REVIEW ARTICLE

An Update on Hypercoagulable Disorders

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Venous thrombosis is a cause of considerable morbidity and is often responsible for chronic venous disorders that frequently lead to visits to dermatologists and others involved in wound healing. Over the past several years, many new causes of thrombophilia have been identified and have dramatically altered the approach to patients presenting with thrombosis. Newly described abnormalities associated with thrombophilia include the syndrome of activated protein C resistance, the prothrombin 20210A mutation, hyperhomocysteinemia, and elevated levels of coagulation factors VIII and XI. Clinicians can now frequently determine causes of thromboses that have previously been deemed idiopathic.

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Venous thrombosis is a cause of considerable morbidity and mortality. It is also responsible for postphlebitic sequelae that lead to visits to primary care physicians, dermatologists, vascular and plastic surgeons, and others. In addition to the risk of embolization, as well as the acute symptoms of pain and swelling, venous thrombosis can cause postphlebitic syndrome, which consists of chronic leg pain, edema, induration, pigmentation, venectasia, varicosis, and venous ulcers. This syndrome, which may occur in as many as 60% of patients with deep venous thrombosis (DVT),[1] is thought to be caused by venous hypertension, damage to venous valves, and abnormal microcirculation.[2]

Venous thrombosis is a common disorder. Approximately 1 in 1000 people per year in the United States will develop clinically apparent venous thrombosis, and there are 250 000 hospitalizations per year attributable to venous thromboembolism (VTE). The incidence of fatal pulmonary embolism, for which venous thrombosis is the major risk factor, is 50 000 people per year.[3]

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Almost 150 years ago, the German pathologist Virchow[4] postulated that thrombosis was due to stasis of the blood, changes in the vessel wall, and changes in the composition of blood. Certain risk factors for VTE have been known for some time, including surgery, trauma, malignant neoplasms, immobility sepsis, congestive heart failure, nephrotic syndrome, obesity, varicose veins, postphlebitic syndrome, oral contraceptives, and estrogens. However, Virchow's "changes in composition of the blood," or thrombophilia (also known as hypercoagulability), was not described until 1965, when Egeberg[5] described inherited antithrombin III deficiency. In the 1980s, deficiencies of the anticoagulant proteins, protein C and protein S, were also found to be causes of inherited thrombophilia.[6,7] However, these inherited deficiencies accounted for only 5% to 15% of VTE[8] (**Table**). Subsequently, the antiphospholipid antibody syndrome has been associated with not only venous thrombosis but also arterial thrombosis.[10] Over the past few years, considerable progress has been made in describing inherited and other causes of this disorder. In this review, we will discuss recent advances in diagnosing these newly described inherited and acquired disorders.

Deficiencies of Antithrombin III and Proteins C and S*

	Protein S Deficiency	Protein C Deficiency	Antithrombin III Deficiency
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant
Prevalence	Unknown	1:200-300	1:2000-5000
Earliest onset			
Homozygotes	Birth	Birth	. . .
Heterozygotes	Adolescence	Adolescence	Adolescence
Levels decreased	Acute thrombosis, warfarin sodium therapy, liver disease, vitamin K deficiency	Acute thrombosis, warfarin sodium therapy, liver disease, vitamin K deficiency	Acute thrombosis, heparin therapy, liver disease

*Adapted from Alving[9] with permission (The McGraw-Hill Companies).

ACTIVATED PROTEIN C RESISTANCE (FACTOR V LEIDEN)

In normal situations, a homeostasis exists between coagulation and fibrinolysis. When coagulation is initiated, the process is regulated by a variety of mechanisms, one of which is the protein C natural anticoagulant system. Thrombin, when bound to thrombomodulin on the endothelial surface, activates protein C into activated protein C (APC). Then APC inactivates activated factors V and VIII. This is one of the mechanisms that inhibits thrombin generation and prevents thrombosis from progressing unchecked.

In 1993, Dahlback et al[11] described a patient with a strong family and personal history of thrombosis who did not possess any of the known hereditary coagulation defects. He found that when APC was added to the patient's plasma, the activated partial thromboplastin time (aPTT) did not prolong as expected. This phenomenon was termed *APC resistance* (APCR).

