to assess the serum levels of hepcidin and to evaluate its relationships with other parameters of iron metabolism in patients with various chronic liver diseases.

Material and Methods: A total of 186 patients with chronic liver disease (CLD), divided into six groups were studied (115 – male, 71 – female; mean age 50.41 ± 12.85 y), and 60 healthy controls were studied. Laboratory parameters of liver function and indices of iron metabolism were evaluated. The presence of iron deposition and the histological grades of steatosis and inflammation, and stage of fibrosis were also evaluated in patients with CLD. The serum level of hepcidin was determined by ELISA test/DRG International Inc. (USA).

Results: Hepcidin was significantly lower in the whole group of patients with CLD (82.9 ± 40.74 ng/ml) compared to the controls (99.14 ± 32.94 ng/ml, p = 0.005). According to the type of liver disease, decreased serum levels of hepcidin were found significantly more frequently in AFLD, NAFLD and CHC in comparison with controls (p = 0.02, p = 0.001 and p = 0.01 respectively), CHB (p = 0.034, p = 0.003 and p = 0.023 respectively) and chronic autoimmune hepatitis and primary biliary cirrhosis (p = 0.023, p = 0.005 and p = 0.022 respectively), with no difference when compared them. There was a reverse relationship between the levels of hepcidin by one hand and the values of iron, ferritin, liver enzymes, some of the parameters of liver function, as well as with degree of the deposition of iron in the liver and severity of steatosis, inflammation and fibrosis (0.01–0.001).

In conclusion, our results show decreased serum levels of hepcidin in patients with nonalcoholic and alcoholic fatty liver diseases, and chronic hepatitis C. The relationships between the parameters of iron metabolism and the severity of liver disease prove the importance of serum hepcidin as a surrogate marker for evaluation of iron overload in patients with chronic liver diseases.

1359 ASSOCIATION BETWEEN LIVER FIBROSIS AND DECREASED APOliprotein-A1 IN A 7-YEARS PROSPECTIVE STUDY. COULD IT EXPLAIN CARDIOVASCULAR-RELATED RISK WITH FIBROSIS PROGRESSION IN TYPE-2 DIABETIC PATIENTS?

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Background: Nonalcoholic fatty liver disease (NAFLD) is frequent in type-2 diabetic (TD2) patients, being associated with more cardiovascular complications (CVC) and liver fibrosis progression. Advanced fibrosis presumed by Fibrotest (FT) was associated with more CVC [1]. Apolipoprotein-A1 (ApoA1) is combined with other markers in FT as the serum level decreases with progression of liver fibrosis; ApoA1 is also the primary protein constituent of HDL-cholesterol, a protective factor against CVC.

Aims were:
1. To evaluate the incidence of CVC according to fibrosis progression
2. To identify the role of ApoA1 in the association of progression of liver fibrosis and CVC.

Methods: TD2-patients without history of liver disease were prospectively followed between 2004–2012 for CVC (myocardial infarction, unstable angina, coronary-bypass, ischemic stroke). CVC-risk was assessed at baseline with the Framingham-risk score (FRS). Liver fibrosis was presumed by FT (Biopredictive), at baseline and
at the end of follow-up (EOF) concomitant with liver stiffness measurements (LSM) by Fibroscan (Echosens). Advanced fibrosis (AF, F2F3F4-META/VIR) was defined by F2>0.48 and LSM>7.1 kPa.

**Results:** 76 TD2-patients without baseline AF were included [55% males, age=58yrs, BMI=29.7Kg/m², median (range) alcohol=0 (0–230) g/day, median follow-up=7 yrs]. There was a significant fibrosis progression as per FT (mean±SD) from baseline to EOF (0.15±0.10 vs 0.25±0.18, p=0.001). 9/76 (12%) TD2-patients progressed to AF [confirmed by LSM in 8/9 (89%)] and 13/76 (18%) developed CVC (20 coronary-heart disease and 1 stroke). Framingham-risk score was similar in TD2-patients who developed or not CVC (10.3vs9.9; p=0.63). TD2-Patients that progressed to AF as per FT presented higher ALT transaminases (48vs22U/L, p<0.01) and lower ApoA1 (1.30vs1.46; p=0.03). Despite similar FRS (9.9vs11.5%, p=0.74), TD2-patients which progressed to AF had higher incidence of CVC (44.4%vs13.4%, p=0.02). In TD2-patients without AF at EOF, similar to baseline ApoA1 serum levels were observed at EOF either in 63 TD2-patients without CVC (1.53vs1.52, p=0.76) or in 9 TD2-patients with CVC (1.41vs1.39, p=0.98).

**Conclusion:** During 7-years prospective follow-up of TD2-patients without advanced fibrosis, the incidence of CVC was 18% with 12% progression to advanced fibrosis. Fibrosis progression was associated with higher incidence of CVC. Lower serum ApoA1 levels that occurred with fibrosis may decrease HDL-cholesterol that could increase the CVC incidence.

Acknowledgements: This research received funding from the European Union Seventh-Framework-Programme (FP7/2007–2013) under grant agreement no. Health-F2–2009–241762, for the project-FLIP.

Reference(s)
[1] Perazzo H, Ngo Y, Munteanu M, et al. Hepatology 2012; 56 (Suppl S1): 4.061, and PNPLA3 GG genotype (OR 2.679) were independently (p=0.02). Multivariate logistic regression analysis showed that older age (OR 1.102), female gender (OR 2.476), type 2 diabetes (OR 4.061), and PNPLA3 GG genotype (OR 2.679) were independently linked to the presence of carotid plaques. In patients aged <50 years, 8/77 cases with PNPLA3 GG genotype (10.9%) had carotid plaques, vs. 6/15 (40%) with PNPLA3 GG genotype (p=0.02). By contrast, in patients ≥50 years the prevalence of carotid plaques was similar in subjects with PNPLA3 CC/GG and GG genotype (33/55, 60% vs. 12/19, 63%; p=0.75). Similarly, PNPLA3 CC/GG cases aged <50 had a lower IMT than PNPLA3 GG patients (0.75±0.17 vs. 0.85±0.25 mm; p=0.05), while this difference was not observed in patients aged ≥50 (0.93±0.25 vs. 1.00±0.26 mm; p=0.32).

**Conclusion:** PNPLA3 GG genotype is associated with a high risk of early carotid atherosclerosis in NAFLD.

1361 LACK OF ASSOCIATION BETWEEN METABOLIC SYNDROME AND NON-ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH PNPLA3 M148M GENE VARIANT

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**Background and Aims:** Patatin-Like phospholipase domain-containing 3 (PNPLA3) 148M variant has been reported to be associated with non-alcoholic fatty liver disease (NAFLD) and with a more severe liver damage. A strong association between NAFLD and metabolic syndrome has been widely reported. Aim of the study was to assess whether PNPLA3 variant may affect clinical phenotype in a large series of patients with NAFLD.

**Methods:** NAFLD was defined by ultrasonographic Hamaguchi’s criteria in 211 subjects with no history of alcohol abuse and chronic liver disease. Patients were genotyped for PNPLA3 148M variant and metabolic syndrome was defined according to the ATPII criteria.

**Results:** PNPLA3 genotype frequencies were II= 45.3%, IM=40.5%, MM=14.2% and the overall prevalence of MS was 68%. MS was more frequently observed in PNPLA3 wild type allele carriers (II=71.6% vs IM=72.1% vs MM=50% [p=0.024]). Odds ratio for MS was 3.3 times lower in MM carriers as compared to IM and II alleles carriers. Median waist circumference (cm) and serum triglycerides (mg/dl) were higher in I allele carriers [110 (103.5/118) vs 105 (101/113.5) vs 106 (96.7/118.5), p=0.065 and 157 (112/193) vs 141 (107.7/185) vs 111.5 (90.7/148.7), p=0.006, respectively], while mean ALT (UI) was lower in M carriers (26 (19/35) vs 30.5 (22/45) vs 30 (22.7/40.5) [p=0.014]). MM carriers had lower median HOMA-IR and higher median HDL-C (mg/dl) compared to wild-type, although not statistically significant level (2.8 vs 3.6 and 50.5 vs 45, respectively). Framingham cardiovascular risk score was significantly higher in II vs MM (9% vs 4% p=0.024 respectively). Body mass index, blood pressure and other biochemical parameters did not differ across genotypes. Fatty liver index sensibility was significantly (p=0.026) lower in MM carriers as compared to wild type (67% vs 86%). Moderate-severe steatosis (Hamaguchi scores 3–6) was more prevalent in MM carriers.

**Conclusions:** PNPLA3 M genotype is associated with a more severe liver disease, but with a lower prevalence of metabolic syndrome and reduced cardiometabolic risk.

1362 RISK OF OBSTRUCTIVE SLEEP APNEA SYNDROME AND ASSOCIATION WITH LIVER DAMAGE IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE WITHOUT SEVERE OBESITY

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**Background and Aims:** A high prevalence of obstructive sleep apnea syndrome (OSAS) has been reported in severely obese
patients with nonalcoholic fatty liver disease (NAFLD), an emerging liver disease associated with obesity and the metabolic syndrome, and it has been postulated that intermittent hypoxia is a cofactor of liver damage progression. Few studies have evaluated the relationship between NAFLD and OSAS in non severely obese patients, and none analyzed liver damage by histology, the gold standard for prognostic assessment. Aim of this study was to determine the risk of OSAS in patients with NAFLD without severe obesity and to evaluate the association with liver damage.

Methods: 85 consecutive patients with histologically proven NAFLD and body mass index (BMI) <35 kg/m². The risk of OSAS was assessed by the Berlin Questionnaire (BQ) and Sleepness Epworth Scale (ESS). Liver damage was evaluated according to Kleiner score.

Results: BQ was positive in 28 (35%), ESS in 11 (13%), and both in 9 (11%) of patients. In patients at high risk of OSAS (as identified by positivity of both BQ and ESS) we observed:

1. A higher prevalence of nonalcoholic steatohapatitis (NASH, the progressive form of NAFLD: 8/9, 89% vs. 2/55, 40%, p = 0.009).
2. A higher severity of NAFLD activity score, reflecting histological liver damage (p = 0.002).
3. C. of hepatic fibrosis (p = 0.015).

Vice versa, a higher prevalence of BQ and ESS positivity was observed in patients with than in those without NASH (8/31, 26% vs. 1/45 2%, p = 0.009). At multivariative logistic regression analysis, BQ and ESS positivity was a risk factor of NASH independently of known clinical confounders, such as BMI and glucose levels (OR 7.46, 95% confidence interval 1.2–146, p = 0.03).

Conclusions: A high proportion of NAFLD patients without severe obesity is at risk of OSAS. High risk of OSAS, as detected by BQ and ESS combined positivity, is independently associated with NAS. Although the association between OSAS and NAS awaits confirmation by polysomnography, data suggest that OSAS may be involved in the pathogenesis of liver disease progression in NAFLD patients without severe obesity, with potential therapeutic implications.

Results: A non-significant reduction in global microbial diversity was observed with increasing histological severity. Twenty-one MGS of interest were identified. A naïve predictor based on 5 MGS showed decreased abundance with histological severity and identified patients with FL with an AUROC of 0.80. High abundance/low abundance cutoffs were determined for some of the MGS. Three MGS identified FL with 83% sensitivity and 64% specificity. Finally, two MGS (digitized as very high/low levels) had 45% sensitivity and 100% specificity for the identification of FL. All metagenomic analyses were repeated 3 months later in 16 patients. The global gene count, diversity and MGS identification were similar to that of the first sample, with a high degree of correlation. Moreover, the naïve predictor issued from the first analysis had an AUROC of 0.81 for the identification of FL on the second sample, showing that the individual metagenotype is a reliable and reproducible trait.

Conclusion: A small number of discriminant MGS identified with high accuracy patients with the most benign form of NAFLD. If confirmed by an ongoing multicentric study, this could translate into a non-invasive diagnostic test with considerable impact on NAFLD patient management.

1364
THE HOMA INDEX IS A USEFUL TOOL FOR RISK STRATIFICATION OF ADVANCED FIBROTIC DISEASE IN A LARGE CohORT OF EUROPEAN NAFLD PATIENTS
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Insulin resistance (IR) and NAFLD are closely linked but some patients have NAFLD despite low levels of IR, as measured by the HOMA index, a simple, surrogate, bed-side marker of IR.

Aim: To understand the relationship between HOMA levels and liver injury in a large population of European NAFLD patients, in particular the risk of advanced disease in those with low HOMA score.

Methods: Patients with suspected NAFLD based on: ultrasonographic steatosis, increased ALT or metabolic risk factors, with daily alcohol <50 g and no other hepatopathy underwent liver biopsy within the FLIP cohort.

Results: 493 patients were included. 65% tee-totalers, 10% former drinkers and 25% current drinkers (median 10 g/day). Median HOMA was 3.2 (25th percentile: 1.97; 75th percentile: 5.37). HOMA was strongly associated with age, BMI, waist circumference, ALT, AST but not with gender, TG, HDL or GGT, alcohol, coffee or soft drinks consumption. HOMA correlated with the amount of steatosis (r = 0.35, p < 0.001), the NAS score (r = 0.34, p < 0.001), ballooning (r = 0.18, p < 0.005), lobular inflammation (r = 0.15, p < 0.001) and fibrosis (r = 0.34, p < 0.001). 126 patients (26%) had a HOMA score <2. They were younger (45 vs. 51 yrs), had lower BMI (26.6 vs. 30.4 kg/m²), waist circumference (94 vs. 103 cm) and ALT (54 vs. 63 IU/L) than those with HOMA≥2 (p < 0.001 for all). Although most HOMA <2 patients were overweight (75%), had increased ALT (85%) and displayed steatohepatitis (60%), the prevalence of advanced disease (bridging fibrosis or cirrhosis) was very low: 7.8% vs. 27.4% in patients with HOMA≥2 (p < 0.001) corresponding to a risk reduction of 4.45 [95% CI 2.2–9.2]. In multivariate analysis, HOMA predicted
advanced disease (β = 0.12, p = 0.001) independent of age (β = 0.04, p = 0.004), gender (β = 0.58, p < 0.04), alcohol consumption (p = 0.27) and waist circumference (p = 0.34). In patients younger than 40 with HOMA <2, advanced disease was exceedingly rare (1/44, 2.3%).

**Conclusion:** In a large European population of NAFLD patients, IR was strongly associated with hepatic inflammation and fibrosis. Low levels of IR (HOMA <2) are associated with reduced risk of advanced disease, especially in younger patients, even if overweight, with high ALT and steatohepatitis.

### 1365 METABOLIC ALTERATIONS OF LIPID KINETICS IN NAFLD PATIENTS AND THEIR CONTRIBUTION TO LIVER DAMAGE

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**Background and Aims:** It has been hypothesized that excessive lipids availability contributes to the necro-inflammatory processes and to fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). The purpose of this study was to evaluate the impact of whole body lipolysis, circulating NEFA levels/composition and insulin resistance (either basically and after a fat challenge) in the pathogenesis of liver damage in NAFLD.

**Methods:** [2H5]glycerol kinetics and plasma levels of Fatty acids (FA), triglycerides (TG) and VLDL-TG, saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) were determined in 15 non-obese, normolipidaemic, non-diabetic patients with biopsy-proven NAFLD and 6 control subjects during the basal state and after an oral fat load (200ml dairy cream and egg yolk). FA composition was assessed by high performance liquid chromatography. Total and incremental post-load area under the curve (AUC and IAUC) of lipids and hormones were calculated. Whole body lipolysis and Adipose tissue IR index were derived from glycerol Ra.

**Results:** In NAFLD patients post-load TG, VLDDL-TG and FA plasma concentrations were increased at all curve time-points (TG p = 0.029, VLDDL-TG p = 0.038 and FA p = 0.036; NAFLD vs controls). Similarly, the corresponding IAUCs resulted increased 2-fold (p = 0.024 for TG and VLDDL-TG, p = 0.022 for NEFA). NAFLD patients had similar levels of SFA, MUFA and PUFA in the basal state, but increased SFA (lauric and myristic acids, p = 0.009 and 0.031) and MUFA (oleic acid, p = 0.023) after load. Basal glycerol Ra and Adipo-IR were significantly increased (p < 0.03 and p < 0.001 respectively vs CT). Among all parameters, higher BMI (p = 0.007), IAUC insulin (p = 0.038), IAUC C-peptide (p = 0.013) and Adipo-IR (p = 0.033 for basal and 0.007 for post-load) were significantly associated with severe fibrosis. However, only BMI (p = 0.05), IAUC insulin (p = 0.05) and IAUC C-peptide (p = 0.027) were associated with a NAS score ≥5.

**Conclusions:** NAFLD patients present multiple qualitative and quantitative alterations in lipid metabolism even in the absence of overt metabolic derangements. Adipose tissue insulin resistance is one of the main determinants of liver fibrosis, but an important contribution is provided by insulin levels and secretion per se.

Funding from the EU’s Seventh Framework Programme (FP7/2007–2013) under grant agreement no. HEALTH-F2–2009–241762 for the project FLIP.

### 1366 BENEFICIAL INFLUENCE OF POLYUNSATURATED PHOSPHATIDYLCHOLINE ENHANCES FUNCTIONAL LIVER CONDITION AND LIVER STRUCTURE IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS. RESULTS OF PROLONGED RANDOMIZED BLINDED PROSPECTIVE CLINICAL STUDY

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From 1998 to 2012 in our prolonged randomized prospective, blinded clinical trial we studied the effect of polysaturated phosphatidylcholine – PUPC (Essential forte N, produced by A. Nattermann & Cie.GmbH) in diabetic patients with NASH. 215 patients with type 2 diabetes of not-complicated course, which was adequately controlled by diet and metformin intake, were recruited for the study. All patients followed the basic treatment scheme included dietary and physical regimen and metformin, daily dosage – 1000 mg. 178 patients of investigational group (IG) additionally were treated by PUPC, daily dosage contained 1368 mg Phosphatidilcholine. Altogether 152 (85.4%) patients of IG and 37 patients of Control Group (CG) were available for follow up. In a subsequent for the remaining patients in the study during above seven years (long-term investigational group (LIG) 114–64.0%), liver function markers and ultrasound studies were measured at least twice per year. A significant reduction of all the liver enzymes was observed after treatment by PUPC, viz. baseline vs. six months after treatment: ALT: 56.5 ± 28.6 IU/L vs. 35.2 ± 18.4 IU/L, p = 0.02, AST: 39.0 ± 9.0 IU/L vs. 26.5 ± 7.2 IU/L, p = 0.04, GGT: 38.2 ± 11.4 IU/L vs. 27.5 ± 8.6 IU/L, p = 0.03). Ultrasound studies were performed on the basis of liver attenuation value assessment and revealed the hepatic echo-texture had become significantly improved after PUPC treatment in 101/152 (66.4%) of patients (p = 0.02), while there was no change in 7/152 (4.6%) individuals. In the group of patients LIG further dynamics of the liver enzymes had no statistically significant differences from CG, but sonograms signs of fatty liver statistically decreased in 93/114 (81.6%) and became more effective control of diabetes in 98/114 (86.0%) patients (significant reduction in HbA1c). The results of liver biopsy (histological examination) and Fibromax test showed, that in patients with NAFLD additionally treated by PUPC, the progress of hepatic fibrosis was significantly slowed then in CG (Fibromax test result: F2 vs. F3) (p = 0.03). In addition after of treatment we found significant increase of steatosis in long-term CG, and its reduction in LIG (p = 0.02).

