The method of \textit{in vivo} evaluation of hemostasis: Spatial thrombodynamics

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Background: Coagulation is a cascade of reactions that eventually leads to formation of thrombin and fibrin. The two most frequently used tests to describe the coagulation system are activated partial thromboplastin time and international normalized ratio. Both tests are performed in vitro by mixing coagulation factors and measuring the time until the clot forms, but neither represents the biology of coagulation in vivo.

Objective: To assess the diagnostic potential of thrombodynamics.

Methods: Publications from potentially relevant journals were searched in Medline and by hand.

Results and discussion: In the spatial clot growth assay, hypercoagulability was characterized by quantitative and qualitative changes. It identified hypercoagulation in rats with induced microvascular thrombosis. The method was used to study coagulation in hemophilia after inhibition of tissue factor pathway inhibitor. It may be used in platelet-free, platelet-poor and platelet-rich plasma. In a small study the assay was able to predict thrombosis in patients with sepsis.

Conclusion: Thrombodynamics is a promising method for measuring coagulation by imitation of in vivo conditions, and is being used in basic research. More work and correlative clinical investigations are still required to determine whether this method will be clinically useful in the future.

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The two main tests currently used to evaluate the coagulation system are prothrombin time (PT) and international normalized ratio (INR), which characterize the extrinsic pathway and activated partial thromboplastin time (aPTT). aPTT characterizes the contact activation pathway. Both tests
are performed in vitro by mixing coagulation factors and measuring the time until clot formation. This is not how coagulation happens in vivo. Coagulation is a spatially heterogeneous process, in which there is no artificial mixing; factors move by diffusion and the coagulation cascade proceeds on cell-surface membranes. PT and aPTT are useful only to identify hypocoagulable conditions and are not sensitive enough to determine the risk of thrombosis.

Tissue factor (TF) is unique among clotting factors as it is expressed on nonvascular cells. Acute inflammation including sepsis may cause hypercoagulation. Inflammation may stimulate expression of TF on endothelial cells and leukocytes. Thrombodynamics is a method for measurement of coagulation by direct visualization of spatial clot formation. In this method, clotting is activated by introducing surface-immobilized TF into the patient’s plasma. The process of clot formation and propagation is recorded by a light-scattering camera taking pictures every 15 seconds. The following parameters are calculated from clot size versus time plots: lag time (delay after which clot starts to form); initial clot growth velocity; and stationary velocity. Figure 1 is a schematic representation of the method of thrombodynamics.

According to the report presented recently at the Congress of the International Society on Thrombosis and Hemostasis in Amsterdam, The Netherlands, this method was able to detect hypercoagulation in rats. Microvascular thrombosis was provoked in rats by administration of epinephrine/collagen solution. Thrombodynamics assay, unlike aPTT, was able to identify hypercoagulability in rats with microvascular thrombosis. Earlier, this method was used in coagulation research after decreasing the activity of tissue factor pathway inhibitor (TFPI), which mostly affects clotting initiation. The experiment was carried out on plasma of patients with hemophilia A and patients without bleeding disorders. TFPI was inhibited by its antagonist BAX499. BAX499 significantly improved spatial fibrin clot formation in normal and hemophilia A plasma initiated with low-density TF in a TFPI-dependent manner, manifested by a lag time.
shortening and an increase in clot size. Compared with homogeneous assays such as thrombin
generation, thrombodynamics was also able to reveal differences in the mode of action between
TFPI antagonism and extrinsic pathway stimulation by recombinant factor VIIa: BAX499 had no
effect on stationary velocity of clot propagation, while addition of recombinant factor VIIa
increased this parameter 5–8-fold. Another study implementing thrombodynamics looked at the
effects of BAX499 in patients with hemophilia A with different concentrations of factor VIII.4
The study questioned if adding two procoagulants, BAX499 and factor VIII, would lead to
hypercoagulation. At a factor VIII activity <30%, BAX499 significantly decreased the lag time
and increased initial clot growth velocity. The clot size increased 200% with addition of BAX499
at a factor VIII concentration <5% but only 30% at concentrations >30%. The likely explanation
for the above phenomenon was different mechanisms of action of factor VIII and BAX499.
Factor VIII acts as a co-factor for factor IXa and improves factor Xa formation via acceleration
of factor-IXa-dependent catalysis (i.e. intrinsic tenase); while BAX499 accelerates factor X
activation by the factor VIIa–TF complex (i.e. extrinsic tenase) by preventing its inactivation by
TFPI. The study suggested the possibility of TFPI inhibitor administration independently of
factor VIII levels, and treatment of patients with hemophilia A of different severity without the
risk of causing thrombosis.