Subsequently, it was found that the defect in APCR does not lie in activated protein C but is actually due to an abnormality in the APC cleavage site on factor V. Approximately 90% to 95% of APCR is due to a single arginine to glutamine mutation at position 506 of factor V, rendering it resistant to degradation by APC.[12] This mutation is known as factor V Leiden, named after the city in which it was described. Since this mutation accounts for 90% to 95% of APCR, a small percentage of patients will harbor APCR in the absence of a factor V Leiden mutation.

One of the most common hereditary causes of thrombophilia, APCR has been found in as many as 50% of selected patients[13] and approximately 20% of unselected patients with thrombosis.[14] Among European whites, the allele frequency in the general population is 4%, but among native populations of Africa, Southeast Asia, and Australia, it is extremely uncommon, if present at all.[15] Among European whites, the frequency of factor V Leiden carriers has been found to be even higher in certain populations. Among the general population, it has been found in 13% in Cyprus,[15] 11% in Sweden,[16] and 9% in Turkey.[17] The relative risk of thrombosis for carriers of the factor V Leiden mutation is thought to be increased 7-fold for heterozygotes and 80-fold for homozygotes in patients younger than 70 years without malignant neoplasms.[18] This is an important cause of thrombosis even in elderly patients.[19]

Since lower extremity venous ulcers have been associated with previous episodes of venous thrombosis, investigators have been interested in whether there is an association between venous ulcers and APCR.[20,21] In a study of 46 consecutive patients with chronic venous leg ulcers with a mean age of 80 years and a median ulcer duration of 4 years, 12 (26%) were found to be associated with APCR. These same investigators found APCR in only 1 (4%) of 23 healthy controls.[22]

There are several ways to test for this abnormality. The original aPTT-based test, in which the aPTT is determined both with and without the addition of APC, has been modified so that it can be performed when patients are receiving anticoagulants. The APC is added to patient's plasma diluted 1:4 in factor V–deficient plasma. An APC ratio is obtained by dividing the aPTT after the addition of APC by the aPTT before the addition of APC; a ratio of less than 2.0 is abnormal. The presence of a lupus anticoagulant may give rise to an abnormal result.[23] A polymerase chain reaction–based test to test for the factor V Leiden mutation can either be done initially, although it would not diagnose non–factor V Leiden causes of APCR, or can be done after the APCR aPTT-based test.

THE PROTHROMBIN 20210A MUTATION

Human prothrombin is a single-chain, vitamin K–dependent protein that is converted by the prothrombinase complex to thrombin during coagulation. In 1996, Poort et al[24] determined that of 28 selected patients with a family history of thrombosis, 5 (18%) possessed a G to A nucleoside transition at position 20210 in the 3′-untranslated region of the prothrombin gene. Of 100 healthy controls, only 1 was found to harbor this mutation. Among patients with a first DVT in the Leiden Thrombophilia Study, 6.2% were found to be heterozygous for this mutation, whereas only 2.2% of healthy controls possessed this mutation. In this study, the presence of this mutation was associated with a relative risk of thrombosis of 2.8.[24] Interestingly, heterozygous carriers were found to have approximately 30% higher prothrombin levels than healthy controls, and presumably, this is the mechanism by which it exerts its thrombotic effects.[25]

Other studies[26-29] have suggested that the prevalence of this mutation among healthy controls varies from 0.7% to 4.0%. Using data from 11 centers, Rosendaal et al[30] found the prevalence to be 2.0%, which was slightly higher in southern Europe (3.0%) than in northern Europe (1.7%). Similar to factor V Leiden, this mutation is extremely uncommon in nonwhite populations.