The study results suggest that polysaturated phosphatidilcholine improves liver function and structures, also help glucose control in diabetic sufferers with NASH.

### 1367 INCREASED GUT PERMEABILITY IN METABOLIC SYNDROME IS NOT ASSOCIATED WITH CHANGES IN BILE ACID PROFILE

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**Background:** High fat diet can lead to metabolic syndrome (MetS) and is associated with changes in gut microbiota. Gut microbiota composition impacts on gut permeability and bile acid composition. The aim of this study was to investigate whether patients with MetS show increased gut permeability, whether this is associated with changes in serum bile acid composition and whether consumption of a probiotic affects gut permeability and serum bile acids.

**Methods:** Patients with MetS were randomized to receive trice daily 6.5x10⁹ CFU Lactobacillus casei Shirota (LcS) (probiotic group)
or not (standard group) for 3 months. Gut permeability was assessed by differential sugar absorption and by diaminooxidase (DAO) serum levels. Serum bile acids (cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid, ursodeoxycholic acid) were determined as unconjugated acids, taurine and glycine conjugates using tandem mass spectrometry.

**Results:** Twenty-eight MetS patients were included in the intervention study and 11 healthy subjects served as controls luced. Gut permeability (Saccharose excretion, Lactulose:Mannitol ratio and DAO serum levels) was significantly increased in MetS compared to controls but did not differ between patient groups (Table 1). No difference in individual serum bile acids or total bile acids was found between controls and patients with MetS (Table 1). Neither gut permeability nor bile acids changed after consumption of a milk drink over three months. Bile acids did not correlate with markers of gut permeability.

**Discussion:** Gut permeability of MetS patients was significantly increased compared to healthy controls. No association between bile acid levels and gut permeability was found. Lcs administration in the MetS patients had no impact on gut permeability or the bile acid profile possibly due to too short study duration or underdosing.

### Table 1

<table>
<thead>
<tr>
<th>Baseline</th>
<th>3 Months</th>
<th>Control</th>
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<tbody>
<tr>
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</tbody>
</table>

**Conclusions:** PIINP discriminates between SS and NASH. The performance of PIINP at the proposed diagnostic thresholds is comparable to that reported in the original publication of this biomarker. Our results suggest that PIINP can be used to detect the minority of patients with NAFLD who have NASH and are at risk of developing progressive fibrosis.

**Reference(s)**


## 1369 FOURTH VALIDATION OF TERMINAL PEPTIDE OF PROCOLLAGEN III (PIINP) FOR THE DETECTION AND ASSESSMENT OF NONALCOHOLIC STEATOHEPATITIS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

**Introduction:** PIINP has recently been shown to discriminate between simple steatosis (SS) and NASH both in patients without advanced fibrosis and in patients with all degrees of fibrosis. In this study we validated PIINP as a biomarker of NASH in a cohort of patients with biopsy proven NAFLD and evaluated its performance at the proposed diagnostic thresholds.

**Methods:** 71 patients with NAFLD and no evidence of other liver disease were included in this study. Liver biopsies were performed on all patients and analysed by a expert liver histopathologist. All liver biopsies were of suitable size for analysis (>12mm and >5 portal tracts) and classified in a dichotomous manner into those with SS or histological NASH. Fibrosis was assessed using the Scheuer classification. Serum samples were taken at the time of liver biopsy.

**Results:** 14 of the 60 patients with non-advanced fibrosis (14 of 148, F1–F2) and all 11 patients with advanced fibrosis (9–F3, 2–F4) had NASH respectively. The AUROC of PIINP in discriminating between SS and NASH in patients with non-advanced fibrosis and all degrees of fibrosis was 0.81 (CI 0.69–0.94) and 0.87 (CI 0.79–0.96) respectively. In comparison, the ability of ALT to discriminate between SS and NASH ranged between 0.43–0.45. Furthermore, amongst patients with non-advanced fibrosis, PIINP discriminated poorly between fibrosis stages (AUROC 0.55–0.56).

**Table: Performance of PIINP in the diagnosis of NASH**

<table>
<thead>
<tr>
<th>PIINP Threshold ng/ml</th>
<th>F0–2: NPV/PPV %</th>
<th>F0–4: NPV/PPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>92/42</td>
<td>88/56</td>
</tr>
<tr>
<td>7.2</td>
<td>85/68</td>
<td>83/85</td>
</tr>
<tr>
<td>11</td>
<td>77/100</td>
<td>72/100</td>
</tr>
</tbody>
</table>

**Figure:** Boxplots of PIINP with respect to SS & NASH.

**Conclusions:** PIINP discriminates between SS and NASH. The performance of PIINP at the proposed diagnostic thresholds is comparable to that reported in the original publication of this biomarker. Our results suggest that PIINP can be used to detect the minority of patients with NAFLD who have NASH and are at risk of developing progressive fibrosis.

**Reference(s)**


## 1369 HIGH-INTENSITY INTERMITTENT EXERCISE THERAPY REDUCES LIVER FAT AND IMPROVES BODY COMPOSITION IN ADULTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

**Aims:** Evaluate a minimally supervised high-intensity intermittent training programme as a therapeutic option in adults with NAFLD.

**Methods:** Following safety screening by ECG monitored graded maximal exercise test, 20 adults with NAFLD were assigned to either twelve weeks of standard care (controls) or high-intensity intermittent training (HIIT) (mean±SD): controls; n=8, 49.8±10.2 years, BMI 31.1±5.1 kg/m²; and HIIT n=12, 52.9±9.6 years, BMI 30.3±4.3 kg/m². The HIIT group exercised at their local fitness facility on cycle ergometers for 30–40 minutes three times per week to a pre-recorded interval training programme delivered via mp3 players. Glucose tolerance was assessed by oral glucose tolerance test, liver fat by proton-magnetic resonance spectroscopy, and body composition by air displacement plethysmography. Comparisons were done for changes between groups by analysis of covariance.

**Results:** Liver fat, total body fat mass, fasting plasma triacylglycerols, alanine aminotransferase, and aspartate aminotransferase declined and lean body mass increased in the HIIT groups vs.

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controls from 10.9±6.6% to 6.6±2.7% vs. 10.1±4.4% to 10.0±3.8% (p < 0.05), 32.8±8.0 kg to 31.2±8.1 kg vs. 31.0±9.9 kg to 31.5±10.3 kg (p < 0.05), 1.8±0.6 mmol/L to 1.6±0.6 mmol/L vs. 1.5±0.6 mmol/L to 1.8±0.9 mmol/L (p < 0.05), 59±30 IU/L to 44±19 IU/L vs. 48±25 IU/L to 53±28 IU/L (p < 0.05), 39±18 IU/L to 33±15 IU/L vs. 31±9 IU/L to 35±13 IU/L (p < 0.05), and 53.9±9.8 kg to 54.9±10.8 kg vs. 59.0±6.9 kg to 58.4±8.0 kg (p < 0.05), respectively. Total bodyweight as well as fasting and 2-hour glucose remained unchanged in both groups.

Conclusions: High-intensity intermittent training three times per week represents a time efficient and effective exercise strategy for reducing liver fat and fasting plasma triacylglycerols, and improving body composition in adults with NAFLD.

1371 HIGH-FIELD MR-SPECTROSCOPY IN PATIENTS WITH NAFLD ALLOWS NOVEL INSIGHTS IN ALTERED HEPATIC LIPID AND ENERGY METABOLISM WITH POTENTIAL DISTINCTION OF NASH AND ADVANCED FIBROSIS

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Background and Aims: Liver biopsy is still the established method for distinction between non-alcoholic fatty liver (NAFL) and steatohepatitis (NASH). We aimed to evaluate magnetic-resonance spectroscopy (MRS) as non-invasive tool permitting novel mechanistic and pathogenetic insights into alterations of hepatocellular metabolism in NAFLD. We therefore quantified hepatocellular lipid content (HCL) by 3.0-T 1H-MRS and 31P metabolites by 7.0-T 31P-MRS to assess whether MRS permits a non-invasive distinction between NAFL, NASH and different fibrosis stages.

Methods: MRS was obtained before liver biopsy in suspected NAFLD/NASH and data were correlated with histology (Kleiner/NAS). Cut-off levels for hepatocellular biopsies (5, 33 and 66%) and 1H-MRS signals (3.1, 5.0 and 6.9%) were established to determine phosphomonoester (PME), phosphoethanolamine, phosphocholine, phosphodiester (PDE), glycerophosphocholine (GPC), glycerophosphoryl-ethanolamine, phosphocreatine (PCr), NADPH, inorganic phosphate (Pi), α-, β- and γ-ATP levels.

Results: Among the investigated students, 127 were normal weight (18.5±BMI<25) and 134 were overweight (25±BMI<30). Among the students overall, NAFLD (39.1%), insulin resistance (36.0%), and hypertension (30.7%) were the main abnormal findings. Liver stiffness determined by ARFI imaging was negatively correlated with the degree of NAFLD diagnosed by ultrasonography (r=−0.522, P < 0.001). ROC analysis showed that the SWV cut-off value for NAFLD was 1.13 (95% CI, 0.75–0.86) with a sensitivity of 76% and a specificity of 73% for separating NAFLD (≥1.13) from non-NAFLD (<1.13). The systolic and diastolic blood pressures were significantly higher in the NAFLD group than in the non-NAFLD group (P < 0.001 and P < 0.001, respectively). Moreover, among the metabolic risk factors, multivariate logistic regression analysis revealed that male gender (OR, 5.09; 95% CI, 2.4–10.6; P < 0.001), decreased SWV (OR, 0.399; 95% CI, 0.3–0.5; P < 0.001) and HOMA-IR (OR, 1.39; 95% CI, 1.0–1.9; P < 0.03) were significantly associated with the presence of hypertension.

Conclusions: In university freshmen, liver stiffness determined by ARFI imaging is considered to be a more sensitive index of minimal to mild liver fat deposition and male gender and decreased liver stiffness are independent risk factors for the presence of hypertension.

Conclusions: MRS signals obtained by high magnetic fields strongly correlate with histological grades of steatosis. In vivo 7.0-T 31P-MRS shows promising results for distinction between histologically verified NAFL and NASH as well as between different degrees of fibrosis indicating alterations in the cell membrane configuration. Altered ATP and NADPH levels may show a modified hepatic energy metabolism in fibrosis. Non-invasive monitoring of NAFLD patients appears feasible but further validation is required.

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Background and Aim: NAFLD (Non-Alcoholic Fatty Liver Disease) is a condition not directly attributable to specific factors. Unhealthy lifestyle and obesity in non-diabetics and without alcohol abuse are commonly considered the most probable causes, even if also lean individuals can be involved. An association of NAFLD with coronary artery disease is currently recognized, but the relationship, if any, with the severity of steatosis and the features of heart function impairment is not yet defined. We aim to challenge if severity of liver steatosis can predict the impairment of heart function in non-diabetic patients.

Patients and Methods: 165 NAFLD and 263 non-NAFLD subjects (total 428, f 273, m 155, BMI 26.76±5.39; age 47.20±12.33), comparable for age and body weight, were studied. The severity of liver steatosis was assessed by UltraSound Bright Liver Score (BLS); heart function was assessed by echocardiography as Ejection Fraction (EF); diastolic relaxation, transmitral E/A Doppler ratio and left ventricular myocardial mass (LVMM). Age, obesity, dietary profile (assessed as Adherence to Mediterranean Diet Score, i.e. AMDS) and insulin resistance (assessed by HOMA) were all taken into account for their possible confounding and additive effect.

Results: By odds ratio NAFLD, Older Age, overweight—greater BMI and insulin resistance were associated with a greater hazard of lower EF and E/A (Fig. 1) and greater LVMM (Fig. 2). Multiple Linear Regression Models, age and gender balanced for reducing these confounding factors, confirm the predictivity (p<0.0001) of NAFLD severity, assessed by BLS, toward lower EF, independent by BMI, AMDS and HOMA and with a variance of 8.1% (Table 1). A linear correlation was observed between BLS and EF (r=−0.183; p<0.0001).

Conclusion: Lower systolic heart function assessed by echocardiography is associated with the severity of NAFLD and unhealthy dietary profile also in lean non-diabetic subjects. NAFLD is a factor that increase also the hazard of lower left ventricular distensibility, assessed as E/A ratio, and of LV hypertrophy, assessed as LVMM by echocardiography.

Table 1. Multiple Linear Regression to EF%

<table>
<thead>
<tr>
<th>Predictors</th>
<th>R</th>
<th>R²</th>
<th>F</th>
<th>Sig.</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.285</td>
<td>0.081</td>
<td>6.212</td>
<td>&lt;0.0001</td>
<td>−0.356</td>
<td>0.002</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.149</td>
<td>0.022</td>
<td>1.578</td>
<td>0.214</td>
<td>−0.232</td>
<td>0.018</td>
</tr>
<tr>
<td>BLS</td>
<td>0.129</td>
<td>0.016</td>
<td>0.401</td>
<td>0.529</td>
<td>−0.444</td>
<td>0.009</td>
</tr>
<tr>
<td>HDL</td>
<td>0.224</td>
<td>0.049</td>
<td>2.298</td>
<td>0.131</td>
<td>0.155</td>
<td>0.004</td>
</tr>
<tr>
<td>AMDS</td>
<td>0.268</td>
<td>0.072</td>
<td>3.934</td>
<td>0.047</td>
<td>−0.638</td>
<td>0.023</td>
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<tr>
<td>Triglycerides</td>
<td>0.098</td>
<td>0.009</td>
<td>0.067</td>
<td>0.794</td>
<td>0.098</td>
<td>0.063</td>
</tr>
</tbody>
</table>

BMI: Body Mass Index; BLS: Bright Liver Score, HDL: High Density Lipoprotein; AMDS: Adherence to Mediterranean Diet Score.

FIGURE

Figure 1. Odds Ratio vs E/A.

Figure 2. Odds Ratio vs. LVMM.
normal liver enzymes in the treatment group, or the possibility to undergo phlebotomy in the controls. Nevertheless, in a per-protocol analysis of patients who completed the study, amelioration of histological damage was demonstrated in 7/11 (64%) phlebotomized patients vs. 1/8 (12%) controls (p = 0.026).

**Conclusions:** Phlebotomy is well tolerated in patients with NAFLD and increased iron stores, effectively reduces iron stores, and results in an improvement in ALT and AST levels. These preliminary results suggest that iron depletion may possibly improve liver damage in patients with NAFLD and increased iron stores.

### 1374

**ANA POSITIVITY IDENTIFIES A SUBGROUP OF NAFLD PATIENTS WITH DISTINCT METABOLIC PROFILE AND IMPAIRED HISTOLOGICAL RESPONSE TO WEIGHT LOSS**

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**Background:** Obesity is often accompanied by comorbidities including metabolic and liver disturbances. Antinuclear (ANA) and anti smooth muscle (ASMA) antibodies can be expressed in various clinical settings including liver diseases.

**Aim:** To evaluate the clinical significance of the expression of ANA and ASMA in the assessment of obese patients.

**Patients and Methods:** 522 obese patients were consecutively enrolled, with a one year follow up in 166 patients. Serum tests, including ANA and ASMA, glucose, insulin and c-peptide levels during oral glucose tolerance test (OGTT), ultrasound and fat measurement at CT scan were performed at baseline and at 12 months follow up. A liver biopsy was performed in 314 patients at baseline and in 86 at follow up.

**Results:** At baseline ANA+ patients presented significantly lower levels of glucose at 180’ (p = 0.023), insulin at 0’ (p = 0.017) and at 180’ (p = 0.039) and c-peptide at 0’ (p = 0.020) and at 180’ (p = 0.010) during OGTT when compared to ASMA+ and ANA−/ASMA− patients. At 12 months a significant decrease of BMI, NAS score and presence of histologically proven NASH was detected in all groups (p < 0.05), with a reduction of BMI significantly higher in ANA+ patients with respect to ANA+ patients (p = 0.006). The extent of BMI reduction was similar in ASMA+ and ASMA− patients. A significant decrease of glucose, c peptide and insulin levels during OGTT, ALT, GGT, grade of steatosis and lobular inflammation at histology was observed in ANA−, ASMA+ and ASMA− patients (p < 0.05) but not in ANA+ patients. Ballooning was significantly decreased in ANA− (p < 0.001) and in ASMA− (p < 0.001) patients but not in ANA+ and ASMA+ patients. Fat measurement at CT scan showed a significant reduction of both total and visceral fat in all groups (p < 0.05) except ANA+ where a significant reduction of total fat (p = 0.021) but not of visceral fat was observed.

**Conclusion:** In our obese population, ANA positivity seems to identify a subset of patients with a lower glucose metabolism disturbance at baseline and an impaired improvement of glucose metabolism, visceral fat and histological features of NAFLD/NASH at follow up, suggesting a different pathogenetic profile.

### 1375

**BARIATRIC SURGERY REDUCES ADIPOCYTE SIZE, IMPROVES LIVER INJURY AND COUNTERACTS LIPOTOXICITY VIA CHANGES IN SERUM FATTY ACID COMPOSITION AND ADIPOGENIC LEVELS**


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**Background and Aims:** Obesity is a growing worldwide pandemic and a risk factor for non-alcoholic fatty liver disease (NAFLD). Recently identified adipokines were found to promote the crosstalk of adipose tissue and the liver, which may play a pivotal role in the development of NAFLD. With the present study, we aimed to explore the interplay of adipose and liver tissue addressing the short-term effects of bariatric surgery.

**Patients and Methods:** Blood, visceral adipose tissue, and liver tissue samples were obtained from 160 morbidly obese patients undergoing bariatric surgery. Liver and adipose tissue were scored histopathologically. Blood samples taken before and 6 weeks after surgery were analyzed for routine parameters of liver injury and lipid metabolism. In a sub-cohort, the composition of free fatty acids and apolipoproteins were assessed at both time points.

**Results:** The 160 severely obese patients (34 male/126 female) had a mean age of 43 years (range 19–65) and mean BMI of 52kg/m² (35–78kg/m²). Adipocyte cell size significantly correlated with AST and ALT blood levels as well as steatosis, ballooning, and NAS score in liver biopsies. Furthermore, serum triglyceride and free fatty acid composition were correlated with liver injury. Bariatric surgery improved levels of stearic acid and dihomo-gamma-linolenic acid as early as 6 weeks after surgery. Several apolipoproteins (ApoAI, ApoAI, ApoCII) were significantly associated with serum and histological parameters of liver injury. Serum markers improved at 6 weeks after surgery, including serum triglycerides, ApoAI, and cell death markers (M30, M65). Plasma levels of adiponectin at baseline correlated with hepatic steatosis, ballooning and NAS-score. 6 weeks after surgery, adiponectin levels were significantly increased independent of the type of surgery.