Dabigatran (Pradaxa) is an oral thrombin inhibitor used to treat atrial fibrillation. The difficulty
of reversing the action of dabigatran may be an issue in clinical practice. Thrombodynamics was
used to study the ability of prothrombin complex concentrate (PCC), activated PCC (APCC) and
recombinant activated factor VII to reverse the action of dabigatran.5 Dabigatran attenuated the
initial clot growth velocity, as predicted. APCC but not PCC or recombinant factor VII increased
the initial clot growth velocity.
In another study, thrombodynamics was used to identify hypercoagulation in patients with paroxysmal nocturnal hemoglobinuria (PNH). This disease is characterized by a hypercoagulable state associated with acute hemolysis, and thrombosis is the main cause of death in PNH. Three patients with PNH were treated with eculizumab, which is a monoclonal antibody directed against complement factor C5, thereby preventing complement-induced lysis of erythrocytes. Thrombodynamics is one of the global tests used to monitor the state of the coagulation system. It revealed hypercoagulation with increased stationary clot growth velocity in all 3 patients who presented with hemolytic crisis before treatment. In the 8 months follow-up period, 2 of the 3 patients had a hemolytic crisis that was accompanied by an increase in the stationary clot growth velocity.

Thrombodynamics was also used to research clot formation in platelet-rich plasma. Clot growth velocity was moderately increased in platelet-rich plasma compared with platelet-free plasma when activated by high TF density, and greatly increased when activated by low TF density. In another study, thrombodynamics was used to evaluate clot formation in plasma after adding activated platelets or platelet microparticles (PMPs). Addition of activated platelets and PMPs increased the clot growth velocity measured by thrombodynamics until saturation was reached. The method was used to show that the surface of PMPs was at least 50–100 times more procoagulant than that of activated platelets. In the same study, a thrombin generation test (another global assay that measures the overall tendency of a plasma sample to form thrombin after initiation of coagulation) provided evidence that the increase in activated platelets or PMPs accelerates the onset of coagulation and increases maximal thrombin concentration.

Recently, thrombodynamics was compared with D-dimer assay to assess patients with sepsis for hypercoagulation. D-dimer is frequently elevated in thrombosis and inflammatory conditions and is a surrogate marker for lysis of already formed clots. In a case of subclavian vein
thrombosis, spatial growth assay identified hypercoagulation 1 day before thrombosis occurred; D-dimer level increased only after thrombosis; and aPTT and PT remained within the normal range. Increased clot growth velocity was associated with a subsequent increase in D-dimer levels. Ninety-two percent of samples that showed hypercoagulation were accompanied by spontaneous clot formation far from introduced TF activator. This spontaneous clot formation could be secondary to soluble TF released in response to inflammation.

Plasmapheresis is known to increase the risk of bleeding in patients and lead to hypocoagulation because of filtering out of clotting factors.\textsuperscript{10} This may lead to increased concentration of clotting factors in plasmapheresis products. Thrombodynamics was used to show hypercoagulation in platelet-free frozen plasma and platelet-poor plasma.\textsuperscript{11} The samples were taken from young male donors and the test was run on the plasma before plasmapheresis and on the products. Plasmapheresis shifted coagulation parameters from a normo- to hypercoagulable state in both platelet-fee and platelet-poor products.

In summary, the spatial growth assay, thrombodynamics, is a way of assessing coagulation through simulation of \textit{in vivo} conditions. Characteristics of the test, lag time, initial clot growth and stationary velocities, represent different phases of clot formation. It is promising to identify the risk of thrombosis in inflammatory conditions including sepsis or to rule out venous thromboembolism. Currently, thrombodynamics is not the only global test for measuring coagulation. Thrombin generation assay is another global test that measures the overall tendency of a plasma sample to form thrombin after initiation of coagulation.\textsuperscript{12} So far, thrombodynamics has not been compared directly with thrombin generation assays. Although thrombodynamics assay is already commercially available and has potential in clinical practice, much additional work and correlative clinical investigations are still required to determine whether this method will be clinically useful in the future.
References


Figure 1: TF introduced to patient’s plasma. The clot formation is recorded by camera and the information is processed by computer.