Several other studies have looked at the prevalence of this mutation among patients presenting

with an episode of venous thrombosis and among the healthy general population. The mutation was found in 4% to 8% of patients with a first episode of thrombosis, representing a relative risk of first venous thrombosis of 2 to 7 compared with the general population.[31]

The prothrombin 20210A mutation was found to be a risk for thrombosis independent of factor V Leiden. Margaglione et al[32] studied 281 patents with a first DVT referred to a center for thrombosis in Italy. The 128 men with a mean age of 38 years and 153 women with a mean age of 35 years were compared with a healthy matched cohort of 850 individuals. In the group with DVT, 14.2% harbored the prothrombin 20210A mutation, whereas the mutation was present in only 4.6% of controls. Factor V Leiden was present in 18.5% of the DVT group and 5.1% of the controls. The adjusted odds ratio for DVT was slightly higher for factor V Leiden (3.4) than prothrombin 20210A (3.1). In this study, 14 patients were found to have both the factor V Leiden and prothrombin 20210A mutations. The average age of their first thrombosis was 31.5 years, which was younger than the 38 years for those with only the prothrombin 20210A mutation and the 39 years for those with only factor V Leiden. This suggests that patients who harbor both abnormalities are at increased risk compared with those with a single abnormality of either factor V Leiden or the prothrombin gene.

Although the prothrombin gene mutation is associated with elevated prothrombin levels, it is not clear that assessing prothrombin levels is an adequate method to test for this mutation. Currently, the recommended test for this mutation is through DNA analysis.

RISK FOR RECURRENT EVENTS IN PATIENTS WITH FACTOR V LEIDEN OR THE PROTHROMBIN GENE MUTATION

Whether the prothrombin gene mutation or factor V Leiden confers risk for recurrent DVT after an initial thrombosis has been an area of investigation. Unfortunately, there are conflicting data. Studies by Ridker et al[33] and Simioni et al[34] have found that patients who tested positive for factor V Leiden are at increased risk for recurrent thrombosis when compared with patients who tested negative for factor V Leiden. However, other studies[35,36] have not been able to corroborate these findings.

The risk of recurrence is increased when patients harbor both the factor V Leiden and prothrombin mutations. In a study of 624 patients with either first or recurrent DVT who were referred to a thrombosis center, an evaluation for inherited thrombophilia, including the prothrombin gene mutation, factor V Leiden, antiphospholipid antibodies, malignant neoplasm, and deficiencies of anti–thrombin III, protein C, or protein S, was performed. The investigators found that 238 patients had no abnormalities and 129 were heterozygous for factor V Leiden. Of these 129 patients who possessed the factor V Leiden, 17 also were heterozygous for the prothrombin gene mutation. At the time of referral, the cumulative incidence of recurrent VTE was not statistically different between those with the factor V Leiden mutation and those without. However, among those with both mutations, the relative risk for recurrent DVT was 2.6-fold increased compared with those with factor V Leiden alone. When only spontaneous (those not occurring during times of increased risk such as immobility, pregnancy, or surgery) recurrent DVTs were evaluated, the relative risk was even higher.[37]

THERAPY

Patients with either factor V Leiden or the prothrombin 20210A mutation who experience a first DVT should receive anticoagulation with warfarin sodium for 3 to 6 months. Patients with recurrent thrombosis or those that harbor both abnormalities and have had a first thrombosis should continue use of warfarin indefinitely. At this time, it is not clear whether patients with a first episode of thrombosis and a single abnormality will benefit from indefinite anticoagulation. Trials looking to answer this question are being planned or are currently under way.[38]

Although some practitioners might prescribe indefinite anticoagulation for those with a single abnormality that experienced a life-threatening thrombosis or thrombosis in an unusual site (cerebral, mesenteric), at present there is no evidence to support or refute this practice.

RISKS ASSOCIATED WITH PREGNANCY AND THE USE OF ORAL CONTRACEPTIVES

It has long been known that the risk of thromboembolism is increased during pregnancy and the puerperium. To evaluate whether the presence of factor V Leiden or the prothrombin mutation increased the risk for thrombosis during this period, investigators in Germany evaluated 119 women with a history of thrombosis during pregnancy or the puerperium and 233 age-matched women. They found factor V Leiden in 44% of patients with a history of thrombosis compared with 8% of healthy women (relative risk, 9.3) and found the prothrombin gene mutation in 17% of those with thrombosis compared with 1% of controls (relative risk, 15.2). Based on their assumption that thrombosis occurs in 1 of 1500 pregnancies, the risk of thrombosis was estimated to be 0.2% among carriers of factor V Leiden, 0.5% among those with the prothrombin mutation, and 4.6% among those with both abnormalities.[39]

Female patients should be counseled that their risk of thrombosis is increased not only during pregnancy and the puerperium but also if they choose to use oral contraceptives. Studies have shown that patients with the prothrombin gene mutation[40] and the factor V Leiden mutation[41,42] who use oral contraceptives are at increased risk for venous thrombosis. Because of its teratogenic potential, warfarin is contraindicated in women who are or may become pregnant.