**Conclusions:** Adipocyte cell size and serum levels of free fatty acids correlate with histological and serological markers of liver injury. Moreover, our findings reveal that bariatric surgery has beneficial effects on liver injury as soon as 6 weeks after the procedure which may be due to the observed changes in lipid metabolism and adipokine levels. Collectively, these findings underline a crosstalk between adipose tissue and the liver which could be therapeutically targeted by bariatric surgery.

### 1376

**GALLSTONE DISEASE IS NOT ASSOCIATED WITH LIVER FIBROSIS AND NONALCOHOLIC STEATOHEPATITIS IN NONALCOHOLIC FATTY LIVER DISEASE**


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**Background and Aims:** GD is the most common disorder of the gastrointestinal tract and it is strongly associated with metabolic...
risk factors. GD and nonalcoholic fatty liver disease (NAFLD) have a similar risk factor profile and frequently coexist. We sought to examine whether the presence of GD in patients with biopsy-proven NAFLD is associated with hepatic fibrosis and the histological nonalcoholic steatohepatitis (NASH) score.

Methods: This is a retrospective review of a prospective database of patients with biopsy-proven NAFLD enrolled from four different gastroenterology clinics in Turkey. A total of 441 Turkish patients were included in the analysis. GD was diagnosed in the presence of sonographic evidence of gallstones, echogenic material within the gallbladder with constant shadowing and little or no visualization of the gallbladder, or absence of gallbladder at ultrasonography, coupled with a history of cholecystectomy.

Results: Fifty-four patients of the 441 NAFLD patients (12.2%) had GD (GD+ subjects). Compared with GD− subjects, GD+ patients were older, had a higher BMI, and showed a higher prevalence of female subjects and the metabolic syndrome. However, GD+ patients did not have a higher risk of advanced fibrosis or definite NASH on liver histology. After adjustment for potential confounders, the prevalence of GD in NAFLD patients was not associated neither with severe fibrosis (≥2) (odds ratio [OR] = 1.06, 95% confidence interval [CI] = 0.53–2.21, p = 0.68) nor with definite NASH (OR = 1.03, 95% CI = 0.495–2.12, p = 0.84).

Conclusions: The results of this multicenter cross-sectional study conducted in Turkey indicated that patients with histology-proven NAFLD and GD were older, had a higher BMI, and showed a higher prevalence of female subjects and the MS compared with those without GD. However, we did not find any significant association of GD with neither liver fibrosis nor definite NASH both at univariate and multivariable analysis. The presence of GD is not independently associated with advanced liver fibrosis and definite NASH in adult patients with biopsy-proven NAFLD.

1377 HYPOTHYROIDISM IS NOT ASSOCIATED WITH SPECIFIC HISTOLOGICAL FEATURES OR SEVERITY OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Recent evidence derived from epidemiological studies has suggested that hypothyroidism can act as a risk factor for non-alcoholic fatty liver disease (NAFLD) as diagnosed by ultrasonography. However, it is currently uncertain whether there is a significant association between hypothyroidism and the severity of liver histology among patients with NAFLD. In this multicenter study, we assessed whether there is a significant relation between liver histology and hypothyroidism among patients with biopsy-proven NAFLD.

Methods: A total of 483 patients with NAFLD (263 males and 220 females, mean age, 45 ± 10 years) were recruited in this study. The NAFLD diagnosis was based on liver biopsy and exclusion of other known etiologic factors of chronic liver disease. An experienced pathologist blinded to clinical data scored the liver biopsies according to the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network scoring system. The diagnosis of hypothyroidism was based on a previous history of hypothyroidism (use of T4 replacement therapy) or according to the TSH value. In multivariable-adjusted linear logistic regression models, each histological feature of NAFLD was considered as the dependent variable.

Results: A total of 64 NAFLD patients (13%) had hypothyroidism. The distribution of subjects with hypothyroidism was not different in NAFLD patients classified according to liver histopathology (steatosis alone, borderline steatohepatitis, definite steatohepatitis). Notably, the presence of hypothyroidism was not associated with the degree of hepatic steatosis (P = 0.31), lobular inflammation (P = 0.52), hepatocyte ballooning (P = 0.74), portal inflammation (P = 0.33), fibrosis (P = 0.33), and the NASH score (0.48) among patients with NAFLD.

Conclusions: This study has shown for the first time that the histological severity of NAFLD is not independently predicted by hypothyroidism. Future follow-up studies are necessary to validate these findings and better estimate the risk of disease progression in relation to hypothyroidism among patients with biopsy-proven NAFLD.
Background and Aims: Non-alcoholic fatty liver disease (NAFLD) has been suggested to be a strong risk factor of colorectal benign adenomas and advanced neoplasms. The aim of this large case-control study was to further investigate the prevalence of colorectal malignant neoplasm (CRMN) in patients with NAFLD and determine whether association between NAFLD and CRMN exists.

Methods: 2315 community subjects (1370 males and 945 females) who underwent a routine colonoscopy according to international colorectal cancer screening guideline were recruited. Nature of colorectal lesions determined by biopsy and NAFLD was diagnosed by ultrasound. Binary logistic regression analysis was applied to explore the related associations.

Results: Prevalence of CRMN was 29.3% (77/263) in patients with NAFLD, which was significantly higher than 18.0% (369/2052) in the control group (P < 0.05). In addition, malignant neoplasm in NAFLD group occurred more frequently at sigmoid colon than in control group (14.3% vs. 11.9%). The incidence of highly-differentiated colorectal adenocarcinoma in NAFLD group was significantly higher than control group (62.3% vs. 9.8%). Univariate analysis showed that NAFLD had strong association with CRMN (OR, 2.043; 95% CI, 1.512–2.761; P < 0.05). After adjusting for metabolic and other confounding factors, NAFLD remained as an independent risk factor for CRMN (OR, 1.868; 95% CI, 1.360–2.567; P < 0.05).

Conclusion: NAFLD was an independent risk factor for CRMN. Sigmoid carcinoma and highly differentiated colorectal adenocarcinoma were more commonly found in NAFLD. (ClinicalTrials.gov number: NCT01657773).

13. GENETIC AND PAEDIATRIC LIVER DISEASES

1381

PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS IN ARABS

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Aim: To review the clinical and biochemical presentation, familial involvement and outcome of patients with progressive familial intrahepatic cholestasis (PFIC) in Arab children.

Methods: This is a retrospective, descriptive study for the referred patients to King Faisal Specialist Hospital and research center in Riyadh with cholestasis who were diagnosed to have PFIC based on clinical presentation, biochemical and gene study been January 2002 and September 2012.

Results: A total of 68 patients were suspected to have PFIC clinically. Gene testing was positive in 48 patients only including PFIC I (5 patients), PFIC II (27 patients), PFIC III (16 patients). Consanguinity is seen in all patients (100%) while 31 patients (64%) have positive family history of liver disease. All the 5 patients with PFIC I present with jaundice before 18 months of age and have poor growth, hepatomegaly and normal hearing. Diarrhea is seen in 4 patients (80%) while 3 patients (60%) have splenomegaly. All patients (100%) have normal GGT. Two patients (40%) underwent liver transplantation. BFIC II patients presented within the first 2 years, jaundice is seen in 22 patients (81%) and itching in 3 patients (11%), six patients (22%) have signs of rickets. Three patients (11%) present with elevated GGT and fifteen patients (55%) underwent liver transplantation and three patients (11%) died awaiting liver transplantation. PFIC III, jaundice present in 5 patients (31%) while itching in four patients (25%) and one patient (6%) with signs of rickets. Normal GGT is seen in one patient only (6%) and one patient (6%) underwent liver transplantation.

Conclusion: PFIC is a common cause of liver disease in Saudi Arabia. PFIC II is the most common. The vast majority of patients with PFIC I and II present within the first two years of life with cholestasis and normal GGT while patients with PFIC III present after two years.
of life with jaundice, itching and high GGT. However, GGT can be high PFIC II (11%) and it can be normal in PFIC III (6%). One third of patients required liver transplantation and patients with PFIC III are the least to required liver transplantation.

1382 CLINICAL FEATURES OF LIVER DISEASE IN PATIENTS AFFECTED BY APOLIPOPROTEIN A-I (Apo-A-I) AMYLOIDOSIS

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Background and Aims: Apo A-I amyloidosis is a rare, autosomal dominant hereditary disease. Leu75Pro Apo A-I is a variant of Apo A-I associated with systemic amyloidosis predominantly involving liver, kidneys, and testis, identified in a large number of cases in the North of Italy. Apo A-I variants deposits can lead to multiple organ failure. Up to October 2012 we evaluated 179 patients affected by Apo A-I amyloidosis. Our analysis is aimed to describe the early manifestations and the features of this type of liver disease.

Methods: At the first observation we evaluated abdominal ultrasound, transient elastography (FibroScan) and cholestatic indexes ($\gamma$GT and ALP). The cut-off values for $\gamma$GT and ALP were 50 IU/ml and 129 IU/ml respectively. We divided all patients into 3 groups according to the ultrasound picture: group A was characterized by a normal abdominal ultrasound, group B by steatosis and group C had a picture of advanced liver disease (i.e. signs of cirrhosis or portal hypertension).

Results: In Table 1 the features of the different groups are described.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients: No (%)</td>
<td>81 (45.3%)</td>
<td>82 (45.8%)</td>
<td>16 (8.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Males: No (%)</td>
<td>38 (46.9%)</td>
<td>50 (61%)</td>
<td>8 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age: mean (± SD)</td>
<td>46.2 (± 16.6)</td>
<td>57.9 (± 11.5)</td>
<td>68.9 (± 6.6)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>$\gamma$GT IU/ml: mean (± SD)</td>
<td>31 (± 46)</td>
<td>169 (± 230)</td>
<td>243 (± 174)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>ALP IU/ml: mean (± SD)</td>
<td>76 (± 49)</td>
<td>171 (± 185)</td>
<td>352 (± 328)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Stiffness KPa: mean (± SD)</td>
<td>4.9 (±2)</td>
<td>13.7 (±10.9)</td>
<td>49.3 (±25.9)</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Conclusions: Our data suggest that there is a significant correlation between the increase in $\gamma$GT or ALP and stiffness and the evolution of the ultrasound picture. Moreover, the difference in the mean age between the 3 groups resulted statistically significant. The analysis of our population leads to the following considerations: (1) the disease tends to appear and advance with age, (2) it is characterized by a cholestatic damage and (3) the progression is directly related to the increase in cholestatic indexes and stiffness. In particular, transient elastography can be considered a good tool for staging and monitoring this type of amyloidosis.

1383 p.Leu75Pro Apo A-I AMYLOIDOSIS CAUSES MACROSCOPICALLY EVIDENT HEPATIC AMYLOIDOSIS IN A PATIENT WITHOUT ANY CLINICAL OR LABORATORY SIGNS OF HEPATIC DYSFUNCTION

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Hereditary amyloidosis is often treated by orthotopic liver transplantation, with the intent to eliminate the main source for the amyloidogenic protein. The lack of hepatic involvement allows to donate the explant liver to another patient in a domino fashion. Most of these cases are associated with mutations of the transthyretin gen. However several patients with amyloidosis associated with the ApoA1-gen have been reported to have hepatic involvement. It has been suggested these patients should not serve as domino liver donors. All cases reported so far have in common that the hepatic involvement was evident only by histological analysis or laboratory markers, with an unremarkable macroscopic picture. Here we present a case of a patient with p.Leu75Pro Apo A-I amyloidosis who underwent a kombined liver-kidney transplantation. The explant liver was intended to be used as a domino organ. The preoperative clinical picture and laboratory markers did not implicate any hepatic dysfunction. On macroscopic inspection however, with 2130g the liver was enlarged, with a grossly nodular surface and firm parenchyma. Histologically, the liver architecture was preserved with areas of moderate to severe steatosis. Large confluent deposits of amyloid were found in a rather unique jig saw like distribution pattern, mainly in zones 2 and 3, often enclosing central veins, differing from the typical perisinusoidal deposition pattern of AL amyloidosis. Polarized light revealed the typical apple green birefringence and immunoreaction for ApoA1 was strong. Portal tracts were morphologically unremarkable, with some lymphohytic infiltrates. On electron microscopy intracellular peroxisomes and myelinosomes were noted. Based on these findings, the explant liver was deemed to be unsuitable for a domino transplantation. This is the first report of a ApoA1-amyloidosis case with macroscopically evident hepatic involvement.

1384 EXTRAHEPATIC MANIFESTATIONS OF CHRONIC HEPATITIS IN CHILDREN

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Background: Besides hepatic manifestations and within the whole digestive system, there is a spectrum of extrahepatic manifestations in all chronic hepatitis, regardless of etiology but closely related to it and pathogenic ways of interrelation with the host organism. Formation and storage of circulating immune complexes in tissues and organs represent the main action of these events. In children, they have a particular character and they can influence the long-term prognosis for the patient, so that should be evaluated and treated early.

Material and Methods: We have investigated a total of 239 patients followed during 2005–2011, with chronic hepatitis of various etiologies and extrahepatic manifestations, using the database for measuring and extracting data used in statistical processing. Extrahepatic manifestations were quantified, explored and correlated with parameters of evolution and prognosis. The authors evidenced extrahepatic manifestations occurred during
treatment with interferon alpha and ribavirin, while assessing prognostic correlations.

Results and Discussion: In patients with chronic HBV, the prevalence of extrapathic manifestations was 21.28%, in children with chronic HCV 15.43%, and 35.55% in those with hepatitis B and D. The most common extrapathic manifestations were haematological (8.78%), including lupus-related phenomena, dermatological (6.76%), rheumatic (5.56%). Also, behavioral disorders and depression were determined in some patients included in the study group. The most common subjective complaints involved arthralgia, myalgia, and asthenia. The most common extrapathic manifestations related to clinical investigations were adenopathies (41%), urticarial manifestations and respiratory pathology being rather rare. Extrapathic manifestations-related comorbidities were represented primarily by acrocyanosis and bruising, followed by obesity and dermatologic disorders.

Conclusions: Most cases were quantified with arthralgia, myalgia and fatigue. The most common extrapathic manifestations were haematological disorders, opposite the respiratory pathology. In some cases, interferon triggered or worsened some extrapathic autoimmune manifestations (mainly cutaneous manifestations), requiring evaluation of the therapeutic options. Specific guidelines are needed for particular situations in order to minimize the side effects and to maximize the pathogenic disease-modifying therapy results.

1385 WILSON'S DISEASE IN CHILDREN: THE RATE OF LIVER CIRRHOSIS
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Introduction: Initial manifestation of WD is variable and is rarely detected in children under 5 years of age, in most cases manifesting with abnormal liver function.

Aim: To determine the rate of liver cirrhosis in children with WD.

Materials and Methods: 101 children (boys – 50; girls – 51; mean age 12.3±2.9 years). The diagnosis of WD was supported by decreased serum ceruloplasmin (SC), increased copper urinary excretion: basal (CuB) and after D-penicillamine challenge (CuC), the presence of Kaiser-Fleischer rings (KF), increased liver copper content and genetic analysis. Cirrhosis is diagnosed based on morphological data or complex laboratory and instrumental investigations.

Results: At initial diagnosis of WD liver cirrhosis was established in 44 (43.6%) children aged 13.3±2.1 years: in children under the age of 12 years – in 14 (31.1%), over 12 years – even in 30 (53.6%) patients (p<0.05). In children with liver cirrhosis were significantly (p<0.05) more often noted the presence of extrapathic neurologic symptoms – 36.4% of cases, the presence of KF – in 68.2% children, acute Coombs-negative hemolytic anemia (HA) – in 20.5% children, compared with patients with WD in the absence of cirrhosis (n=57), in which the rate of neurological symptoms was – 10.5%, the presence of KF – 24.6%, the presence of HA – 3.5% children. In children with WD at the stage of liver cirrhosis: CuB 189.5 [111.1: 492.9] mcg/24h, CuC 2015.5±1206.7 mcg/24h, which significantly exceeds that in children with WD in the absence of liver cirrhosis: CuB 100.8 [74.7, 133.0] mcg/24h, CuC 1529.0±866.9 mcg/24h (p<0.05). SC does not depend on the stage of WD: in liver cirrhosis SC was 9.1±5.2 mg/dl, and in its absence – 11.3±7.6 mg/dl (p>0.05).

Conclusion: In pediatric practice there is late diagnosis of WD, which leads to the diagnosis of the disease in 43.6% at the stage of liver cirrhosis. Liver cirrhosis in children with WD is associated with the presence of KF (68.2%), neurological symptoms (36.4%) and higher levels of copper urinary excretion.

1386 EVEROLIMUS DOES NOT FURTHER REDUCE POLYCYSTIC LIVER VOLUME WHEN ADDED TO LONG ACTING OCTREOTIDE: RESULTS FROM A RANDOMIZED CONTROLLED TRIAL IN POLYCYSTIC LIVER DISEASE PATIENTS
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Background and Aims: Polycystic liver disease (PLD) is caused by autosomal dominant polycystic kidney disease (ADPKD) or autosomal dominant polycystic liver disease (PCLD), and affects quality of life by the mass effect of the enlarged liver on surrounding organs. Somatostatin analogues reduce liver volume in PLD patients, however the overall effect is modest. A pilot trial in 16 ADPKD patients has shown a potentially beneficial effect of mTOR inhibitors on polycystic liver volume. The aim of this study was to assess whether everolimus and octreotide give a larger reduction of liver volume than octreotide monotherapy.

Methods: In this randomized, open-label, single-center clinical trial, 44 PLD patients were treated with either everolimus 2.5 mg daily combined with octreotide 40 mg intramuscularly every 4 weeks or octreotide monotherapy during 48 weeks. Both PCLD and ADPKD patients were included. Exclusion criteria were MDRD-GFR <60 ml/min/1.73m2 and liver volume <2500 ml. The primary outcome was change in liver volume measured with CT-volumetry.

Results: We randomized 44 PLD patients (28 PCLD, 16 ADPKD, 90% female) to treatment with octreotide (n=21) or octreotide-everolimus (n=23). We analyzed 43 patients, as one CT scan could not be measured because of technical difficulties. Liver volume decreased by 4.5% (p<0.01) in the combination therapy arm, while in the monotherapy arm, liver volume decreased by 3.5% (p<0.01). The difference between treatment arms was not significant (p=0.86). Diagnosis significantly influenced the treatment effect, as PCLD patients had larger reduction of liver volume than ADPKD patients (P<0.01).

Conclusions: The combination of everolimus and octreotide gives a similar reduction of polycystic liver volume compared to octreotide monotherapy, indicating that they do not act synergistically. Furthermore, PCLD patients respond better to treatment than ADPKD patients.

