Commentary
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Risk factors for thromboembolism: pathophysiology and detection

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An association between the risk of venous thromboembolism and a hypercoagulable state has been recognized for some years. More recent advances in thrombosis research and laboratory medicine have provided an ever-expanding list of specific laboratory anomalies that may predispose people to venous thromboembolism. This article will review hypercoagulability with emphasis on laboratory risk markers and will provide some practical guidelines concerning the potential usefulness and limitations of such data in the context of patient management. An overview of procoagulant and anticoagulant reactions is shown in Fig. 1, and the conditions that promote a hypercoagulable state are summarized in Table 1.

Hemostasis involves a concerted and complex series of reactions, integrating vascular, endothelial cell, platelet and plasma factor responses that regulate thrombus formation. In response to injury, or other prothrombotic stimuli, rapid changes in vascular endothelial cells will lead to both the release of intracellular proteins that participate in the hemostatic process and an alteration of cell-surface properties, promoting a thrombogenic environment. Platelet adhesion and activation and concurrent activation of the
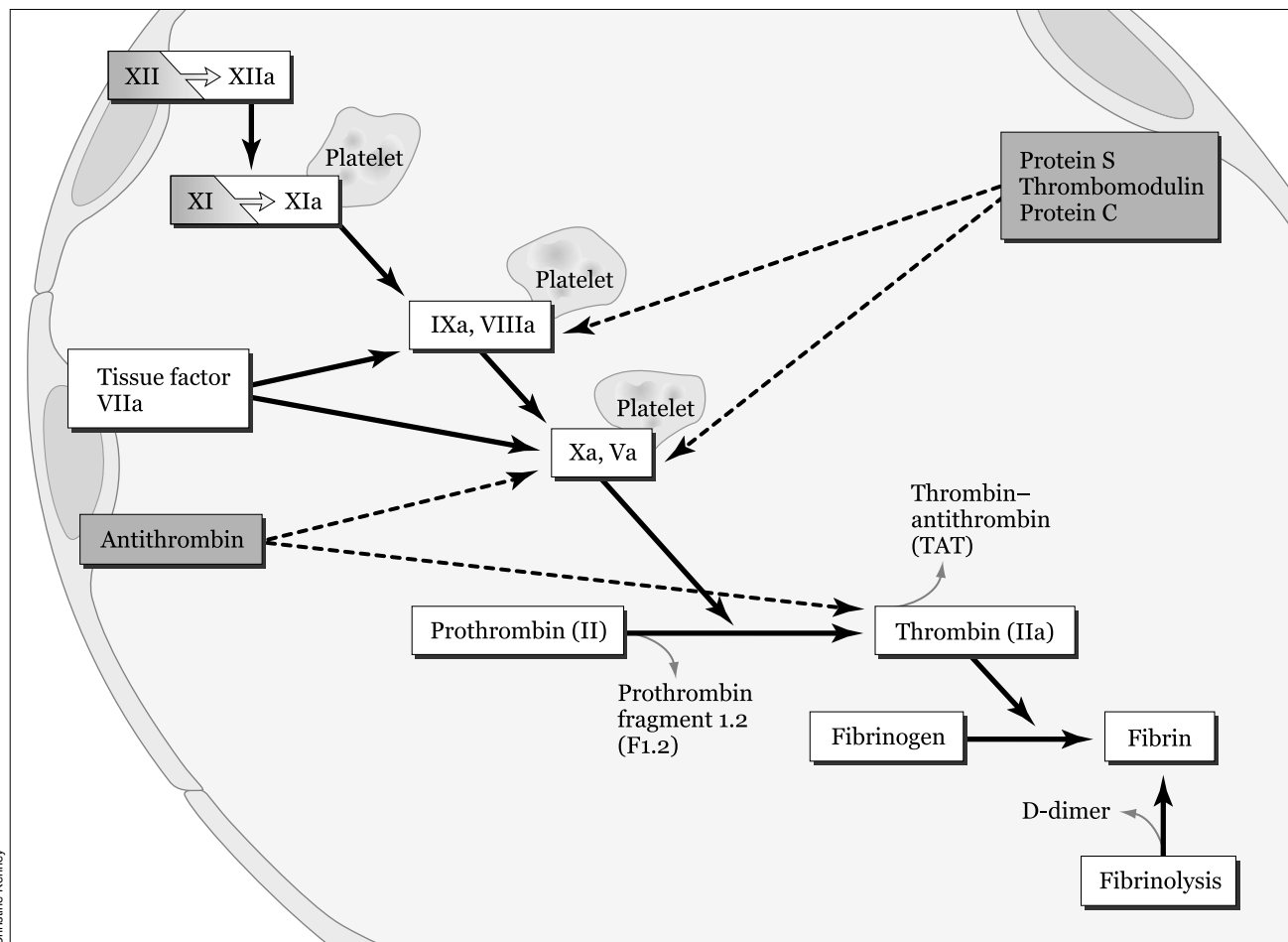

