LABORATORY MARKERS OF COAGULATION ACTIVATION

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For years, clinicians have sought to employ blood tests to predict thrombotic events in high-risk patients. Advances in our knowledge of the biochemistry of coagulation in the last two decades have facilitated the development of sensitive and specific assays that are able to detect the generation of coagulation enzymes in vivo. It has thus far not been possible to measure directly the levels of most hemostatic enzymes in vivo. Many of the enzymes are not available for quantification in blood as they are neutralized rapidly by naturally occurring protease inhibitors or bound to cellular receptors in the locale in which they are generated. Faced with these obstacles, investigators have resorted to developing immunochemical assays for peptides that are liberated with the activation of coagulation enzymes.

To establish a relationship between the levels of activation markers of coagulation and thromboembolic disease, a clinical study must be carefully designed and executed. First, the selected assays should be specific for the activation products of interest, possess sufficient sensitivity, and be properly standardized to perform in a reproducible manner. Second, care must be taken to ensure that technical factors do not introduce in vitro artifacts that can significantly alter results. These include the quality of the venipuncture procedures, the choice of anticoagulant cocktail for blood specimens, the sample processing procedure, and the plasma storage conditions. Third, objective endpoints must be used to establish a diagnosis of thrombosis. Fourth, bias in patient selection should be avoided and an appropriate control group of patients must be chosen for comparison with thrombosis cases. Prospectively designed studies must have sufficient power to detect significant differences between cases and controls.

Assays for Coagulation Activation

It is now appreciated that the factor VII-tissue factor mechanism is important in the physiologic activation of factor IX, which can be monitored by measuring the levels of the factor IX activation peptide ($t^1/2$ in the circulation ~15 min).[1, 2] This assay also reflects the action of factor XIa upon factor IX. Factor X activation mediated by the extrinsic or intrinsic pathways can be monitored by measuring the factor X activation peptide ($t^1/2$ in the circulation ~30 min).[3]

Thrombin generation takes place at an appreciable rate under physiologic conditions only in the presence of factor Xa, factor Va, calcium ions, and activated platelets. During this process, the amino terminus of the prothrombin molecule is released as the inactive F_{1+2} fragment ($t^1/2$ in the circulation ~90 min). Immunoassays have been developed for this fragment,[4-6] which serves as a measure of prothrombin activation in vivo, and several are now available commercially in kit form.[7-9] Once evolved, thrombin converts fibrinogen into fibrin releasing fibrinopeptide A ($t^1/2$ in the circulation ~3B5 min), which can also be measured by immunoassay.[10-13]

Alternatively, thrombin can also rapidly activate protein C by binding to thrombomodulin on vascular endothelial cells, and an immunoassay has been developed for the activation peptide liberated from the zymogen during this transformation.[14]

Coagulation Factor Deficiencies

The investigation of patients with hereditary coagulation factor deficiencies using activation peptide assays has generated information regarding the pathways responsible for coagulation activation in vivo under basal conditions (i.e., the absence of thrombosis or provocative stimuli). Patients with factor VII deficiency but not factor XI deficiency have reduced levels of factor IX activation,[1] whereas patients with deficiencies of factor VIII or factor IX have normal levels of factor X and prothrombin activation.[3] The infusion of relatively small doses of recombinant factor VIIa (10B20 :g/kg of body weight) in factor VII-deficient patients results in substantial elevations in the plasma concentrations of the factor IX activation peptide, factor X activation peptide, and prothrombin fragment F_{1+2}.[15] Thus these data demonstrate that the factor VII-tissue factor pathway is largely responsible for the activation of factor IX as well as factor X in the basal state.[1, 3, 15]

It has also been shown that administration of a monoclonal antibody-purified factor IX concentrate to individuals with hemophilia B increases plasma factor IX activation peptide levels that are initially greatly decreased, but does not change factor X activation peptide or F_{1+2} measurements.[15] Infusion of highly purified factor VIII concentrates in hemophilia A patients results in no significant change in the plasma concentrations of factor X activation peptide and F_{1+2}.[15] These observations indicate that the factor IXa-factor VIIIa-cell surface complex is unable to activate factor X under basal conditions. In response to vascular injury or thrombotic stimuli, it is surmised that increased formation of free thrombin or factor Xa via the action of the factor VII-tissue factor pathway generates factor VIIIa or a natural surface (e.g., activated platelets) on which assembly of the factor IXa-factor VIIIa complex takes place. This hypothesis is consistent with the severe bleeding tendency of most patients with factor VIII or factor IX deficiency, and the insensitivity of the factor X activation peptide and F_{1+2} assays to deficiencies of these two proteins.