1387 IDENTIFICATION OF A NOVEL GENE ASSOCIATED WITH POLYCYSTIC LIVER AND KIDNEY DISEASES
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Background and Aims: Polycystic liver disease (PLD) can arise in association with polycystic kidney (ADPKD) or as an isolated liver disease (PCLD). Mutations in PRKCSH or SEC63 cause PCLD, while mutations in PKD1 or PKD2 cause ADPKD. There is considerable genetic heterogeneity and ~80% of polycystic liver patients lack mutations in hitherto identified genes. We set out to discover a novel gene responsible for hepatic cystogenesis.

Methods: We assessed a 40-member family with polycystic liver disease. Mutation analysis for the 4 disease-related genes was prioritized and validated by sanger sequencing.
Results: We identified a private variant c.3562C>T at a highly conserved amino acid residue in the PLD3 gene in all affected relatives. This mutation was absent in >1,500 healthy controls and predicted to affect protein configuration by homology modeling. There was highly significant linkage with the PLD3 locus (LOD score: 4.62).

We replicated our findings in a cohort of polycystic liver patients. In 3 unrelated PCLD families and 1 ADPKD family we identified 4 additional PLD3 mutations. All variants were predicted to be damaging with profound structural effects on protein domains.

Conclusions: Germline PLD3 mutations cause a broad phenotype of polycystic liver and kidney disease. This study provides new insights for the study of hepatic cystogenesis and a link for signaling pathways in ADPKD and PCLD.

1388 ROLE OF LIVER BIOPSY IN ASSESSMENT OF LIVER DYSFUNCTION IN HCV-INFECTED CHILDREN FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background and Aim: Liver dysfunction occurring in 50–72% of patients undergoing hematopoietic stem cell transplantation (HSCT) is often multi-factorial with iron overload (IO), chronic graft-versus-host-disease (C-GVHD) and hepatitis being the main causes. We performed histopathologic liver evaluation in this setting with overlapping etiologies.

Methods: Thirty HCV-infected (genotype 4) children (mean age: 7.5±4.4; M:F 22:1) transplanted (6 auto-transplant and 24 allogeneic transplant) in a single institution underwent liver biopsies a median of +1099 days after HSCT. Association between liver histology and multiple variables, including number of red cell transfusions, HCV viremia, HBV co-infection, frequency of ALT flares (>3UNL), hepatic GVHD, C-GVHD, and biochemical parameters (serum ferritin, albumin and fibrinogen) was assessed.

Results: Hepatic GVHD was diagnosed in 10/31 (32.2%) (7 isolated and 3 had hepatic plus extra-hepatic GVHD). All patients experienced ALT flares postransplant compared to 17 (56.6%) during transplant and only 4 (13.3%) pretransplant (p=0.016). Ferritin was >500 ng/ml in 3/6 (50%) of autologous and 10/24 (41.6%) of allogeneic HSCT recipients. HCV viremia was mild in 8/30 (26.6%), moderate in 13/30 (43.3%) and high in 9/30 (30%). All patients except one had varying degrees of fibrosis, 26 (86.6%) were Metavir F1 and 3 (10%) were F2; none had advanced fibrosis or cirrhosis. Two (6.6%) had mild steatosis and 4 (13.3%) had mild hemosiderosis (grade 1/4). Advanced hepatic siderosis [grades 2/4 in 13 (43.3%) & 3/4 in 4 (13.3%)] correlated with higher ferritin levels (>1000–3000 ng/ml & >3000 ng/ml respectively (p=0.009) and number of transfusions (p < 0.01). Regression analysis showed significant correlation between ferritin levels and histological grade of hepatocytic iron. Ferritin levels were higher in children with C-GVHD & isolated hepatic GVHD (p=0.007&0.004 respectively) compared to patients without GVHD. C-reactive protein and fibrinogen were not different in patients with or without IO, or GVHD or in those with concomitant HBV infection. Thyroid hormones, ASMA, ANA were normal in all the patients & none developed signs of ESLD.

Conclusion: Histopathologic liver assessment is essential for HSCT recipients with HCV infection with IO being the most frequent finding in the post-HSCT setting. IO contributes to hepatic dysfunction and should be considered in the differential diagnosis, particularly in patients with high serum ferritin levels.

1389 PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS: DETECTION OF NEW MUTATIONS AND RARE FORMS OF TRANSMISSION

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Background and Aim: Progressive familial intrahepatic cholestasis (PFIC) comes in three types of autosomal recessive disease, which usually present in infancy or childhood, with cholestasis of hepatocellular origin. Liver histology is important, but not specific, for diagnosis. Genotyping is conclusive. To exclude rare forms of transmission, the study of parents is required. To sort out the meaning of the novel mutations a molecular study of healthy controls and protein modelling are mandatory.

Methods: Between 2008 to 2012, 4 cases of PFIC1, 11 of PFIC2 and 2 of PFIC3 were identified. To examine the meaning of new mutations, homology modelling with the program MODELLER was done in 4 cases.

Results: Genotyping revealed 18 mutations including 9 known and 9 new. Six novel mutations were in BSEP. In this group, molecular analysis of parents led to the identification a rare type of transmission, uniparental paternal disomy (UPD), and one de novo mutation in PFIC2. In the last case, the model of BSEP structure showed that R52W and H615R mutations involved sites that affect, respectively, the transmembrane domain and one of the two ATP binding pocket of the protein.

Figure: Model of the structure of BSEP.

This seems to be consistent with the hypothesis of a glycoprotein capable of arriving at the canaliculus, but unable to guarantee the bile salt export activity.

Conclusion: PFICs diagnosis can be suspected on clinical manifestations and serology, but only full gene testing is able to offer a conclusive identification. Protein modelling studies can contribute to investigate the functional consequence of novel mutations.
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$\alpha_1$-ANTITRYPSIN PI*MZ-MUTATION AND ALCOHOLIC STEATOHEPATITIS ARE BI-DIRECTIONALLY AGGRAVATING AMPLIFIERS IN CHRONIC LIVER DISEASE

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**Background**: Heterozygous $\alpha_1$-antitrypsin (AAT) deficiency type PI*MZ (PiMZ) results in chronic liver injury and predisposes to hepatocellular carcinoma. This retrospective case-control-study focuses on the impact of PiMZ-genotype on the development of chronic liver disease in alcohol consuming patients.

**Methods**: 6886 consecutive liver specimens were immunohistochemically tested for PiZ-deposits. On the basis of 254 PiZ-positive patients, 59 PiMZ-adults without concomitant liver disease other than ALD were extracted and matched to PiMM- (wild type) patients with respect to age, gender and lifetime alcohol consumption. Histomorphological changes and routine laboratory parameters were examined.

**Results**: Comparative analysis of PiMZ- and PiMM-patients indicates that PiMZ-genotype significantly aggravates alcohol-toxic liver injury: The extent of liver fibrosis and necroinflammatory activity as assessed by Ishak and HAI Scores were significantly higher in PiMZ- than in PiMM-patients ($p=0.001$, and $p=0.007$ respectively). Vice versa, alcohol consumption exacerbates hepatocellular PiZ-deposition in PiMZ-patients ($r=0.45$, $p=0.00033$) as a factor of disease progression.

**Conclusion**: Since prevalences of PiMZ-heterozygosity range between 1–5% in Western populations, there is a substantial risk of coincidence with chronic alcohol abuse. PiMZ-genotype and ALD are mutually reinforcing pathogenetic components contributing to liver injury. We therefore recommend advising patients with heterozygous PiMZ-mutation and concurrent alcohol of their increased risk for disease progression.

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PHENOTYPE–GENOTYPE CORRELATIONS IN SIBLINGS OF INDEX PATIENTS WITH WILSON DISEASE

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**Background and Aim**: The clinical presentation of Wilson disease (WD) is highly variable. The aim of this study was to evaluate the disease presentation in siblings of index patients in order to reveal possible genotype-phenotype correlation.

**Patients and Methods**: 165 affected siblings of 1077 index patients (mostly from Central and Eastern Europe) identified by genetic testing were studied with complete data available in 137. Age and symptoms at onset, serum ceruloplasmin, presence of Kayser-Fleischer rings and if available, results of liver biopsy were analyzed. H1069Q was assayed by PCR. H1069Q compound heterozygotes and H1069Q negative patients were further examined by denaturating gel HPLC using exon specific primers. Mutations were identified by direct sequencing on an ABI Prism 310 Genetic Analyzer (Perkin Elmer, Norwalk, USA).

**Results**: 131 of the 137 siblings were brothers or sisters, 5 were children and 1 a second degree relative of an index case. 73 index patients had liver disease (age: 17.2±8.9; 41 female, including 6 cases of fulminant WD and 2 with hemolytic anemia) and 54 neurologic disease (age: 21.0±7.5 years, 28 female). 92 siblings (67%, 47 female) were asymptomatic, 44 had symptoms which were discordant in seven. Ceruloplasmin was normal in 9. 81 had no KF-rings. In 93 and 33 siblings, mutations on both (including 46 H1069Q homozygotes) or one chromosome/s were identified, respectively. At diagnosis, 37 siblings were older and 99 younger than the index patients, one pair were monozygous twins. 52 siblings of patients with hepatic WD were asymptomatic, 24 had liver disease. During follow up, 2 siblings not taking the prescribed medication developed neurologic symptoms. 40 siblings of patients with neurologic WD were asymptomatic (17 of them had no KF-rings, 4 normal CPL), 13 had neurologic symptoms, 5 had liver disease (including the homozygous twin) and one hemolytic anemia.

**Conclusion**: Of the symptomatic siblings of patients with WD, only a minority (15%) had discordant symptoms, including the monozygous twin sister of a patient with severe neurologic disease. Nevertheless the discordant cases indicate that genetic factors other than ATP7B mutations apparently modify the phenotypic presentation of WD.

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THE COMMON ADIPONUTRIN (PNPLA3) VARIANT IS ASSOCIATED WITH SUBCLINICAL LIVER INJURY ALREADY IN YOUNG AGE: ANALYSIS OF A COHORT OF PAEDIATRIC PATIENTS

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**Background**: Previous studies showed that the common PNPLA3 (encoding adiponutrin) variant p.I148M is a risk factor for severe forms of fatty liver disease (NAFLD) in adults and children [1,2]. It also increases liver stiffness in patients with chronic liver diseases [3]. Here we analyse the effects of this polymorphism on the liver status in a cohort of very young paediatric patients without any apparent chronic liver disease.

**Methods**: Overall, we recruited 142 children (100 Caucasians, 42 Asians age range 5–9 years, boys=64, mean standard deviation score (SDS)-BMI=1.45). NAFLD was assessed by abdominal ultrasound. Other chronic liver diseases were excluded. Blood samples were drawn at inclusion; the PNPLA3 variant was genotyped with PCR-based 5-nucleotide and fluorescence detection assays (TaqMan).

**Results**: We achieved 100% genotyping success and observed the following genotype frequencies: [II] = 75 (52.8%), [IM] = 57 (40.1%), [MM] = 10 (7.1%). Overall, there was no association between the PNPLA3 genotype and presence of fatty liver (N=15, Armitage’s trend test $P>0.05$). However, we detected a significant difference in serum ALT levels between carriers of the adiponutrin genotypes (non-parametric ANOVA $P=0.021$). In particular, homozygous risk allele carriers presented with significantly (P=0.009) higher median serum ALT activities (27 IU, range 17–40 IU) as compared to carriers of genotype [II] (median 19 IU, range 10–60 IU). In the univariate linear regression analysis, both PNPLA3 polymorphism and SDS-BMI were associated with ALT levels ($P=0.049$ and $P<0.001$, respectively). In the multivariate model, we detected a significant association with the SDS-BMI ($P<0.001$) and a trend for the PNPLA3 variant ($P=0.06$). Other liver enzymes were not affected by adiponutrin genotypes (all $P>0.05$).

**Conclusions**: This genetic association study in a paediatric cohort encompassing very young children demonstrates that the PNPLA3 variant p.I148M is associated with early subclinical liver injury. This
points to a novel screening strategy to detect individuals prone to PNPLA3-associated liver disease.

Reference(s)

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FACILITATION OF BLOOD DONATION AMONGST HAEMOCHROMATOSIS PATIENTS: A UK PILOT
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Background and Aim: The standard medical therapy for haemochromatosis is removal of iron by regular phlebotomy1. Current EASL guidelines recommend that blood taken from uncomplicated haemochromatosis patients should be made available through national blood transfusion services1. However, this practice varies widely across Europe and is often hindered by administrative difficulties. Here, we aim to describe a pilot facilitating the process of blood donation amongst haemochromatosis patients in the UK.

Methods: A dedicated haemochromatosis clinic was established. At this clinic, patients with uncomplicated haemochromatosis interested in becoming blood donors were offered a simple information leaflet. One page provided information about eligibility; the second formed a self-referral application to be countersigned by the responsible physician. Upon receipt of referral, patients were contacted by members of the local Blood Service. Data on clinical characteristics including genotype, alcohol consumption, BMI, co-morbidities and previous blood donation was collected.

Results: Patients attending (n=100) since the introduction of this service (Aug 2011) are included. The median age was 57 (22–82) and the majority (69%) were male. Most (89%) were C282Y homozygotes; the remainder were H63D/C282Y compound heterozygotes. The majority (91%) had uncomplicated haemochromatosis; however many were ineligible for blood donation by virtue of age (20%), co-morbidity (17%), or induction therapy (15%). Prior to the introduction of this service, there were 3 regular blood donors. Since the introduction of this service, of those potentially eligible 21 (54%) showed interest in blood donation, 17 (44%) applied, 15 (38%) have registered. In total, there are now 13 (33%) regular blood donors. Since the introduction of this facilitation process, we have significantly increased the number of regular blood donors amongst this cohort. If this process was undertaken nationally or more widely across Europe, this could have a significant impact on the availability of this precious resource.

Reference(s)
higher prevalence of bile duct obstruction in these mice (number of mice with obstructed/patent bile ducts: 9/2 in ABCB4−/− vs 3/11 in wt, p = 0.005). Furthermore, frequencies of hepatic CD8 and of NK lymphocytes were both higher in ABCB4−/− compared with wt mice (%CD8+CD3+/lymphocytes: 10.3 vs 6.2, p = 0.03; %NK+CD3−/lymphocytes: 12.7 vs 7.3%, p = 0.002), as determined by flow cytometry. Interestingly, enhanced lymphocyte activation was accompanied by 25-fold up-regulation of hepatic iNOS mRNA expression in these mice.

**Conclusion:** An infant with EHBA had anatomical and molecular features of ABCB4/MDR3 deficiency. In a murine model, ABCB4 heterozygosity enhances hepatic CD8 and NK lymphocyte responses to virus infection and increases the frequency of ductal obstruction. Whether this activation is causally linked to hepatic up-regulation of iNOS and low phospholipid “toxic bile” requires further investigation.

**1396 THEORETICAL EFFICACY OF D-PENICILLAMINE ENCAPSULATED ALGINATE/CHITOSAN NANOPARTICLES IN RAT MODEL OF COPPER TOXICITY WITH NEUROBEHAVIORAL IMPAIRMENTS**

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Animal models of Wilson’s disease (WD) viz. Long evans cinnamon rats, rarely exhibit neurological symptoms impedig the development of novel therapeutic approaches to treat neurological manifestations in WD patients. The aim of this study was to

1. examine the effect of intraperitoneally injected copper lactate for 90 days especially on copper and zinc levels in liver, kidney & brain tissues; expression of hepatic metallothionein-I (MT-I) and Atp7b gene; and MT-III and acetylcholine esterase (AChE) gene in brain, biochemical parameters, and neurobehavioral functions of male Wistar rats, and

2. therapeutic evaluation of orally administered D-penicillamine encapsulated alginate/chitosan nanoparticles for 90 days on Cu intoxicated Wistar rats.

Reverse transcription-PCR and Morris water maze test were used for expression and neurobehavioral studies. Copper intoxicated animals showed significantly increased ceruloplasmin, serum & urine copper levels and decreased serum acetyl choline esterase (AChE) activity, increased expression of hepatic MT-I gene with impaired neuromuscular coordination and spatial memory. However, no changes were observed on the expression levels of hepatic Atp7b gene; and MT-III and acetylcholine esterase (AChE) gene in brain, biochemical parameters, and neurobehavioral functions of male Wistar rats, and

**Conclusion:** This report illustrates examples of atypical JH in which cardiomyopathy was absent. The beta-thalassemic trait was likely an important cofactor influencing iron overload and liver disease in case 1, but iron overload could hardly be explained by simple heterozygosity for HVJ mutations in both cases. Other genetic factors should be investigated, and further studies are needed to understand genotype-phenotype correlation in JH.

**1397 ATYPICAL JUVENILE HEMOCHROMATOSIS ASSOCIATED WITH HETEROZYGOSITY FOR NOVEL HEMOJUVELIN MUTATIONS**

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Background and Aims: Juvenile hemochromatosis (JH) is a rare autosomal recessive disorder characterized by severe iron overload presenting in the 2–3 decade, whose common features are hypogonadism and cardiomyopathy. JH causing mutations have been identified in the hemojuelin (HVJ) and in the hepcidin genes. Defective forms of HVJ result in decreased hepcidin and consequent iron overload. Here we describe two atypical presentations of JH. Case 1: a 12 year-old male from Central Italy was discovered high levels of aminotransferases (x3 UNL), while abdominal ultrasonography demonstrated hepatosplenomegaly. Blood tests showed a beta-thalassemic trait, ferritin 9035 ng/ml and transferrin saturation 84% with negative HFE mutations and no other known liver disease. Histopathological liver evaluation demonstrated massive parenchymal siderosis and bridging fibrosis. Cardiac ecocolor doppler was normal. Case 2: A 12 year-old female from Northern Italy was incidentally discovered with increased iron parameters (ferritin 467 ng/ml, transferrin saturation 87–95% at two different evaluations, normal blood count and aminotransferases). Evaluation of hepatic iron by T2 MRI revealed mild iron overload.

**Methods:** Genetic tests with direct sequencing of hemochromatosis causing genes (HFE, TIR2, HVJ, Hepcidin and Ferroportin-1) was performed in the children and their parents.

**Results:** In case 1, we detected a newly described mutation in the HVJ gene (g.3659_3660insG) present in heterozygosity, which was inherited together with the beta thalassemia trait from the father, who (like the mother) had normal iron parameters. In case 2, genetic tests pointed out another novel mutation in the HVJ gene (g.2297delC) present in heterozygosity. The mutation was inherited from the mother, which had mild iron deficiency. The father had normal iron stores. Both mutations caused a frameshift in the coding sequence determining premature stop codons. No other disease causing variants were detected in both cases.

**Conclusion:** This report illustrates examples of atypical JH in which cardiomyopathy was absent. The beta-thalassemic trait was likely an important cofactor influencing iron overload and liver disease in case 1, but iron overload could hardly be explained by simple heterozygosity for HVJ mutations in both cases. Other genetic factors should be investigated, and further studies are needed to understand genotype-phenotype correlation in JH.

**1398 HAEMOCHROMATOSIS – FREQUENCY OF HFE MUTATIONS AND IMPORTANCE OF SECONDARY CAUSES**

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Hereditary iron overload, implicated in most cases of hereditary hemochromatosis, is mainly due to mutations of the HFE gene (C282Y/C282Y and C282Y/H63D). The role of the HFE S65C mutation in the development of hepatic iron overload is unknown, and the importance of clinical cofactors on iron overload has been recognized.