Fig. 1: An overview of the coagulation pathway.

plasma clotting cascade will rapidly promote thrombin formation and fibrin deposition. Almost simultaneously, reactions are set in motion to limit the thrombotic process. The dynamic balance between procoagulant reactions and their downregulation by natural anticoagulants in conjunction with the fibrinolytic system should function within normal parameters to prevent abnormal thrombus propagation. However, in some instances, alteration of just one variable in this complex series of interacting components will bring about a significant hypercoagulable state, which can manifest clinically as venous thromboembolism. More often, changes in multiple variables are required in a combined fashion to lead to a clinically significant hypercoagulable state. In addition to laboratory alterations, clinical factors will also affect a patient's overall thrombotic risk.

A shift of the hemostatic balance toward promoting clot formation may be because of an increase in the concentration of a procoagulant, such that altered reaction kinetics would favour thrombin formation. High levels of prothrombin, Factor VIII and Factor XI have all been recently identified as increasing the risk of venous thromboembolism.[1–3] These are all factors that participate in the clotting cascade. The plasma concentration of such proteins will in large part be determined by genetic factors. For example, some genetic polymorphisms are known to be associated with increased factor concentrations.[4] Specific mutations can affect either the rate of transcription or stability of a protein, resulting in increased steady-state levels of the particular factor.[1] Nonhereditary variables, for example, pregnancy, hormonal therapy, an acute phase reaction and aging have also been shown to be associated with increased levels of procoagulants.[5,6] In the case of prothrombin, an altered genetic sequence in the promoter region of the gene, known as the prothrombin G20210A mutation, is the marker associated with thrombotic risk.[1] The increased risk associated with high Factor VIII and Factor XI is based on epidemiologic data for thrombotic risk of individuals in the highest quartile of these factor levels measured as a continuous variable.[2,3]

Hypercoagulability may also result if there is an increase in the function of a procoagulant factor. The classic example of a mutation causing an increase in the function of a procoagulant is Factor V Leiden, the most frequent heritable risk for thrombosis in whites.[7] This is a point mutation of Factor V (FV R506Q). Factor V activation proceeds normally, however, the mutation significantly impairs Factor Va inactivation by protein C, thus preventing the removal of Factor Va from the clotting cascade in a timely manner. This persistence of activated Factor V can be measured in terms of its function and is known as resistance to activated protein C.[8] Acquired resistance to activated protein C in the absence of Factor V Leiden has also been described. It is probably caused by a variety of mechanisms, however, the association of this condition with a risk of thrombosis may not be as pronounced, as discussed in this issue (page 1016) by Shannon Bates and colleagues.[9]

The hemostatic balance is also dependent on the normal function of anticoagulant pathways. A decrease in natural anticoagulants, namely, circulating proteins whose concerted action results in decreasing thrombin production, has been shown in numerous studies to be a marker of thrombotic risk.[10,11] Evidence for the role of diminished natural anticoagulants in thrombosis comes mostly from studies of kindred with thrombophilia, a familial predisposition to venous thromboembolism. Numerous studies have identified decreased function of antithrombin, protein C or protein S to be associated with an increased thrombotic tendency when detected in families with a history of venous thromboembolism. Mutations in these natural anticoagulants are heterogeneous; they can either decrease the concentration or interfere with the anticoagulant function of the factor, and they are relatively rare. Patients with more than one abnormality, such as those with homozygous mutations or compound heterozygotes, are generally at greater risk of clinical manifestations.[12]

Additional acquired or environmentally influenced hypercoagulable conditions suitable for laboratory testing in-