The above mechanistic findings derived from studies of patients with coagulation factor deficiencies have significant potential implications with regard to the utility of basal coagulation system markers in diagnosing prethrombotic patients.[15] It follows that the conversion of a prethrombotic state to a thrombotic event occurs due to small increases in the generation rates of hemostatic enzymes that exceed the inhibitory threshold of an individual=s endogenous anticoagulant mechanisms as well as the sequestration of these proteases on specialized cell surfaces. It remains to be determined whether persons with elevated basal levels of coagulation system markers are more likely to respond in a hypersensitive fashion to environmental stimuli. Because the activity of the blood coagulation mechanism in such individuals is closer to the threshold of normal inhibitory processes, such individuals may generate slightly more thrombin via the factor VII-tissue factor mechanism via the extrinsic pathway. This thrombin could then be used

to ignite the dormant intrinsic cascade, which could ultimately result in the generation of large amounts of free thrombin and the development of arterial or venous thrombosis.

Deficiencies of Natural Anticoagulants

Hereditary deficiencies of protein C, protein S, and resistance to activated protein C have all been associated with hypercoagulable states, and coagulation activation has been investigated in patients with deficiencies of each of these critical anticoagulant proteins. In asymptomatic people with heterozygous deficiencies of protein C, protein S, and resistance to activated protein C, the mean F_{1+2} concentration is significantly increased as compared to age-matched controls.[16-18] Approximately one-third of patients have levels greater than the upper normal limit of normal controls (defined as the mean + 2 standard deviations).[16, 17] The elevations in F_{1+2} measurements are not due to diminished clearance of the fragment.[16] Fibrinopeptide A levels were elevated in approximately 20% of subjects.[16, 17]

In asymptomatic persons with heterozygous protein C deficiency, protein C activation as measured by the protein C activation peptide assay is reduced to about 50% of normal.[16] In two adult patients with homozygous protein C deficiency, it has been shown that protein C activation as well as F_{1+2} can be normalized by administration of a monoclonal antibody purified protein C concentrate.[19] Thus it has been shown that the protein C anticoagulant pathway can inhibit prothrombin activation in vivo, and that the activation of protein C by the thrombin-thrombomodulin complex is a tonically active mechanism in the regulation of coagulation system activation.

Coronary Artery Disease

Elevated levels of fibrinopeptide A have been reported early after the onset of acute transmural myocardial infarction,[20-24] and the values then decrease over the subsequent 24 hours.[22] The increased fibrinopeptide A levels rapidly return to normal after the administration of heparin,[22, 23] suggesting that the generated thrombin is readily inactivated by heparin-antithrombin III complexes.

Using assays for F_{1+2} and fibrinopeptide A, the extent of hemostatic mechanism hyperactivity has been quantitated in patients presenting with unstable angina or acute myocardial infarction and compared to control patients with stable angina or normal individuals matched for age and sex.[25] At the onset of acute coronary syndromes, patients with unstable angina or acute myocardial infarction have significantly elevated concentrations of F_{1+2} and fibrinopeptide A, which reflects the presence of ongoing intracoronary thrombosis. At six months, patients who did not experience additional cardiac events, including silent ischemia, were reinvestigated and found to manifest increased concentrations of F_{1+2} with virtually normal plasma levels of fibrinopeptide A.