**Objective:** Determine the frequency of C282Y, H63D and S65C mutations on HFE gene in patients with iron overload, attending our Hepatology clinic. Additionally, verify the presence of nonalcoholic fatty liver disease (NAFLD), hepatitis C and excessive alcohol
consumption and to evaluate the influence of these variables on body iron deposits.

**Methods**: We considered iron overload, transferrin saturation (TS) >50% in females, >60% in males and/or serum ferritin (SF) >200 ng/mL in females or >300 ng/mL in males. Demographic and clinical data including alcohol intake were collected. Biochemical iron parameters, blood count, glucose, liver function tests, lipid profile, serology for hepatitis B and C, and HFE mutation analysis (C282Y/H63D/S65C) were done. T test was used to compare the different groups.

**Results**: We studied 230 patients with iron overload, 175 men (76%). The frequency of the mutation in HFE gene was 125/230 (54.3%), 10 (8%) with genotype C282Y/C282Y, 14 (11.2%) C282Y/H63D, 15 (12%) H63D/H63D, 21 (17%) C282Y/wild type, 59 (47%) H63D/wild type, 2 (1.6%) C282Y/S65C and 4 (3.2%) S65C/S65C. Those in which genetic mutations were found, C282Y homozygotes had TS (average 87%) and SF (average 2442 ng/mL) higher than the others: C282Y/H63D homozygotes (SF 513 ng/mL, p<0.06, TS 55%, p<0.01), heterozygous C282Y/wild type (SF 452 ng/mL, TS 51% p<0.012) and homozygous H63D (SF 546 ng/mL, p<0.021, TS 49% p<0.018). In the remaining patients, 36 (43.8%) had NAFLD (average SF 560.5 ng/mL, TS 44%), 38 (36%) excessive alcohol consumption (average SF 920 ng/mL, TS 73%) and 16 (15.2%) HCV infection (average SF 487.6 ng/mL, TS 58%).

**Conclusion**: The frequency of HFE gene mutations in patients with iron overload was 125/230 (54.3%). The TS and SF levels were significantly higher in individuals homozygous for the C282Y mutation, confirming the correlation between genotype C282Y/C282Y and higher risk of iron overload. Hepatitis C, excessive alcohol consumption and NAFLD are implicated in increased iron deposits and constitute additional risk factors for iron overload.

**1399 ORTHOTOPIC LIVER TRANSPLANTATION FOR HEREDITARY HAEMOCHROMATOSIS IN IRELAND**

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Ireland has the highest reported prevalence of Hereditary Haemochromatosis (HH) worldwide. In HH, hepatic iron accumulation can lead to fibrosis, cirrhosis and hepatocellular carcinoma (HCC) formation. Approximately 5% of patients with HH progress to cirrhosis, which is associated with a significantly increased risk of HCC development. In this study, we sought to characterise the association between HH and liver transplantation in Ireland. Data was obtained from the transplant database at the National Liver Transplant Unit, St Vincent’s Hospital, Dublin.

A total of 634 patients underwent liver transplantation between January 1995 and April 2012. 27 of these had a diagnosis of HH. 93% of HH patients were male. HCC was the primary indication in 15 patients (56%) while significant risk factors for liver disease were present in 11 (41%); alcoholic liver disease or hepatitis C infection in 9 and 2 patients, respectively). Only 5 patients (19%) had undergone successful therapeutic venesection prior to transplantation. The one year and 5 year survival for individuals with HH was 83.3% and 61.1%, respectively.

Despite the prevalence of HH in Ireland, it remains an uncommon indication for liver transplantation, particularly in females. The vast majority of HH patients have concomitant HCC or other risk factors for liver disease. Early detection and treatment to prevent complications such as HCC remains the cornerstone of HH management.

**1400 DECLINING PREVALENCE OF DIABETES MELLITUS IN PATIENTS WITH HEREDITARY HAEMOCHROMATOSIS IN THE ERA OF HFE GENE TESTING**

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**Background and Aims**: Diabetes Mellitus (DM) is a pandemic with a large healthcare burden. Hereditary haemochromatosis (HH) also has a high prevalence, particularly in Ireland. The classical association between advanced HH and DM is well described – so called “bronze-diabetes”. Most HH patients no longer present with advanced disease. How this impacts on prevalence of DM in the context of a spectrum of HH associated iron-overload is poorly characterised. We describe DM prevalence in patients with homozygous C282Y HFE gene (C282Y/C282Y) attending a dedicated haemochromatosis clinic in University Hospital Galway, Ireland.

**Method**: This is a retrospective cohort study. Two researchers performed data abstraction and updated haemochromatosis patient database. 682 patients with the C282Y/C282Y genotype attended the Haemochromatosis Clinic, and patients with a chart-record of DM or HbA1C ≥48 mmol/mol were identified. Patients were classed as severe iron overload (serum ferritin >1000 µg/L), mild-moderate iron overload (200/300 µg/L to 1000 µg/L; female/male respectively) or no iron overload (<200/300 µg/L).

**Results**: 14% of patients presented with severe iron overload and 54.4% with mild-moderate iron overload. Median serum ferritin on presentation in males was 551.7 µg/L and 220.7 µg/L in females. The prevalence of DM was 9.3%; with Type 2 DM in particular having a prevalence of 7.5%. The proportion of patients with Type 2 DM was higher for males (9.5%, n=36) than females (5%, n=15) p=0.027; and was higher in those presenting with severe (15.1%, n=14) versus mild-moderate (7.4%, n=26) or no iron overload (3.9%, n=8), p<0.001.

**Conclusions**: This study demonstrates a decline in prevalence of DM compared with previous studies. This may be as a result of few patients now presenting with markedly elevated serum ferritin.

**1401 THE PREVALENCE OF TYPE 2 DIABETES MELLITUS IN PATIENTS WITH COMPOUND HETEROZYGOUS HAEMOCHROMATOSIS**

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**Background and Aims**: Diabetes Mellitus (DM) is a known complication of hereditary haemochromatosis (HH). Although it has been well-described in patients with HH (C282Y homozygous) who presented with severe iron overload, minimal data are available regarding patients with compound heterozygous haemochromatosis. Furthermore, with the availability of HFE gene testing, how this impacts on prevalence of DM in the context of a spectrum of HH associated iron-overload has not been described. We aim to determine the prevalence of DM in patients with compound heterozygous genotype (C282Y/H63D) attending a dedicated haemochromatosis clinic in University Hospital Galway, Ireland.

**Method**: This is a retrospective cohort study. Two researchers performed data abstraction and updated haemochromatosis patient database. 304 patients with C282Y/H63D genotype attended the Haemochromatosis Clinic, and patients with a chart-record of DM
or HbA1c ≥48 mmol/mol were identified. Patients were classed as severe iron overload (serum ferritin >1000 μg/L), mild-moderate iron overload (200/300 μg/L to 1000 μg/L; female/male respectively) or no iron overload (<200/300 μg/L).

**Results:** 5% of patients presented with severe iron overload and 47.5% with mild-moderate iron overload. Median serum ferritin on presentation in males was 345.3 μg/L and 108.8 μg/L in females. The prevalence of DM was 9.2%; with Type 2 DM in particular having a prevalence of 8.9%. The proportion of patients with Type 2 DM was higher for males (11.8%, n= 24) than females (3.1%, n= 3), p = 0.013; and was higher in those presenting with severe (37.5%, n= 6) versus mild-moderate (9.3%, n= 13) or no iron overload (5.8%, n= 8) p < 0.001.

**Conclusions:** This study demonstrates a Type 2 DM prevalence of 8.9% in patients with compound heterozygous haemochromatosis, with a lower rate in those with no iron overload.

**1402**

**HYDRODYNAMICS-BASED, LIVER-TARGETED NONVIRAL GENE THERAPY APPROACH FOR MONOGENIC DISEASES**

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**Background and Aims:** An autologous cell modification alleviating a specific pathology is an ideal therapeutic approach for monogenic disorders. In terms of simplicity and biological safety, in vivo application of nonviral methods is preferable. We have proved the concept of hydrodynamic gene delivery (HGD) in large animals by means of interventional radiology under a control of a special injection system, which is propelled by high-pressure gas (Hydrojector). The aim of this study is to develop a new version of Hydrojector, which avoids risks using high-pressure gas and consists of items conforming to good-clinical-practice guideline, and to show a feasibility of this approach in clinic.

**Methods:** A new system, Hydrojector-EM, was constructed by implementing an electromotor as a driver instead of gas and by only employing clinically approved stuffs that directly contact with the injection solution. A regional HGD targeting entire liver was performed from a catheter tip, which was placed at the conjunction of IVC and hepatic veins with temporal blockades both above and below the tip in a rat. A portal vein pressure was continuously monitored over the liver. Hydrojector-EM controlled the injection through a negative feedback circuit from the portal vein pressure to the electromotor so as to follow a predefined pressure-surge in a certain time frame. The association between gene delivery efficiency and time-pressure curves was analyzed.

**Results:** A regional HGD was completed in twelve rats applying four different time-pressure curves, and none of them was suffered from technical aspects except for a transient increase of liver enzymes, which recovered in a few days. The highest transgene expression of luciferase, 272 pg luciferase/mg of proteins, was obtained 3 hours after the injection, when a pressure profile was applied reaching 30 mmHg above the basal pressure in 10 seconds. Consistent results were obtained by immunohistochemistry for luciferase in the liver specimens. A delivery of pBS-HCRHP-FIXIA plasmids using the optimal condition lead to a sustained expression of human factor IX protein more than 500 ng/ml in rat plasma over 2 months.

**Conclusions:** These results suggest that Hydrojector-EM can enhance the robustness of HGD toward clinical utility of this technology.

**1403**

**ASSOCIATION OF VITAMIN D BINDING PROTEIN GENE POLYMORPHISM rs222020 AND THERAPEUTIC OUTCOME IN ASIAN PATIENTS WITH CHRONIC HEPATITIS C INFECTION**

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**Background and Aims:** Vitamin D deficiency and Vitamin D binding protein (GC), rs7041G>T and rs4588C>A polymorphisms were demonstrated to be associated with negative outcome of interferon-alfa based therapy in patients chronic hepatitis C (CHC). Another polymorphism, rs222020 of GC gene was found to be strongly associated with vitamin D deficiency in general population. This study aimed to assess the association between the GC gene polymorphism, rs222020G>A, and therapeutic outcome in patients with CHC treated with pegylated interferon-alfa and ribavirin.

**Methods:** 250 Asian patients with CHC treated with pegylated interferon-alfa based regimen were genotyped for the polymorphisms of the GC gene. The definitions of rapid viral response (RVR), early viral response (EVR), end of treatment viral response (EOT) and sustained viral response (SVR) were classified according to the European Association For The Study of The Liver (EASL) clinical practice guideline.

**Results:** Of these, 182 patients achieved SVR. Baseline characteristics including age, sex, alcohol consumption, baseline HCV RNA viral load and advanced fibrosis were not different in patients with SVR and non-SVR. Frequencies of GC rs222020G>A polymorphisms were 37 (14.8%), 128 (51.2%) and 85 (34.0%) for GG, GA and AA, respectively. SVR rate was not different between GG, GA and AA genotypes (73.0%, 68.8% and 78.8%, respectively; p = 0.27) same as in subgroup HCV genotype 1 or 4. The SVR rate was 55.6%, 63.3% and 73.3%, respectively, p = 0.43. GC polymorphism was also not associated with RVR, EVR and EOT.

**Conclusions:** Although vitamin D binding protein gene polymorphism rs222020 affected serum Vitamin D level, this polymorphism had no impact on therapeutic outcome in Asian patients with chronic hepatitis C treated with Pegylated interferon alfa based regimen.

**1404**

**THE PNPA3 I148M MUTATION SIGNIFICANTLY INCREASES THE RISK OF DEVELOPING ALCOHOL-RELATED CIRRHOSIS IN ALCOHOL DEPENDENT INDIVIDUALS**

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The common single nucleotide polymorphism, rs738409, in PNPLA3 encodes a non-synonymous (I148M) mutation which has been associated with risk of developing significant liver injury in response to a variety of noxious agents, including alcohol. Why this mutation causes this effect at a functional level is still largely unknown. The frequency of this allele was studied in a UK sample which included: 744 control individuals; 633 alcohol dependent individuals who had not been screened for liver disease and 387 patients who had misused alcohol for a minimum of 25 years and had biopsy-proven alcohol-related liver disease of varying severity including: minimal steatosis (n = 67); cirrhosis (n = 206), and intermediate biopsy changes (n = 114). All patients and control subjects were of white Irish, Welsh, Scottish or English ancestry. KASP genotyping was performed on genomic DNA extracted from all samples.

The primary finding was a strong association with cirrhosis when allele frequencies were compared with either the alcohol-dependent group or the controls.
This mutation is strongly associated with an increased risk of developing cirrhosis in alcohol dependent individuals in this UK sample. Future work includes genotyping in a larger control population and studies to understand the mutations effect at a functional level.

1405
THE ZNF699 GENE IS NOT ASSOCIATED WITH ALCOHOL DEPENDENCE IN A UK SAMPLE BUT MAY ALTER RISK FOR ALCOHOL-RELATED CIRRHOSIS

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The zinc-finger protein 699 (ZNF699) gene has previously been associated with alcohol dependence (Riley et al, 2006). This protein, a transcription factor of unknown physiological function, was initially identified as the most likely orthologue of Hang, a gene crucial for ethanol tolerance in Drosophila. Riley and co-workers, genotyped several ZNF699 SNPs in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPASD) cohort, and found significant single-marker and haplotypic associations based on the markers rs7254880, rs12460279, rs7252865 and rs10854142. In an attempt to replicate this finding these alleles were genotyped in a UK cohort comprising 1052 alcohol dependent individuals and 956 controls all of whom were of white Irish, Welsh, Scottish or English ancestry. There was no evidence of an association between any of the markers and alcohol dependence per se. Within in the alcohol dependent population were a subgroup who had misused alcohol for a minimum of 25 years and had either minimal steatosis (n = 78) or cirrhosis (n = 193); in this subgroup an association was found between the SNP rs7254880 and alcohol-related cirrhosis compared with the minimal liver injury group (allelic p = 0.00426) (Table 1). These findings do not explain the lack of replication but may suggest that ZNF699 increases susceptibility to significant alcohol-related liver injury.

The marker rs7254880 was the one most strongly associated with alcohol dependence in the IASPASD cohort and was also the marker associated with cirrhosis in this study. It seems likely that the two studies are discrepant because of different inclusion criteria or heterogeneity in the frequency of susceptibility haplotypes between the two samples.

1406
POLYCYSTIC LIVER DISEASE PATIENTS HAVE LOW QUALITY OF LIFE WHICH IS ASSOCIATED WITH CONCURRENT ABDOMINAL SYMPTOMS

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Background and Aims: Polycystic liver disease (PLD) carries a progressive course ultimately leading to severe hepatomegaly and mechanical complaints. It is still unknown whether these symptoms compromise quality of life. Our aim was to determine the quality of life in PLD patients compared to the general population and to investigate its relation with concurrent abdominal symptoms.

Methods: The baseline data of 54 PLD patients from a randomized clinical trial (NCT00565097) were used for our cross-sectional analysis. The health-related quality of life was assessed using the generic short-form health survey (SF-36) and physical (PCS) and mental component scores (MCS) were compiled. Differences between the mean scores of PLD patients and the general Dutch population were tested for statistical significance using the student’s t-test.

The severity of symptoms was measured with a standardized, 7-points scale gastro-intestinal symptoms questionnaire. Symptoms reported in ≥33% of patients were included for further analysis. Finally, the component scores of quality of life were compared between categories for severity of symptoms using the non-parametric Kruskal-Wallis-test.

Results: The PCS and all physical subdomains were significantly lower compared to the control population (p < 0.01). In contrast, the MCS was similar to controls, although the mental subdomains vitality and social functioning were significantly lower in PLD patients (p < 0.01). We found that abdominal and epigastric pain, dyspnea and early satiety were significantly associated with a reduced PCS (p < 0.01), but not with MCS (p = 0.4489).

Conclusion: PLD patients have a significantly lower quality of life in the physical dimension compared to the general population, whereas the mental dimension remains unaffected. Symptoms of abdominal and epigastric pain, dyspnea and early satiety had a significant impact on physical domains, and should be targets for therapy.

Table 1 (abstract 1405). Analysis of co-morbid liver disease
TTR MUTATIONS IN GERMAN PATIENTS WITH FAMILIAL AMYLOID POLYNEUROPATHY

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Background: Familial amyloid polyneuropathy (FAP) is a heterogeneous group of diseases transmitted as an autosomal dominant trait. Most commonly it is associated with a mutation of the transthyretin-gene (TTR). To date, more than 80 different TTR-mutations are known to form amyloid. With the differing genotypes varying clinical manifestations are being observed, significantly influencing the outcome of liver transplantation – currently the only curative treatment option. To optimize patient selection as well as patient's age for transplantation emphasis should be placed on the specific gene mutation involved. Countries such as Japan, Sweden and Portugal have certain high frequency mutations. Common mutations in Germany have yet to be identified.

Aim: The mutational pattern of TTR-amyloidosis in Germany was studied.

Methods: 65 patients with TTR-amyloidosis from 40 unrelated families were included. Diagnosis was confirmed histologically. The mutation was identified by genomic DNA extraction from patient's blood and direct sequencing. Patients were examined for clinical signs and symptoms.

Results: The most prevalent mutations detected were Val30Met and Gly47Ala (Gly67Ala), identified in 36/70 and 17/65 patients, respectively. The Val30Met mutation was associated mainly with polyneuropathy and patients with the Gly47Ala mutation presented primarily with cardiac symptoms, but had PNP as well. Further mutations identified were Leu58His, Ile107Val, Gly53Ala, Asp39Val, Arg34Thr and Glu89Gln. 16/65 patients received a liver transplantation and 21/65 patients are currently being treated with Tafamidis. Outcomes are still being followed.

Conclusions: This is the first study of the mutational pattern of patients with TTR-amyloidosis in Germany. Similar to the largest loci of FAP – Portugal, Sweden and Japan, Val30Met was found to be the most common mutation. However a second single mutation, the Gly47Ala was detected in 17 (26%) of the studied patients with 14 of those belonging to one family. This may be a frequent mutation across the country. Our ongoing analysis of a larger cohort of patients will help to categorize the predominant mutations in Germany. Furthermore it will help us to identify prognostic factors for patients treated with Tafamidis or liver transplantation.

HFE GENE MUTATIONS: RELATIONSHIP WITH THE PRESENCE OF COLORECTAL POLYPS AND CANCER

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Introduction: The pilot study for colorectal cancer (CRC) screening in the population of the Basque Country has obtained a high incidence of polyps and CRC. Its cause is unknown. There seems to be a relationship between high iron deposits and increased risk of colorectal cancer. The H63D mutation in the HFE gene (Hereditary Hemochromatosis) contributes to iron overload, especially the H63D/H63D mutation, but with considerable variability in the phenotypic expression. The Basque Country has this mutation with a higher prevalence than other countries in the world.