Table 1: Hypercoagulable states associated with venous thromboembolism

Variable	Alteration	Test for detection
Procoagulant		
Factor XI	Increased level	Factor assay
Factor VIII	Increased level	Factor assay
Factor II	Increased level	Prothrombin 20210 DNA test
Factor V Leiden	Increased function	APC resistance test or FVR506Q DNA test
Anticoagulant		
Antithrombin	Decreased level or function	Functional assay
Protein C	Decreased level or function	Functional assay
Protein S	Decreased level or function	Functional assay or free protein S level
Miscellaneous		
Acquired APC resistance	Decreased response to protein C	APC resistance test
Antiphospholipid	Acquired autoantibody	Anticardiolipin and lupus anticoagulant
Homocysteine	Increased level	Homocysteine level

clude the anticardiolipin antibody, an autoantibody with antiphospholipid properties that is at times associated with lupus anticoagulant activity,[13] and the presence of hyperhomocysteinemia.[14] Both of these conditions have been clearly linked with an increase in the risk of venous thromboembolism in population-based studies, though their specific mechanism of action remains the subject of continued debate. A decrease in fibrinolytic activity due to the combined effect of factors inhibiting or enhancing fibrinolysis could also influence the overall thrombotic risk. To date, however, no clear evidence exists from clinical studies to confirm such an effect.[15]

Alterations leading to hypercoagulability probably involve not only plasmatic but also cellular elements, however, the latter are much less amenable to laboratory study and are not as well understood at present. Several potential candidates, such as thrombomodulin, the endothelial-surface cofactor for protein C activation, and other endothelial- and platelet-surface receptors involved in hemostasis, are currently under investigation.[15]

The conditions described here that predispose patients to hypercoagulability promote procoagulant reactions, ultimately resulting in excess thrombin generation. There is convincing laboratory evidence that thrombin generation can vary significantly because of alterations in the concentration of coagulant and anticoagulant factors at physiologically relevant levels. In a synthetically reconstituted "plasma" system, a 28-fold variation in thrombin availability was observed depending on the variation in the concentration of plasma clotting and anticoagulant factors. The greatest increase in thrombin formation was found by this method when prothrombin and antithrombin concentrations were maximized and minimized respectively.[16] Such laboratory data directly support the clinical evidence that the alteration of these factors promotes a hypercoagulable state. The ability to vary systematically one or multiple reactants under experimental conditions will continue to enhance our understanding of the global impact of these changes on hypercoagulability.

Evidence of an increase in procoagulant reactions in vivo can be obtained by attempting to measure the "activation state" of the clotting system. Cleavage products such as prothrombin fragment 1.2, a peptide released when prothrombin is converted to thrombin, or factor-inhibitor complexes, for example, thrombin-antithrombin, can be measured in the laboratory. Although such measures of the activation of coagulation have been the subject of many research studies, these tests are not routinely available and are expensive to perform. In the absence of data from large prospective clinical trials, these measures have limited usefulness at present for either the detection or treatment of hypercoagulability.[17] Currently, we perform a battery of tests looking for specific anomalies in patients suspected of having an underlying hypercoagulable state. It is, however, possible that global screening tests may become available for the assessment of these patients sometime in the future.

As Bates and coworkers indicate,[9] the laboratory tests for hypercoagulability have to be performed in a reliable and reproducible manner. The appropriate ordering of tests is also a very important aspect of the pre-analytical variables that may affect the reliability of the results. As is true for all diagnostic tests, the higher the pretest probability of a suspected condition, the more likely that a confirmatory diagnosis will be detected in the laboratory. Based on this premise, deficiencies in antithrombin, protein C and protein S should be searched for in individuals who have suffered venous thromboembolism at a young age, to an unusual extent or in an unusual location, or in the context of a positive family history. The presence of Factor V Leiden will most often be found in patients such as these, however, it may also be detected in up to 20% of unselected patients with venous thrombosis and in approximately 5% of healthy individuals.[10] In addition, checking for the prothrombin mutation, anticardiolipin antibody or lupus anticoagulant, and homocysteine levels would complete the battery of tests that should be carried out for patients strongly suspected of thrombophilia on clinical grounds. Additional patients who could be considered for screening are those with recurrent, unexplained thrombotic events and, once a family proband has been identified with an abnormality, screening asymptomatic family members for this defect would be appropriate. Detecting one abnormality does not preclude the possibility of a second defect, as is now well documented for those with a particularly strong tendency to venous thrombosis. It is unclear at present how best to integrate Factor VIII and Factor XI measures in patients with venous thromboembolism. Further studies are required to define their role, if any, in patient assessment.