The virtual normalization of fibrinopeptide values in most patients has been widely interpreted as demonstrating that coagulation system hyperactivity in these disorders is restricted to the time period during which the coronary thrombus is generated. However, the aforementioned investigation, using the F_{1+2} assay that monitors an earlier point in the coagulation cascade, supports the view that abnormalities of the hemostatic

mechanism frequently occur in patients with acute coronary syndromes long after clinical stabilization. The occurrence of a persistent hypercoagulable state in patients with unstable angina or myocardial infarction[25] as well as stable angina appears to be independent of the severity of coronary artery atherosclerosis.[26] The above findings raise the interesting possibility that increased activity of the hemostatic mechanism may predate the onset of acute coronary syndromes, and this hypothesis is currently under examination in a large prospective trial (Northwick Park Heart Study II).[27, 28]

Conclusion

Studies employing activation peptide assays indicate that a biochemical imbalance between procoagulant and anticoagulant mechanisms can be detected in the blood of patients prior to developing thrombosis and that coagulation activation is suppressed by oral anticoagulant therapy.[29-32] Properly designed prospective studies will be required to evaluate whether these assays will improve our ability to identify individuals destined to develop a clinically relevant hypercoagulable state. It also remains to be determined whether such assays can pinpoint patients most likely to benefit from prolonged anticoagulant therapy or provide a better means for determining the optimal amount of drug to administer.

References

1. Bauer KA, Kass BL, ten Cate H, Hawiger JJ, Rosenberg RD. Factor IX is activated in vivo by the tissue factor mechanism. Blood 76:731, 1990.
2. Boisclair MD, Lane DA, Philippou H, et al. Mechanisms of thrombin generation during surgery and cardiopulmonary bypass. Blood 82:3350, 1993.
3. Bauer KA, Kass BL, ten Cate H, Bednarek MA, Hawiger JJ, Rosenberg RD. Detection of factor X activation in humans. Blood 74:2007, 1989.
4. Lau HK, Rosenberg JS, Beeler DL, Rosenberg RD. The isolation and characterization of a specific antibody population directed against the prothrombin activation fragments F_2 and F_{1+2}. J Biol Chem 254:8751, 1979.
5. Teitel JM, Bauer KA, Lau HK, Rosenberg RD. Studies of the prothrombin activation pathway utilizing radioimmunoassays for the F_2/F_{1+2} fragment and the thrombin-antithrombin complex. Blood 59:1086, 1982.
6. Boisclair MD, Lane DA, Philippou H, Sheikh S, Hunt B. Thrombin production, inactivation and expression during open heart surgery measured by assays for activation fragments including a new ELISA for prothrombin fragment F_{1+2}. Thromb Haemostas 70:253, 1993
7. Shi Q, Ruiz JA, Perez LM, et al. Detection of prothrombin activation with a two-site enzyme immunoassay for the fragment F1.2 (abstract). Thromb Haemostas 1989;62:165, 1989.
8. Pelzer H, Schwart A, Stuber W. Determination of human prothrombin activation fragment 1+2 in plasma in plasma with an antibody against a synthetic peptide. Thromb Haemostas 65:153, 1991.