Objective: To study the prevalence of the different HFE mutations, in particular, the H63D/H63D mutation, in the population of the Basque Country (Gipuzkoa) with CRC or polyps detected during the CRC screening and compare these results with those obtained in cases arising from the screening in which no polyps were detected.

Methods: Prospective study. Correlation of HFE mutations with the risk for polyps or CRC in the population of the Basque Country detected during the CRC screening program in Gipuzkoa.

Results: We have studied 432 patients, 263 men (61%), 169 women (39%), mean age 60 years. We found polyps-CRC in 221 patients (51.16%) (12 adenocarcinomas, 25 in situ carcinomas: 37 (16.74%) and 211 patients (48.84%) without polyps. The study revealed the following HFE mutations: C282Y/wt mutation in 18 patients (15polyp-CRC group, 3 group without polyps); H63D/wt mutation in 140 (76 polyp-CRC group, 64 group without polyps); H63D/H63D in 16 (6 polyp-CRC group: 10 group without polyps); C282Y/H63D 2 (2 group polyp-CRC); C282Y/C282Y, 1 group without polyps. In 12 patients S65C mutation was present: 9 were S65C/wt (6 in group without polyps), 2 H63D/S65C (1 in each group), and 1 C282Y/S65C (in polyp-CRC group). Only one HFE mutation presented statistically significant differences: C282Y/wt genotypic frequency was 6.79% in polyp-CRC group and 1.42% in non polyp group (p < 0.05); allelic frequency was 3.85% vs 1.18% (p < 0.05).

Conclusions: The H63D/H63D mutation is not related to the presence of polyps or CRC in the Basque Country. C282Y/wt mutation could play a role in the genesis of polyps or CRC.

NEW CLINICAL AND GENETIC PROFILES OF GLYCOGEN STORAGE DISEASE TYPE IIIa IN A CHINESE FAMILY

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Background and Aims: Glycogen storage disease type III (GSD-III) is a rare autosomal recessive disorder. Here we report the clinical and genetic findings of two siblings from a Chinese family to expand our knowledge of GSD-III.

Methods: Acylcarnitines in blood spots were analyzed by tandem mass spectrometry. The coding and promoter regions of the AGL gene were analyzed by direct sequencing. Agilent whole human genome oligo microarrays (4x44K) were applied to analysis of gene expression profiling.

Results: Sequencing of the AGL gene showed that both patients were compound heterozygotes for c.118C>T (p.Gln40X) and c.753_756 delCAGA (p.Asp251GlufsX29) which inherited from the parents. AGL protein expression couldn't be detected in patients, while it decreased in the parents who carried a single mutated allele. Interestingly, both patients showed an aberrant acylcarnitine profile during fasting. Several pathways were affected by the absence of AGL, including PPARG signaling pathway, fatty acid biosynthesis, lipid synthesis and visceral fat deposits, and the metabolic syndrome are the most prominent affected molecules. A higher expression of PPARG, ACSL6, ACACB, MCAT, HSD11B1, and CCL2 was observed in GSD-III patients than healthy controls. Annotation of the interconnected functional network of these genes provided a unique representation of lipid and fatty acid metabolism in GSD-III patients.
Conclusions: We identified one novel AGL mutation (p.Asp251Glufsx29). GSD-III can give rise to pathological acylcarnitine profile. AGL mutation induced up-regulation of gene expression in PPAR signaling pathway, lipid/fatty acid biosynthesis and chemokines, which might be the cause of hyperlipaemia and hepatocytes steatosis.

1410
THE STUDY OF Th17 CELLS AND CLINICAL CHARACTERS IN PEDIATRIC PATIENTS WITH CHRONIC HEPATITIS B
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Background: Interleukin-17 (IL-17) mediated immune response has been shown to play a critic role in inflammation-associated disease. However, its role in the pediatric patients with chronic hepatitisB (CHB) remains unkown. We aim at investigating the frequency and cytokine production of Th17 cells in the pediatric patients with CHB, and evaluated the association between the Th17 cytokine and clinical characters.

Methods: The study subjects were enrolled from 65 pediatric patients with CHB and 9 healthy controls. The frequency of Th17 cells was carried out by intracellular cytokine staining analysis. The degree of hepatic inflammation was grated using the histological activity index (HAI).

Results: Compared with the healthy controls, frequency of Th17 cells in peripheral blood was significantly higher in the pediatric patients with CHB. The proportion of Th17 cells was higher in the patients with higher HAI score (G2-G3) compared to those subjects with lower HAI score (G0-G1), but the frequency of Th17 cells had no correlation with serum HBVDNA loads or ALT levels. Compared with the younger age group (age 1–6), Th17 cells frequency was higher in the older age group (age 7–18).

Conclusion: Peripheral Th17 cell frequency is closely associated with the inflammation activity of liver tissue in the pediatric patient with CHB.
1411 SOFOSBUVIR + PEGINTERFERON + RIBAVIRIN FOR 12 WEEKS ACHIEVES 90% SVR12 IN GENOTYPE 1, 4, 5, OR 6 HCV INFECTED PATIENTS: THE NEUTRINO STUDY

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Introduction: Sofosbuvir (SOF) is a novel pangenotypic nucleotide inhibitor of the HCV NS5B polymerase. Results from NEUTRINO, a Phase 3, open-label study evaluating the efficacy and safety of 12 weeks of SOF + peginterferon alfa-2a (PEG-IFN) + ribavirin (RBV) are reported.

Methods: Treatment-naïve genotype 1, 4, 5, or 6 HCV-infected patients received SOF 400 mg daily and RBV 1000–1200 mg daily with PEG-IFN 180 µg weekly for 12 weeks. The primary endpoint was SVR12. Secondary objectives included safety, tolerability and resistance.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>SVR12 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>327</td>
<td>90%</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>292</td>
<td>89%</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>28</td>
<td>96%</td>
</tr>
<tr>
<td>Genotype 5/6</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>54</td>
<td>80%</td>
</tr>
<tr>
<td>No Cirrhosis</td>
<td>273</td>
<td>92%</td>
</tr>
<tr>
<td>IL28B CC</td>
<td>95</td>
<td>98%</td>
</tr>
<tr>
<td>IL28B non-CC</td>
<td>232</td>
<td>87%</td>
</tr>
<tr>
<td>Black</td>
<td>54</td>
<td>87%</td>
</tr>
</tbody>
</table>

Results: 327 patients (292 genotype 1, 28 genotype 4, 7 genotype 5/6) were enrolled and received study drug; 64% were male, 23% Black, 17% had compensated cirrhosis, and 29% carried the IL28B CC genotype. The SVR12 rate for SOF+PEG-IFN+RBV (295/327, 90%) was superior to a historic control rate of 60% (p<0.001). SVR12 rates for subgroups are shown in the table. All patients had HCV RNA<1LLOQ by treatment week 6 and relapse accounted for all virologic failures. No NS5B S282T variants were detected following relapse. Five patients (2%) discontinued treatment due to AEs and 4 patients (1%) experienced SAEs. AEs and laboratory abnormalities were consistent with the profile for PEG-IFN+RBV. AEs observed in ≥20% included fatigue, headache, nausea, insomnia and anemia.

Conclusions: 12 weeks of treatment with SOF + PEG-IFN + RBV achieved 90% SVR12 in treatment naïve genotype 1, 4, 5, or 6 HCV-infected patients with no viral resistance detected in failures. This regimen was well tolerated and is a short, simple and effective treatment option for patients with genotype 1, 4, 5 or 6 HCV infection.

1412 COMPARATIVE GENETIC ANALYSES POINT TO HCP5 AS SUSCEPTIBILITY LOCUS FOR HCV-ASSOCIATED HEPATOCELLULAR CARCINOMA

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Introduction: Recently, genetic variations in MICA (lead single nucleotide polymorphism [SNP] rs2596542) were identified by a genome-wide association study (GWAS) to be associated with hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) in Japanese patients. In the present study, we sought to determine whether this SNP is predictive for HCC development in the Caucasian population as well.

Methods: An extended region around rs2596542 was genotyped in 1924 HCV-infected patients from the Swiss Hepatitis C Cohort Study (SCCS). Pair-wise correlation between key SNPs was calculated both in the Japanese- and the European populations (HapMap3: CEU and JPT), followed by a detailed comparison of haplotype structures between both populations. Logistic and Cox regression analyses were performed to assess the association between SNPs and occurrence of HCV-related HCC.

Results: To our surprise, the minor allele A of rs2596542 in proximity of MICA appeared to have a protective impact on HCC development in Caucasians, which represents an inverse association as compared to the one observed in the Japanese population. Hence, if there is a shared causal variant across Japanese and European populations in this region, it cannot be rs2596542. If such shared variant exists it must have opposite Pearson correlation in Japanese and European samples. Detailed fine-
mapping analyses revealed a new SNP in HCP5 (rs2244546) upstream of MICA as strong predictor for HCV-related HCC in the SSCS (univariate \( P = 0.027 \); multivariate \( P = 0.0002 \), odds ratio = 3.96, 95% confidence interval = 1.90–8.27). This newly identified SNP had a similarly directed effect on HCC in both Caucasian and Japanese populations, suggesting that rs2244546 may better tag a putative true variant than the originally identified SNPs.

**Conclusion:** Our data confirms the MICA / HCP5 region as susceptibility locus for HCV-related HCC and identifies rs2244546 in HCP5 as a novel tagging SNP with a similarly directed effect in both European and Japanese populations. In addition, our data exemplify the need for conducting meta-analyses of cohorts of different ethnicities in order to fine-map GWAS signals.

### 1413 SIMEPREVIR (TMC435) WITH PegIFN/ribavirin IN TREATMENT-NAIVE PATIENTS: RESULTS FROM QUEST-2, A PHASE III TRIAL.

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**Background and Aims:** Simprevir is a potent, once-daily, oral, investigational HCV NS3/4A protease inhibitor. QUEST-2 (NCT01290679) is a Phase III, randomised, double-blind, placebo-controlled trial assessing the efficacy, safety and tolerability of simprevir versus placebo as part of a regimen including peginterferon α-2a (pegIFN-α2a) or pegIFN-α2b/ribavirin (PR) in treatment-naive patients chronically infected with genotype-1 HCV. SVR12 results from a primary (Week 60) analysis are presented.

**Methods:** Patients \((n = 391)\), randomised 2:1 and stratified by HCV genotype-1 subtype and host IL28B genotype, received simprevir (150 mg QD) + PR or placebo + PR for 12 weeks, followed by PR alone. Total treatment duration was 24 or 48 weeks (simprevir group) based on response-guided therapy (RGT) criteria (HCV RNA < 25 IU/mL Week 4 and undetectable Week 12) or 48 weeks (placebo group).

**Table 1**

<table>
<thead>
<tr>
<th>Percentage of patients achieving SVR12</th>
<th>Placebo/PR</th>
<th>Simprevir/PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>50%</td>
<td>81%</td>
</tr>
<tr>
<td>Treated with pegIFN-α2a/pegIFN-α2b</td>
<td>62%/42%</td>
<td>88%/78%</td>
</tr>
<tr>
<td>Patients who met RGT criteria</td>
<td>not applicable</td>
<td>86%</td>
</tr>
<tr>
<td>HCV subtype 1a or other/1b</td>
<td>46%/53%</td>
<td>80%/82%</td>
</tr>
<tr>
<td>META ViR score F0–F2/F3–F4</td>
<td>51%/47%</td>
<td>85%/66%</td>
</tr>
</tbody>
</table>

**Results:** Simprevir/PR was superior to placebo/PR; SVR12: 81 vs 50%, respectively \((p < 0.001)\). The majority \((91\%)\) of simprevir-treated patients met RGT criteria and completed treatment at Week 24. Overall, 79% of simprevir and 13% of placebo-treated patients achieved RVR. Treatment with simprevir/PR led to lower rates of on-treatment failure and relapse compared to placebo/PR \(7 \text{ vs} 32\% \text{ and} 13 \text{ vs} 24\%, \text{respectively}\). The incidence of AEs was similar between groups, regardless of the pegIFN used. The most common AEs were fatigue, influenza-like illness, pruritus and headache. A slightly higher proportion of simprevir patients experienced rash and photosensitivity, compared to placebo \((27 \text{ vs} 20\% \text{ and} 4 \text{ vs} 1\%, \text{respectively})\). There was no difference in the proportion of patients experiencing anaemia.

**Conclusions:** Simprevir 150 mg QD was well tolerated, leading to a high SVR12 rate of 81% when administered with either pegIFN-α2a or pegIFN-α2b. The majority of patients \((91\%)\) receiving simprevir was able to shorten therapy to 24 weeks.

### 1414 THE CLIF-CONSORTIUM SCORE PREDICTS MorTALITY MORE ACCURATELY THAN THE MELD AND MELD-SODIUM SCORES IN PATIENTS HospitalIZED WITH DECOMPENSATED CIRRHOSIS with and without ACUTE-ON-CHRONIC LIVER FAILURE (ACLF).


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**Background:** Patients from CANONIC study (Prospective observational investigation defining ACLF). The objective was to develop a novel prognostic score for patients hospitalized for decompensated cirrhosis.

**Methods:** 302 patients with and 1047 without ACLF were assessed.

1. Original CLIF-SOFA score, to define organ failures was simplified to a 3-point sub-score. Second: Proportional-Hazards model for Competing-Risks was used to assess predictors of mortality. Coefficients for independent predictors of mortality were used as relative weights for CLIF-Consortium score (CLIF-C score).

**Third:** Discrimination ability of the CLIF-C score was assessed with the Concordance Index and compared to that of MELD and MELD-
sodium. Fourth: CLIF-C score coefficients were validated internally and externally \((n=297)\).

**Results:** First: Predictive ability of the simplified CLIF-SOFA was similar to that of the original score. Second: Independent predictors were the simplified CLIF-SOFA score, age, serum sodium, leucocyte count and ascites. The CLIF-C score was computed as the linear combination of the products of each predictor by the corresponding weight. Third: In patients without ACLF the CLIF-C score was superior to MELD and MELD-sodium scores in predicting mortality (Table). In patients with ACLF the CLIF-C score was superior to MELD and MELD-sodium scores in predicting mortality (Table). Fourth: Bootstrap estimates of model coefficients were very close to the estimates obtained in the model. In the external cohort, CLIF-C score was validated and performed better than MELD and MELD-sodium.

**Conclusions:** The CLIF-C score is superior to MELD and MELD-sodium scores in predicting prognosis of patients with acute decompensation of cirrhosis.

Table: Comparison of Harrell’s Concordance Index (C-Index)

<table>
<thead>
<tr>
<th>No ACLF group ((n=1047))</th>
<th>C-Index</th>
<th>C-Index</th>
<th>C-Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-Day mortality</td>
<td>0.777 (0.681–0.882)</td>
<td>0.699 (0.628–0.771)</td>
<td>0.726 (0.651–0.801)</td>
</tr>
<tr>
<td>90-Day mortality</td>
<td>0.726 (0.683–0.770)</td>
<td>0.644 (0.596–0.692)</td>
<td>0.680 (0.633–0.727)</td>
</tr>
<tr>
<td>1-Year mortality</td>
<td>0.665 (0.632–0.699)</td>
<td>0.592 (0.558–0.626)</td>
<td>0.618 (0.584–0.652)</td>
</tr>
</tbody>
</table>

**Background and Aims:** Improving surveillance of hepatitis B and C was identified as a priority for the European Centre for Disease Prevention and Control and in 2011, an enhanced surveillance programme was implemented. The programme aims to improve the epidemiological understanding of these diseases by differentiating cases as either acute or chronic and collecting data on a range of epidemiological variables. This presentation aims to present the key findings from this first data collection with a focus on 2011 data.

**Methods:** Revised EU case definitions were developed for both diseases which differentiate cases by acute and chronic disease status. The enhanced surveillance for hepatitis B and C collects data on 33 demographic and specific epidemiological variables. This presentation aims to present the key findings from this first data collection with a focus on 2011 data.

**Background:** Faldaprevir (FDV) is a once-daily (QD) NS3/4A protease inhibitor. This double-blind, placebo-controlled Phase III study (STARTVerso1) assessed the efficacy and safety of FDV plus pegylated interferon alfa-2a and ribavirin (PegIFN/RBV).

**Methods:** Treatment-naïve patients with chronic HCV genotype-1 (GT-1) infection were randomised 1:2:2 to receive 24 weeks' PegIFN/RBV with: placebo for 24 weeks (arm 1); FDV 120 mg QD for 12 or 24 weeks (response guided; arm 2); or FDV 240 mg QD for 12 weeks (arm 3). Patients with early treatment success (ETS, HCVRNA <25 IU/mL at Week 4 and undetectable at Week 8) in arms 2 and 3 stopped all treatment at Week 24. Patients without ETS and those in arm 1 received PegIFN/RBV for 48 weeks. Randomisation was stratified by HCV GT-1 subtype and race. The primary endpoint was sustained virological response 12 weeks after planned end of treatment (SVR12).

**Results:** 652 patients were treated: mean age 48 years, 52% male, 78% Caucasian, 20% Asian, 17% grade ≥3 fibrosis, 39% IL28B CC, 66% GT-1b.

**Table 1**

<table>
<thead>
<tr>
<th>Virological results</th>
<th>Arm 1</th>
<th>Arm 2</th>
<th>Arm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PegIFN/RBV N=132</td>
<td>52 (69)</td>
<td>63 (29/46)</td>
<td>52 (69)</td>
</tr>
<tr>
<td>SVR12, % (n)</td>
<td>79 (204)</td>
<td>90 (96/107)</td>
<td>80 (210)</td>
</tr>
<tr>
<td>PegIFN/RBV N=259</td>
<td>47 (40/86)</td>
<td>71 (107/151)</td>
<td>71 (114/160)</td>
</tr>
<tr>
<td>SVR12 in IL28B (rs1297980)</td>
<td>22 (29)</td>
<td>87 (226)</td>
<td>89 (233)</td>
</tr>
<tr>
<td>SVR12 in patients with ETS, % (n)</td>
<td>90 (26/29)</td>
<td>86 (194/226)</td>
<td>89 (208/233)</td>
</tr>
</tbody>
</table>

\*p<0.0001 vs placebo, based on Cochran–Mantel–Haenszel test, adjusted for genotype and race.

**LATE BREAKING ABSTRACTS**
All study medications were discontinued due to adverse events (AEs) in 4%, 4% and 5% of patients, and FDV only was discontinued in 0%, 1% and 3% of patients, respectively. Serious AEs occurred in 6%, 7% and 7% of patients. Grade 3 rash occurred in <1% of patients in each arm; no patients had Grade 4 rash. Up to Week 24, haemoglobin ≤8.5 g/dL occurred in 2%, 3% and 3% of patients, respectively.