Once an appropriate patient has been selected for laboratory screening, it is important to optimize the timing of the test. Treatment- or patient-related variables may transiently alter many of the test results. When a patient is taking heparin therapy for an acute event, antithrombin levels may decline transiently, as can free protein S owing to an alteration in its binding equilibrium. Warfarin therapy will cause a decrease in both protein C and protein S because these are both vitamin K–dependent proteins. Activated protein C resistance, the functional assay for the presence of Factor V Leiden, is also subject to variation, as discussed by Bates and colleagues.[9] Testing should be done when the patient is not taking anticoagulants, is in a nonacute phase and is not pregnant. Once an abnormality is detected, it may be prudent to confirm the finding and exclude specimen or technical errors. Genetic testing, such as looking for the presence of Factor V Leiden or prothrombin A20210G, is not prone to variation due to treatment or concurrent medical conditions. Clearly, however, the validity of all test results will also remain dependent on appropriate specimen handling and the expertise of the laboratory performing the testing.

If evidence of a hypercoagulable state is found through

laboratory testing, it is then important to consider how, if at all, this will alter patient management. It is common practice to recommend prolonged anticoagulation prophylaxis to patients with multiple venous thrombotic events, regardless of the presence or absence of an underlying thrombophilic state. If the thrombotic event occurs in the absence of a clinical risk, it may also be more prudent to maintain longer term anticoagulation therapy, regardless of laboratory findings.[18] For those patients with a single thrombotic event and evidence of a hypercoagulable state, long-term anticoagulation therapy should be offered if there is a significantly increased risk of recurrent events, such as for patients with a high level of anticardiolipin or in the case of antithrombin deficiency. In the absence of a high risk of recurrence, and in asymptomatic carriers of a biochemical defect, appropriate patient education and prophylaxis are essential elements of good patient care.[19]

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References

1. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996;88:3698-703.
2. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep vein thrombosis. *Lancet* 1995;345:152-5.
3. Meijers JCM, Tekelenburg WLH, Bouma BN, Rogier BM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med* 2000;342:696-701.
4. Humphries SE, Cook M, Dubowitz M, Stirling Y, Meade TW. Role of genetic variation at the fibrinogen locus in determination of fibrinogen concentrations. *Lancet* 1987;I:1452-5.
5. Meade TW, Brozovic M, Chakrabarth R, Howarth DJ, North WRS, Stirling Y. An epidemiological study of the haemostatic and other effects of oral contraceptives. *Br J Haematol* 1976;34:353-64.
6. Bauer KA, Weiss LM, Sparrow D, Vokonas PS, Rosenberg RD. Aging-associated changes in indices of thrombin generation and protein C activation in humans. Normative Aging Study. *J Clin Invest* 1987;80:1527-34.
7. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994;369:64-7.
8. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to protein C. *Proc Natl Acad Sci USA* 1993;90:1004-8.
9. Bates SM, Ginsberg JS, Straus SE, Rekers H, Sackett DL. Criteria for evaluating evidence that laboratory abnormalities are associated with the development of venous thromboembolism. *CMAJ* 2000;163(8):1016-21
10. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: part 1. *Thromb Haemost* 1996;76:651-62.
11. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: part 2. *Thromb Haemost* 1996;76:824-34.
12. De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G202210A prothrombin mutation. *N Engl J Med* 1999;341:801-6.
13. Santoro SA. Antiphospholipid antibodies and thrombotic predisposition: underlying pathogenetic mechanisms. *Blood* 1994;83:2389-91.
14. Den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996;334:759-62.
15. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* 2000;95:1517-32.
16. Butenas S, van't Veer C, Mann KG. "Normal" thrombin generation. *Blood* 1999;94:2169-78.
17. Tripodi A, Mannucci PM. Markers of activated coagulation and their usefulness in the clinical laboratory. *Clin Chem* 1996;42:664-9.
18. Kearon C, Gent M, Hirsh J, Weitz J, Kovacs MJ, Anderson DR, et al. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. *N Engl J Med* 1999;340:901-7.
19. Sanson BJ, Simioni P, Tormene D, Moia M, Friederich PW, Huisman MV, et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. *Blood* 1999;94:3702-6.
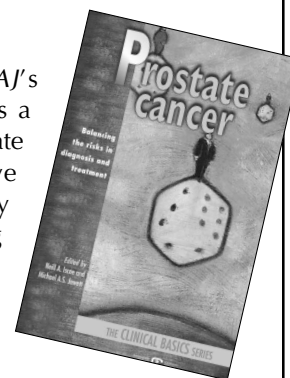
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