9. Hursting MJ, Butman BT, Steiner JP, et al. Monoclonal antibodies specific for prothrombin fragment 1.2 and their use in a quantitative enzyme-linked immunosorbent assay. Clin Chem 39:583, 1993.
10. Nossel HL, Younger LR, Wilner GD, Procupez T, Canfield RE, Butler VP Jr. Radioimmunoassay of human fibrinopeptide A. Proc Natl Acad Sci USA 68:2350, 1971.
11. Nossel HL, Yudelman I, Canfield RE, et al. Measurement of fibrinopeptide A in human blood. J Clin Invest 54:43, 1974.
12. Nossel HL, Ti M, Kaplan KL, Spanondis K, Soland T, Butler VP Jr. The generation of fibrinopeptide A in clinical blood samples: evidence for thrombin activity. J Clin Invest 58:1136, 1976.
13. Cronlund M, Hardin J, Burton J, Lee L, Haber E, Bloch KJ. Fibrinopeptide A in plasma of normal subjects and patients with disseminated intravascular coagulation and systemic lupus erythematosus. J Clin Invest 58:142, 1976.
14. Bauer KA, Kass BL, Beeler DL, Rosenberg RD. Detection of protein C activation in humans. J Clin Invest 74:2033, 1984.
15. Bauer KA, Mannucci PM, Gringeri A, et al. Factor IXa-factor VIIIa-cell surface complex does not contribute to the basal activation of the coagulation mechanism in vivo. Blood 79:2039, 1992.
16. Bauer KA, Broekmans AW, Bertina RM, et al. Hemostatic enzyme generation in the blood of patients with hereditary protein C deficiency. Blood 71:1418, 1988.
17. Mannucci PM, Tripodi A, Bottasso B, et al. Markers of procoagulant imbalance in patients with inherited thrombophilic syndromes. Thromb Haemostas 67:200, 1992.
18. Greengard JS, Eichinger S, Griffin JH, Bauer KA. Variability of thrombosis among homozygous siblings with resistance to activated protein C due to an Arg to Gln mutation in the gene for factor V. N Eng J Med 331:1559, 1994.
19. Conard J, Bauer KA, Gruber A, et al. Normalization of markers of coagulation activation with a purified protein C concentrate in adults with homozygous protein C deficiency. Blood 82:1159, 1993.
20. Johnsson H, Orinius E, Paul C. Fibrinopeptide A (FpA) in patients with acute myocardial infarction. Thromb Res 16:255, 1979.
21. van Hulsteijn H, Kolff J, Briet E, van der Laarse A, Bertina R. Fibrinopeptide A and beta thromboglobulin in patients with angina pectoris and acute myocardial infarction. Am Heart J 107:39, 1984.
22. Eisenberg P, Sherman LA, Schechtman K, Perez J, Sobel BE, Jaffee AS. Fibrinopeptide A: a marker for acute coronary thrombosis. Circulation 71:912, 1985.
23. Mombelli G, Im Hof V, Haeberli A, Straub PW. Effect of heparin on plasma fibrinopeptide A in patients with acute myocardial infarction. Circulation 69:684, 1984.
24. Rapold HJ, Grimaudo V, Declerck PJ, Kruithof EKO, Bachmann F. Plasma levels of plasminogen activator inhibitor type 1, $-thromboglobulin, and fibrinopeptide A before, during, and after treatment of acute myocardial infarction with alteplase. Blood 78:1490, 1991.
25. Merlini PA, Bauer KA, Oltrona L, et al. Persistent activation of the coagulation mechanism in unstable angina and myocardial infarction. Circulation 90:61, 1994.

26. Kienast J, Thompson SG, Raskino C, et al. Prothrombin activation fragment 1+2 and thrombin antithrombin III complexes in patients with angina pectoris: Relation to the presence and severity of coronary atherosclerosis. Thromb Haemostas 70:550, 1993.
27. Miller GJ, Wilkes HC, Meade TW, Bauer KA, Barzegar S, Rosenberg RD. Haemostatic changes that constitute the hypercoagulable state (letter). Lancet 338:1279, 1991.
28. Miller GJ, Bauer KA, Barzegar S, et al. The effects of quality and timing of venipuncture on markers of blood coagulation in healthy middle-aged men. Thromb Haemostas 73:82, 1995.
29. Conway EM, Bauer KA, Barzegar S, Rosenberg RD. Suppression of hemostatic system activation by oral anticoagulants in the blood of patients with thrombotic diatheses. J Clin Invest 80:1535, 1987.
30. Mannucci PM, Bottasso B, Tripodi A. Prothrombin fragment 1+2 and intensity of treatment with oral anticoagulants (letter). Thromb Haemostas 66:741, 1991.
31. Millenson MM, Bauer KA, Kistler JP, Barzegar S, Tulin L, Rosenberg RD. Monitoring Amini-intensity@ anticoagulation with warfarin: comparison of the prothrombin time using a sensitive thromboplastin with prothrombin fragment F_{1+2} levels. Blood 79:2034, 1992.
32. Elias A, Bonfils S, Daoud-Elias M, et al. Influence of long term oral anticoagulants upon prothrombin fragment 1+2, thrombin-antithrombin III complex and D-dimer levels in patients affected by proximal deep vein thrombosis. Thromb Haemostas 69:302, 1993.