**Conclusions:** FDV plus PegIFN/RBV significantly increased SVR12 rates in HCV GT-1 patients in Europe and Japan compared with PegIFN/RBV alone and was well tolerated. In total, 88% of patients treated with FDV were eligible to stop all treatment at Week 24.

### Table 1

<table>
<thead>
<tr>
<th>HCV RNA &lt; 25 IU/mL, mITT</th>
<th>GT1a/1b, prior TVR or BOC failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>DCV + SOF (n=21)</td>
<td>J</td>
</tr>
<tr>
<td>× 24 weeks</td>
<td>DCV + SOF + RBV (n=20)</td>
</tr>
<tr>
<td>SVR4</td>
<td>21 (100)</td>
</tr>
<tr>
<td>End Of Treatment (Week 24)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>SVR24</td>
<td>21 (100)</td>
</tr>
</tbody>
</table>

1* missing.

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**LATE BREAKING ABSTRACTS**

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1417 SUSTAINED VIROLOGIC RESPONSE WITH DACLATASVIR PLUS SOFOSBUVIR ± RIBAVIRIN (RBV) IN CHRONIC HCV GENOTYPE (GT) 1-INFECTED PATIENTS WHO PREVIOUSLY FAILED TELAPREVIR (TVR) OR BOCEPREVIR (BOC)


**Background and Aims:** Daclatasvir (DCV) plus sofosbuvir (SOF) ± RBV achieved high rates of sustained virologic response in HCV GT1–3-infected previously untreated patients (arms A-H). Additional arms (I,J) evaluated the efficacy of DCV + SOF ± RBV in patients who failed TVR or BOC.

**Methods:** A total of 41 GT1 non-cirrhotic patients with previous breakthrough (n=15), relapse (n=13), or nonresponse (n=14) to pegIFN/RBV+TVR or BOC (n=9) (patients who discontinued TVR or BOC due to adverse events were excluded; 1 patient received TVR and BOC) were randomized: 1:1 to DCV+SOF with or without RBV for 24 weeks. DCV and SOF were dosed orally at 60mg QD and 400 mg QD, respectively. RBV was dosed BID at 1000–1200mg/d. The primary end point was HCV RNA <25 IU/mL at 12 weeks post-treatment (SVR12). SVR4 is reported; SVR12 will be presented at the Week 24 End Of Treatment (Week 24) 21 (100) 19 (95)*

**Results:** Most patients had HCV GT1a (83%), were IL28B non-CC (98%), and had estimated METAVIR stage ≥F2 (83%). Mean HCV RNA was ≥6 log IU/mL. HCV RNA <25 IU/mL was achieved in 40/41 patients by week 4 and in all patients by end of treatment. None had breakthrough or relapse and all patients achieved SVR4 (Table). The most common adverse events (>30% total) were fatigue and headache. There were no grade 3–4 hematoletic or hepatic laboratory abnormalities.

**Conclusion:** The all-oral, once-daily combination of DCV+SOR with or without RBV for 24 weeks achieved 100% SVR4 in non-cirrhotic GT1 prior TVR/BOC treatment failures. These data provide proof-of-concept that the combination of two potent direct-acting antivirals with different viral targets is effective in patients who failed pegIFN/RBV + a protease inhibitor.

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1418 DACLATASVIR COMBINED WITH PEGINTERFERON ALFA-2A AND RBIVARIN FOR 12 OR 16 WEEKS IN PATIENTS WITH HCV GENOTYPE 2 OR 3 INFECTION: COMMAND GT2/3 STUDY

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**Background:** Daclatasvir (DCV) is a first-in-class NSSA replication complex inhibitor, active against HCV genotype (GT) 1–6 in vitro. Study AI444–031 evaluated DCV 60mg QD combined with peginterferon-alfa-2a (peg-alfa-2a) 180mcg weekly and ribavirin (RBV) 400 mg BID in patients with chronic HCV GT2 or GT3 infection to determine whether this regimen enhances virologic responses, potentially allowing shorter treatment than current 24-week peg-alfa/RBV regimens.

**Methods:** Adult treatment-naïve patients were randomly assigned to DCV/peg-alfa-2a/RBV for 12 or 16 weeks or placebo/peg-alfa-2a/RBV for 24 weeks. DCV/peg-alfa-2a/RBV recipients without protocol-defined response (PDR; HCV RNA < LLOQ week-4 and undetectable week-10) discontinued DCV at week 12 and received 12 additional weeks of peg-alfa-2a/RBV. The primary efficacy endpoint was SVR12.

**Results:** Baseline characteristics were well-balanced in the DCV 12-week (N=50), DCV 16-week (N=50) and placebo (N=51) arms; more patients with GT3 (18/80, 22.5%) than GT2 (17/11, 14.5%) were cirrhotic. 78%–88% of DCV recipients achieved PDR. SVR12 rates were higher in GT2 than GT3 with all regimens; within each genotype, SVR12 rates were similar in DCV arms and higher than placebo/peg-alfa-2a/RBV. In DCV arms, one GT2 and 12 GT3 patients relapsed. In GT3, relapse was higher among cirrhotics (3/7, 43%) than non-cirrhotics (3/19, 16%) in the 12-week arm but similar in the 16-week
arm (1/4, 25% vs 5/20, 25%). There were 7 on-treatment serious AEs (DCV: 4; placebo: 3); no deaths. AEs were typical of those associated with peg-alfa/RBV.

**Conclusion:** Shorter treatment duration (12 or 16 weeks) with DCV/peg-alfa-2a/RBV demonstrated higher SVR rates than 24 weeks of peg-alfa-2a/RBV in patients with GT2 or GT3 infection, with higher SVR rates in GT2 with all regimens. These results support further evaluation of DCV-containing regimens for different HCV genotypes.

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>GT2</th>
<th>GT3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DCV</td>
<td>DCV</td>
</tr>
<tr>
<td>12 wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 wk</td>
<td></td>
<td></td>
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<tr>
<td>PBO</td>
<td></td>
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</tbody>
</table>

Protocol-defined response: 21 (85) 18 (78) - 12 (50) 22 (85) 22 (82) 21 (78) 16 (69) 16 (67) 16 (59) 13 (45) 15 (63) 18 (69) 18 (67) 16 (59) 13 (45)

End of treatment response: 23 (96) 21 (91) 22 (92) 25 (96) 24 (89) 21 (78) 23 (96) 21 (91) 22 (92) 25 (96) 24 (89) 21 (78) 23 (96) 21 (91) 22 (92) 25 (96) 24 (89) 21 (78) 23 (96) 21 (91) 22 (92) 25 (96) 24 (89) 21 (78)

SVR12 (HCV RNA < LLOQ 12 weeks posttreatment): 21 (88) 19 (83) 17 (71) 18 (69) 21 (78) 14 (52) 21 (88) 19 (83) 17 (71) 18 (69) 21 (78) 14 (52)

SVR24 (HCV RNA undetectable 24 weeks posttreatment): 20 (83) 19 (83) 16 (65) 19 (69) 15 (67) 16 (59) 20 (83) 19 (83) 16 (65) 19 (69) 15 (67) 16 (59)

*Failure to achieve SVR reflects relapse or missing data at posttreatment week 24.

**LATE-BREAKER POSTER ABSTRACTS**

**1419**

**IL28B RNA EXPRESSION DEPENDS ON A NOVEL TT/G POLYMORPHISM ASSOCIATED WITH IMPROVED HCV CLEARANCE PREDICTION**


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**Background and Aims:** Despite the availability of new antiviral agents, the treatment of chronic hepatitis C remains suboptimal and burdened with adverse effects. Genome-wide association studies identified rs12979860, a single nucleotide polymorphism (SNP) nearby IL28B, as an important predictor of spontaneous and treatment-induced HCV clearance. However, the mechanisms underlying the association of rs12979860 with viral clearance remain elusive. We looked for other polymorphisms in the IL28B region upstream of rs12979860 that could be in linkage disequilibrium with rs12979860. Furthermore, their functional relevance was determined by measuring IL28B and IP-10 mRNA expression in peripheral blood mononuclear cells (PBMCs) from selected patients.

**Results:** We identified a novel TT/G polymorphism in the CpG region upstream of IL28B a better predictor than rs12979860 for both spontaneous (P = 8.6E-09 versus P = 1.6E-08) and treatment-induced (P = 2.7E-07 versus P = 2.4E-05) HCV clearance. We demonstrated that TT/G promotes the methylation of the adjacent cytosine residue. By using polyclonal-stimulated PBMCs from individuals carrying different allelic combinations of TT/G and rs12979860, we showed that induction of IL28B and IP-10 mRNA relies on TT/G, but not rs12979860, making TT/G the only functional variant identified so far.

**Conclusion:** Reduced IL28B and IP-10 production seems sufficient to explain the reduced ability of TT/G carriers to clear HCV compared to Wild Type individuals. However, a recent study suggested that reduced HCV clearance in such patients may result from the transient expression of an interferon analogue (IFNL4) with immunomodulatory properties. Further investigations will be needed to understand the respective contribution of IL28B and IFNL4 expression to HCV clearance.

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**1420**

**ASUNAPREVIR WITH PEGINTERFERON-ALFA AND RIBAVIRIN IN TREATMENT-NAIVE PATIENTS WITH GENOTYPE-1 OR -4 CHRONIC HEPATITIS C: SVR24 RESULTS FROM A RANDOMIZED PHASE 2B STUDY (AI447016)**

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**Background:** Asunaprevir (ASV; BMS-650032) is a selective inhibitor of HCV NS3 protease with activity in vitro against genotypes-1 (GT-1) and -4 (GT-4). Study AI447016 stage 2 is a randomized phase 2b evaluation of ASV with peginterferon alfa-2a/ribavirin (alfa/R) in treatment-naïve patients with chronic HCV GT-1 or GT-4.

**Methods:** Patients (N = 238) were randomized 3:1 to receive ASV (200 mg BID) or placebo (PBO) with alfa/R. At Week 12, ASV-treated patients with a protocol-defined response (PDR), HCV RNA < LLOQ [25 IU/mL] Week 4 and undetectable Week 10 were re-randomized 1:1 to continue ASV+alfa/R or receive PBO+alfa/R through Week 24. Patients without PDR and the PBO arm continued alfa/R through Week 48.

**Results:** Efficacy (modified ITT) and safety are shown in the tables. Among GT-1 patients, 71% (27/38) who were IL28B (rs12979860) CC and 60% (69/115) non-CC achieved SVR24. Among PDR-GT-1 patients (N = 133), 73% (49/67) with 12 weeks of triple therapy and 67% (44/66) with 24 weeks achieved SVR24. Grade 3–4 amniontransferase elevations occurred in 10% on ASV and 2% on PBO. Other AEs were comparable between arms.

**Conclusions:** Triple-therapy with ASV+alfa/R results in higher SVR24 rates than PBO+alfa/R in GT-1 and GT-4 infection. Among GT-1 PDR patients, 12 and 24 weeks of triple therapy resulted in similar SVR24 rates. Further studies of ASV-containing regimens are underway.
Table 1

<table>
<thead>
<tr>
<th>Safety, % (n)</th>
<th>ASV 200 mg BID + alfa/R (N=177)</th>
<th>PBO + alfa/R (N=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia (all grades)</td>
<td>20% (35)</td>
<td>23% (14)</td>
</tr>
<tr>
<td>Neutropenia (all grades)</td>
<td>16% (28)</td>
<td>18% (11)</td>
</tr>
<tr>
<td>Rash (all grades)</td>
<td>17% (30)</td>
<td>25% (15)</td>
</tr>
<tr>
<td>Grade 3–4 ALT increases</td>
<td>10% (18)</td>
<td>2% (1)</td>
</tr>
<tr>
<td>SAEs</td>
<td>8% (14)</td>
<td>5% (3)</td>
</tr>
<tr>
<td>AE-related discontinuations</td>
<td>6% (10)</td>
<td>5% (3)</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Efficacy</th>
<th>GT1a</th>
<th>GT1b</th>
<th>GT4</th>
<th>PBO</th>
<th>GT1</th>
<th>GT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASV 200 mg BID + alfa/R</td>
<td>(N=159)</td>
<td>(N=94)</td>
<td>(N=63)</td>
<td>(N=18)</td>
<td>(N=53)</td>
<td>(N=7)</td>
</tr>
<tr>
<td>SVR12</td>
<td>72%</td>
<td>70%</td>
<td>73%</td>
<td>78%</td>
<td>53%</td>
<td>29%</td>
</tr>
<tr>
<td>SVR24</td>
<td>64%</td>
<td>59%</td>
<td>71%</td>
<td>89%</td>
<td>45%</td>
<td>43%</td>
</tr>
</tbody>
</table>

SVR12 responders missing at Wk24, 5 failures (2 <LOQ), 1 SVR12 failure/ SVR24 responder; 2 SVR24 responders missing at Wk12; 3 SVR12 responders missing at Wk24; 4 SVR12 failure/SVR24 responder.

1421 ALISPOR VIR (ALV) PLUS PEGINTERFERON/RIBAVIRIN (PR) ACHIEVES HIGH SVR12 RATES AMONG NULL RESPONDERS, IL28BCT/TT AND CIRRHOTIC HCVG1 PATIENTS (FUNDAMENTAL STUDY INTERIM ANALYSIS)

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Background: ALV inhibits cyclophilin A, an essential host protein for HCV replication. We investigated the efficacy and safety of ALV+PR vs ALV placebo+PR in HCVG1 subjects with previous PR failure.

Methods: 461 patients stratified by BMI, IL28B genotype and previous PR response, were randomized to ALV 400mg BID+PR (N=18) vs ALV 800mg QD+PR (N=53) vs PR (N=10). In future, ALV is intended to be developed as IFN free treatment in combination with DAAs.

Highest efficacy was among those who received ALV 400mg BID+PR for 40–48 weeks of treatment with (n=18) 89% SVR12 ALV 400mg BID+PR, (19) 74% ALV 600QD+PR, (20) 75% ALV 800QD+PR vs (10) 30% PR. Viral breakthrough on ALV was lowest in ALV 400BID+PR (2.8% vs 5.5% with P/R). SAEs in 9.9% ALV+PR vs 5.3% P/R and were most common with ALV 400BID+P/R (15.7%). 10.5 discontinued ALV+PR therapy vs 5.3% PR. One case of pancreatitis (fully recovered) occurred with ALV+PR. Most common AEs were headache, fatigue, anaemia, neutropenia, nausea and hyperbilirubinemia. The incidence of confirmed hypertension was low, but was more common with ALV.

Conclusion: All ALV+PR doses achieved high rates of on treatment viral response and SVR12 despite shorter than planned ALV treatment. The greatest benefit was among Null and Cir vs PR patients. In future, ALV is intended to be developed as IFN free treatment in combination with DAAs.

1422 END OF TREATMENT RESPONSE AFTER PROTEASE INHIBITOR (PI)-BASED THERAPY FOR HEPATITIS C RECURRENT AFTER LIVER TRANSPLANTATION: A MULTICENTRIC EUROPEAN EXPERIENCE


Background: Telaprevir or boceprevir in combination with peg-interferon/ribavirin improved sustained virological response (SVR) rate in hepatitis C (HCV) genotype 1 patients, naive or previously treated. We describe in this large European cohort, the results of PI after liver transplantation (LT) for HCV recurrence.

Patients and Methods: This cohort study enrolled 98 liver transplant patients (male: 87%, mean age 56±10 years [31–75]), with an active genotype 1 hepatitis C, in 17 centers between March 2011 and November 2012, treated with boceprevir (n=41) or telaprevir (n=57) immediately or after a 4-weeks lead-in phase (n=23). The meantime between LT-PI was 60±63 months [2–300]. Indication was HCV recurrence (>F1), including cirrhotic patients (n=22), and cholestatic hepatitis (n=11). Thirty-four (33%) patients were non-responders to a previous course of dual therapy post-LT; 91% of patients received cyclosporine (n=51) or tacrolimus (n=42).

Results: The mean follow-up was 36±19 weeks (W) [12–95]. At baseline, HCV viral load, GGT and hemoglobin levels were 6.48±0.95 log_{10} IU/mL [2.66–8.28], 293±349 IU/L [22–2179],
13.6:1.8 g/dL [8.8–17.5], respectively. After 12W, a complete early virologic response was obtained in 34 (83%) boceprevir patients and in 35 (61%) telaprevir patients (p = 0.026). Among 17 boceprevir and 16 telaprevir patients, 14 (82%) and 7 (43%) achieved an end of treatment response (EOT) with an undetectable viral load, respectively (p = 0.032). Among 9 boceprevir and 5 telaprevir patients, 6 and 1 achieved SVR12, respectively. Among 6 patients in the boceprevir group, 3 achieved SVR24. In the telaprevir group, 29 patients discontinued therapy (serious adverse events, n = 13; virological breakthrough, n = 6; non-response, n = 9). In the boceprevir group, 14 patients discontinued therapy (serious adverse events, n = 5; virological breakthrough, n = 2; non-response, n = 4; retransplantation, n = 1). Four patients died in a context of infectious disorders: boceprevir, n = 2 (W20/W24); telaprevir, n = 2 (W2/W9).

The most common side effect was anemia in 85% of patients: 95% in boceprevir and telaprevir groups received erythropoietin alone or combined with ribavirin dose reduction.

Conclusion: In liver transplanted patients, EOT rate was 82% and 38% with boceprevir and telaprevir, respectively. Among the overall population, 44% of patients discontinued therapy because of treatment failure or occurrence of serious adverse events.

1423 INTERIM ANALYSIS OF AN INTERFERON (IFN)- AND RIBAVIRIN (RBV)-FREE REGIMEN OF DACLATASVIR (DCV), ASUNAPREVIR (ASV), AND BMS-791325 IN TREATMENT-NAIVE, HEPATITIS C VIRUS GENOTYPE 1-INFECTED PATIENTS

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Background and Aims: The IFN- and RBV-free regimen of DCV (NS5A inhibitor), ASV (protease inhibitor) and BMS-791325 (non-nucleoside NS5B inhibitor, 75mg BID) for 24 or 12 weeks was well tolerated and achieved SVR4 and SVR12 >90% in treatment-naive, hepatitis C virus (HCV) genotype (GT) 1 patients. We present safety and SVR following 24 or 12 weeks of this treatment using two BMS-791325 doses (75 vs 150 mg BID).

Methods: This phase 2 study randomized treatment-naive, HCV GT1, non-cirrhotic patients (N = 32) 1:1 to DCV 60 mg QD, ASV 200 mg BID, and BMS-791325 75 mg BID for 24 (Group 1) or 12 (Group 2) weeks. Following safety evaluation, 34 additional patients were randomized to DCV, ASV, and BMS-791325 150 mg BID for 24 (Group 3) or 12 (Group 4) weeks. The primary end point was HCV RNA <25 IU/mL at 12 weeks post-treatment (SVR12). Safety and SVR from Groups 1 (SVR12), 2 (SVR24) and 4 (SVR4) are described, Groups 3 (SVR4) and 4 (SVR12) will be presented.

Results: Patients were mainly GT1a (74%), white race (79%), and IL28B non-CC (70%). 64 of 66 patients had HCV RNA <25 IU/mL by Week 4 (Table). In this interim analysis, there was no difference in virologic responses between 12 and 24 weeks of treatment. Overall, patients achieved SVR4 92% (46/50), SVR12 94% (30/32), and SVR24 94% (15/16) (Table). Three failures (Groups 3–4) were observed in patients receiving BMS-791325 150 mg 2-viral breakthrough, 1-relapse). No patients discontinued due to adverse events (AEs) related to DCV+ASV+BMS-791325. Most common AEs (≥10% total) were headache, asthenia, and gastrointestinal. Two serious AEs have been reported, both unrelated to DCV+ASV+BMS-791325. No hepatotoxicity or Grade 3/4 elevations of ALT/AST or bilirubin were reported.

Conclusion: DCV+ASV+BMS-791325 achieved high rates of SVR4, SVR12, and SVR24 in treatment-naive GT1 patients, characterized by GT1a and IL28B non-CC. This regimen was well tolerated with no apparent safety signals. Expansion of the current study is underway to better define the efficacy and safety of this regimen.

Table: Virologic response during and after treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration</th>
<th>BMS-791325 Dose</th>
<th>Week 4</th>
<th>Week 12</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 weeks</td>
<td>150 mg</td>
<td>16 (100)</td>
<td>16 (100)</td>
<td>16 (100)</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>75 mg</td>
<td>15 (94)</td>
<td>15 (94)</td>
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<tr>
<td></td>
<td>12 weeks</td>
<td>150 mg</td>
<td>15 (94)</td>
<td>15 (94)</td>
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<td></td>
<td>12 weeks</td>
<td>75 mg</td>
<td>16 (89)</td>
<td>16 (89)</td>
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<tr>
<td></td>
<td>12 weeks</td>
<td>150 mg</td>
<td>17 (94)</td>
<td>17 (94)</td>
<td>17 (94)</td>
</tr>
</tbody>
</table>

HCV RNA <25 IU/mL, n (%) was 94% (15/16) (Table). Three failures (Groups 3–4) were observed. Among 17 boceprevir and 16 telaprevir patients, 14 (82%) and 7 (43%) achieved an end of treatment (EOT) with an undetectable viral load, respectively (p = 0.032). Among 9 boceprevir and 5 telaprevir patients, 6 and 1 achieved SVR12, respectively. Among 6 patients in the boceprevir group, 3 achieved SVR24. In the telaprevir group, 29 patients discontinued therapy (serious adverse events, n = 13; virological breakthrough, n = 6; non-response, n = 9). In the boceprevir group, 14 patients discontinued therapy (serious adverse events, n = 5; virological breakthrough, n = 2; non-response, n = 4).

Conclusion: In liver transplanted patients, EOT rate was 82% and 38% with boceprevir and telaprevir, respectively. Among the overall population, 44% of patients discontinued therapy because of treatment failure or occurrence of serious adverse events.

1424 SYNERGISTIC INTERACTIONS OF HCV NSSA REPLICATION COMPLEX INHIBITORS SENSITIZE RESISTANT VARIANTS AND ENHANCE THE EFFICACY OF DACLATASVIR (DCV, BMS-790052) IN VITRO AND IN VIVO


Background: Daclatasvir (DCV, BMS-790052) is an HCV NSSA replication complex inhibitor (RCI) with pM to low nM potency for genotypes 1 to 6. The in vitro potency translated into robust antiviral effects in Phase I monotherapy studies; however, some variants with high levels of resistance (>1,000 fold) were observed.

Methods: Replicon studies of inhibitor-binding, inhibitor combinations, colony elimination and an HCV-infected chimeric mouse study were conducted to investigate the synergistic effects of a pair of NSSA inhibitors.

Results: The observation that an NSSA RCI can bind to resistant NSSA without inhibiting HCV replication led us to test whether the inhibitor-binding could sensitize NSSA and induce DCV inhibition of resistant variants. The potency (EC50) of DCV on GT-1a resistant replicon cells (Y93N) was tested in the presence of increasing concentrations of a 2nd NSSA-targeting molecule BMS-128. Both DCV and BMS-128 exhibit poor activity towards the Y93N variant, with EC50 values >400nM. However, concentrations of BMS-128 that show no significant inhibition (200 nM) enhance DCV’s potency by ~7,000 fold to 0.07 nM (see Figure). This synergistic enhancement of potency was observed with multiple resistant variants and genotypes. The synergistic effect has been validated in vivo using an HCV-infected chimeric mouse model. Colony elimination assays comparing combinations of inhibitors of NSSA, NS5B, NS3 protease, and the synergist BMS-128 demonstrated that the synergist could replace either the NS3 protease or NS5B inhibitors in combination therapy.

Conclusions: Our results suggest that NSSA molecules in the replication complex communicate with each other, providing...
a working hypothesis whereby BMS-128 binds resistant NSSA causing a conformational change that is transmitted to adjacent NSSA molecules, re-sensitizing resistant NSSA to DCV inhibition. This unprecedented synergistic anti-HCV activity with two NSSA-targeting molecules enhances the resistance barrier and expands sub-genotype coverage of DCV, providing additional options for HCV combination therapy.

### 1425 SIMEPREVIR (TMC435) WITH PEGINTERFERON/RIBAVIRIN FOR CHRONIC HCV GENOTYPE-1 INFECTION IN TREATMENT-NAÏVE PATIENTS: RESULTS FROM QUEST-1, A PHASE III TRIAL

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**Background and Aims:** Simeprevir (TMC435) is a potent, once-daily, oral, investigational, HCV NS3/4A protease inhibitor. QUEST-1 (TMC435-C208; NCT01289782) is a Phase III, randomised, double-blind, placebo-controlled trial assessing simeprevir plus peginterferon α-2a/ribavirin (PR) versus placebo plus PR in treatment-naïve patients with genotype-1 infection. Safety and SVR12 results from a primary (Week 60) analysis are presented.

**Methods:** HCV genotype-1-infected patients with METAVIR score F0–F4 (n=394), stratified by HCV subtype and host IL28B genotype; and 56% infected with HCV genotype-1a.

**Background:** Licensed hepatitis B vaccines with alum require 3 doses over 6 months in healthy adults. Vaccine adjuvants such as Toll-like receptor 9 (TLR9) agonists have the potential to induce higher rates of protection with fewer doses and be equally safe.

**Methods:** Two multicenter, observer-blinded, randomized, phase 3 studies were conducted in 4867 persons 18–70 years of age, comparing two doses of HEPLISAV (20 mcg rHBsAg combined with 3000 mcg 1018 ISS, a TLR9 agonist) given at 0 and 1 month (placebo at 6 months) to three doses of Engerix-B (EB) (20 mcg rHBsAg combined with 500 mcg alum) given at 0, 1 and 6 months. Peak seroprotection rates (SPR = % with anti-HBs ≥ 10 mIU/mL) were compared in analyses pooling the modified intent-to-treat (mITT) populations of the two trials. The mITT population included all subjects who received a study injection and had an anti-HBs result. The safety of the vaccines was also evaluated in pooled analyses.

**Results:** Among the 4815 subjects in the mITT population (HEPLISAV 3736; EB 1079), the mean age (HEPLISAV 47.2 years; EB 46.0 years) was similar in both groups. The peak SPR in the HEPLISAV group of 76.7% (95% CI: 74.0%, 79.2%) at month 7 (p<0.0001) were similar in both groups. The relative risks of autoimmune events (EB 0.35%; HEPLISAV 0.27%), rare serious autoimmune events (EB 0.09%; HEPLISAV 0.05%), and ANCA+ vasculitis (EB 0.09%; HEPLISAV 0.026%) were higher in the EB group than in the HEPLISAV group. An analysis of all autoimmune events adjusted for time since last vaccine dose demonstrated a significantly higher rate in the EB group (1.35 per 100 person-years) than in the HEPLISAV group (0.4 per 100 person-years; relative risk = 3.4, 95% CI 1.1, 10.9).

**Conclusion:** HEPLISAV given as 2 doses over 1 month induced significantly higher rates of seroprotection with lower rates of autoimmune diseases than Engerix-B given as 3 doses over 6 months.

### 1427 THE BILE ACID RECEPTOR TGR5 PROMOTES CHOLANGIOCYTE PROLIFERATION THROUGH A cSRC–EGFR–ERK SIGNALLING PATHWAY

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**Introduction and Aims:** TGR5 (Gpbar-1) is a membrane-bound, G-protein-coupled bile acid receptor, expressed in different types of cells.
liver cells, including cholangiocytes [1]. Bile acids can promote cholangiocyte secretion and proliferation [2], however, the function of TGR5 in cholangiocytes is largely unknown. Aim of the present study was to elucidate the role of TGR5 for cholangiocyte proliferation and to identify TGR5-dependent signalling pathways in cholangiocytes.

**Methods:** TGR5 knockout and wildtype mice were fed a cholic acid diet (0.5%) for 7 days. Ductular proliferation was assessed by cytokeratin (CK)-19 staining. In isolated cholangiocytes proliferation was measured by BrDU incorporation after stimulation with bile acids or specific TGR5 agonists. TGR5-dependent pathways were studied with different kinase inhibitors (SU6656, PP1, PP2, AG1478, U0126). Western blotting was used to confirm the phosphorylation of the EGFR and ERK1/2.

**Results:** While the amount of CK19-positive bile ducts was similar between TGR5 wildtype and knockout mice on chow diet, a significant increase of bile duct proliferation was observed in wildtype mice after 7 days of cholic acid diet as compared to the TGR5 knockout mice on the same diet. Treatment of isolated cholangiocytes from TGR5 wildtype and knockout mice with tauroliothocholic acid (TLC 10 and 25 μM) or a TGR5 specific agonist resulted in an increased cholangiocyte proliferation exclusively in wildtype-derived cells. Preincubation of the wildtype cholangiocytes with inhibitors of ERK1/2, EGFR and cSrc abolished TLC- and TGR5 agonist-induced BrDU incorporation. Treatment with inhibitors of ROS-formation such as N-acetylcystein or apocynin also reduced TLC-mediated BrDU incorporation. In contrast inhibition of adenylate cyclase (SQ22536/dideoxyadenosine) had no effect on TLC/TGR5 agonist-dependent cholangiocyte proliferation. Stimulation of wildtype cholangiocytes with TLC and a TGR5 agonist significantly elevated tyrosine phosphorylation of the EGFR at positions 845 and 1045. Furthermore, TLC and the TGR5 agonist led to a significant increase in ERK1/2 phosphorylation exclusively in TGR5 wildtype-derived cholangiocytes.

**Conclusion:** TGR5 mediates bile acid-induced cholangiocyte proliferation through activation of a cSrc-EGFR-ERK signalling pathway, which is independent of adenylate cyclase activation.

**Reference(s)**


**1428**

**INVERSE CHANGES IN HEPATIC EXPRESSION OF INTERFERON LAMBDA AND ALPHA AND IFNL4 GENOTYPE ARE ASSOCIATED WITH TREATMENT RESPONSE IN SOFOSBUVIR/RIBAVIRIN TREATED HCV GENOTYPE-1 SUBJECTS**

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**Background:** Mechanisms of relapse with directly acting antiviral (DAA) therapy for HCV are unclear. The recently identified IFNL4-AG genetic variant is associated with slower HCV viral kinetic decline and poorer treatment outcome with interferon-alpha based therapy, and predicts expression of a novel interferon protein IFNL4. We explored endogenous interferon balance in the liver and effect of IFNL4 genotype on response to DAA therapy.

**Methods:** Sixty chronic HCV genotype-1, treatment naïve subjects were treated with the NS5B RNA polymerase inhibitor Sofosbuvir with ribavirin for 24 weeks. Subjects had a high prevalence of negative treatment predictors including black race (83%) and unfavorable IFNL4-AG genotype (85%). Core liver biopsies were obtained in eight subjects before and at end of treatment to measure expression changes in interferons and interferon stimulated genes (ISGs). Genotyping of IFNL4 was performed with custom TaqMan assays. Viral kinetic (VK), pharmacokinetic (PK), and pharmacodynamic (PD) data for 25 subjects was fitted using a VK-PK-PD model.
Results: Of 55 subjects who completed the study, 38 achieved SVR12 and 17 relapsed. In 8 subjects with paired liver biopsies, 7 achieved SVR12 and 1 relapsed; 6 of 8 carry at least 1 IFNL4–ΔG allele. In liver, we find treatment-related decrease in expression of IFNL1, IFNL2, IFNL3, IFNL4–ΔG, and IFNG (Figure 1), which correlates with decrease in hepatic expression of ISGs. Inversely, IFNA2 expression increased in most subjects who achieved SVR12, while expression decrease in hepatic expression of ISGs. Inversely, IFNL2, IFNL3 liver, we find treatment-related decrease in expression of HCV-infected cirrhotics are in urgent need of therapy yet are often under-represented in clinical trials. Efficacy...
and safety of BOC/P/R was evaluated in compensated (no history of ascites, UGI bleeding, encephalopathy) cirrhotic patients using meta-analysis of five Phase 3 trials.

**Methods:** All analyzed patients received 4 weeks of P/R lead-in followed by BOC/P/R or P/R for 24, 32 or 44 weeks. Metavir scores (centrally-read) of liver biopsies from 2415 patients showed: 2074 F0–F2; 129 F3; 212 F4. Multivariate logistic regression (MLR) models were used to identify baseline and on-treatment predictors of sustained virologic response (SVR), combining F3/F4 patients to increase power to identify predictors. Safety was evaluated by serious adverse event (SAE) rates. Anemia was defined as a hemoglobin <10g/dL.

**Results:** Of 180 F4 patients treated with BOC/P/R, 62% were male, mean age 53 yrs, log10 viral load 6.39 and 43% with <150,000 platelets/mm3. Pooled meta-estimates for SVR rates in F4 patients were 55% (treated with BOC/P/R) vs. 17% (treatment with P/R) (Table). MLR identified 4 predictors of SVR in F3/F4 patients: undetectable HCV-RNA at treatment week (TW) 8 (i.e., after 4 weeks of P/R lead-in + 4 weeks of BOC/P/R; odds ratio (OR)=10.57; p<0.0001); ≥1 log10 decline in HCV-RNA at TW4 (i.e., after 4 weeks of P/R lead-in; OR=2.64; p=0.0053); male sex (OR=2.23; p=0.0141) and baseline HCV-RNA ≥800,000 IU/mL (OR=2.55; p=0.0383). No F3 (0/5) or F4 (0/17) patients with <3 log10 decline and detectable HCV-RNA at TW8 achieved SVR. SAEs occurred in 18% (33/180) of F4, 12% (13/107) of F3 and 12% (189/1638) of F0–F2 patients on BOC/P/R. More F4 patients (113/178; 63%) on BOC/P/R developed anemia than F3 (60/107; 56%) or F0–F2 (870/1629; 53%).

**Conclusions:** Based on an overall favorable benefit-risk profile, BOC/P/R can be safely and effectively used in compensated cirrhotic patients. Efficacy is particularly high in cirrhotic patients achieving SVR-12. With PEG-IFN+RBV treatment, patients with SVR-12 had significantly improved HRQL scores in Bodily-Pain ($\Lambda = 18.5 \pm 5.2$, $p = 0.02$), Vitality ($\Lambda = 11.7 \pm 4.7$, $p = 0.04$) and Mental-Health ($\Lambda = 11.5 \pm 4.5$, $p = 0.05$) than patients without SVR-12. With PEG-IFN+RBV treatment, patients with SVR-12 had no significant improvement. In comparison to general population, SOF+RBV patients achieving SVR had higher scores in Vitality ($p=0.009$) and General-Health ($p=0.005$) and similar scores in all the other SF-36 domains. In POSITRON, all SF-36 domain scores remained similar between SOF+RBV and placebo groups after 12 weeks of treatment ($p>0.05$). At follow-up week 4, patients receiving SOF+RBV who achieved SVR had higher General-Health scores as compared to placebo ($p=0.02$). Multivariate analyses showed that depression and anemia predicted HRQL impairment in both studies ($\beta = -4.06$ to $-8.96$, $p<0.001$ and $\beta = -4.6$ to $-5.01$, $p<0.05$; respectively), and in FISSION, SOF+RBV was independently associated with better HRQL than PEG-IFN+RBV ($\beta = 5.36$, $p=0.003$).

**Conclusions:** SOF+RBV is associated with better HRQL than PEG-IFN+RBV and similar to patients not receiving active treatment. Achieving SVR on SOF+RBV is associated with improvement in HRQL.

**Table: Meta-analysis**

<table>
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<th>Treatment</th>
<th>% SVR (95% CI), no. of patients</th>
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<tbody>
<tr>
<td></td>
<td>F4</td>
</tr>
<tr>
<td>BOC/P/R</td>
<td>55% (43.66), n=180</td>
</tr>
<tr>
<td>P/R</td>
<td>17% (0.41), n=32</td>
</tr>
</tbody>
</table>

1431

**MINIMAL IMPACT OF SOFOSBUVIR+RIBAVIRIN ON HEALTH RELATED QUALITY OF LIFE (HRQL) COMPARED TO PEGYLATED INTERFERON + RIBAVIRIN FOR CHRONIC HEPATITIS C (CH-C): RESULTS FROM FISSION+POSITRON STUDIES**

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**Background:** Interferon-based treatment for CH-C causes substantial HRQL impairment. Interferon-free regimens are currently being developed.

**Aim:** Assess HRQL in GT2-GT3 CH-C patients treated with sofosbuvir (SOF) and ribavirin (RBV) in two phase-3 studies.

**Methods:** Short Form-36 (SF-36) was administered at baseline, end-of-treatment [week-12 for SOF and week-24 for pegylated-interferon (PEG-IFN) containing regimens] and post-treatment to treatment-naïve subjects receiving either SOF+RBV (N=105) for 12-weeks or PEG-IFN+RBV (N=110) for 24-weeks (FISSION) and to IFN-ineligible, -tolerant, or -unwilling subjects receiving SOF+RBV (N=207) or placebo (N=71) for 12 weeks (POSITRON). Clinical-laboratory data were collected. Virologic data were blinded.

**Figure 1. HRQL from FISSION study.**

**LATE BREAKING ABSTRACTS**

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Rifaximin. The potential association of rifaximin treatment with CDAD and pseudomembranous colitis (PMC) cannot be ruled out. Caution is advised.

Interactions:

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Dosage and Administration:

Recommended dose: 550 mg twice a day.

For episodes of hepatic encephalopathy in patients 18 years of age.

For End-Stage Liver Disease score >25.

Side Effects:

Common adverse reactions include: abdominal distension, diarrhoea, nausea, vomiting, ascites, peripheral oedema, peripheral neuropathy, dermatological reactions (rash, pruritus, arthralgia).

Rare adverse reactions include: jaundice, elevation of blood bilirubin, elevation of liver enzymes, elevation of blood urea nitrogen, elevation of creatinine, elevated creatine kinase, hyperglycaemia, hypertriglyceridaemia.

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