ASSOCIATION BETWEEN THE FACTOR V LEIDEN AND PROTHROMBIN GENE MUTATIONS AND ARTERIAL THROMBOSIS

Several studies[43-45] have failed to show an association between either the factor V Leiden or the prothrom-

bin gene mutation and arterial disease, such as stroke or myocardial infarction. However, there is a suggestion that these defects may contribute to myocardial infarction in young women[46,47] or in those with other risk factors for atherosclerosis.[48] At this time, these 2 mutations are not thought to be a major cause of arterial thrombosis.

HYPERHOMOCYSTEINEMIA

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. Levels of homocysteine can be elevated through a variety of genetic and environmental mechanisms, such as hereditary enzyme deficiencies (such as cystathionine β-synthase deficiency and methylene-tetrahydrofolate reductase deficiency), chronic renal failure, hypothyroidism, certain malignant neoplasms, or the use of methotrexate, phenytoin, or theophylline. However, deficiencies of folate or vitamins B_{12} and B_6 account for two thirds of cases of hyperhomocysteinemia.[49]

There is strong evidence linking elevated homocysteine levels and arterial disease. The Physicians' Health Study[50] followed up more than 14 000 physicians without known coronary artery disease. Those with plasma homocysteine levels 12% above normal had a 3-fold increased risk for the development of coronary artery disease than those with lower levels. Furthermore, other studies[51-56] have also shown an increased risk of myocardial infarction, stroke, and death in patients with elevated homocysteine levels.

In addition to being a risk factor for arterial disease, hyperhomocysteinemia has been shown to be a risk for venous thrombosis. Of 269 patients younger than 70 years with a first episode of DVT participating in the Leiden Thrombophilia Study, homocysteine levels above the 95th percentile were found in 10%, which corresponds to an odds ratio of 2.5 when compared with a healthy matched control group.[57] This increased risk was independent of other known risk factors for venous thrombosis, such as protein C, protein S, antithrombin deficiencies, and APCR. Similarly, in a meta-analysis of 10 case-control studies, den Heijer et al[58] found an odds ratio of 2.5 for fasting homocysteine levels above the 95th percentile. Furthermore, elevated homocysteine levels have also been shown to be associated with a higher rate of recurrent thrombosis.[59,60]

The diagnosis of hyperhomocysteinemia can be made by measuring fasting homocysteine plasma levels. Whereas the normal level is less than 15 µmol/L, patients with mild disease have levels in the 15- to 30-µmol/L range, patients with moderate disease have levels in the 30- to 100-µmol/L range, and those with severe disease have higher levels. Methionine loading stresses the homocysteine metabolic pathways and is thought to be more sensitive than fasting levels in detecting homocysteine metabolic abnormalities.[61] Homocysteine is measured before and 4 to 8 hours after 100 mg/kg of oral methionine is given to a fasting patient. Hyperhomocysteinemia is diagnosed if the plasma homocysteine level is more than 2 SDs above the mean after the methionine load.[49]

Similar to patients with venous thrombosis who harbor no mutations or either of the factor V Leiden or prothrombin 20210A mutations, those with hyperhomocysteinemia and a first episode of thrombosis should be treated with anticoagulation for 3 to 6 months. However, if a patient is deficient in folate or vitamins B_6 or B_{12}, those vitamins should be administered in sufficient doses to achieve normal levels. In the absence of a specific vitamin deficiency, plasma homocysteine levels can be reduced up to 50% using folic acid alone.[62,63] Typically, folic acid is given orally in doses of 1 to 2 mg/d. It is uncertain whether the addition of vitamins B_6 (pyridoxine hydrochloride)or B_{12} (cyanocobalamin) is of additional benefit to folic acid in the absence of a specific vitamin deficiency. Although homocysteine levels have been shown to decrease with folic acid supplementation, to date, we do not know whether this will ultimately lead to a decreased frequency of adverse outcomes, such as arterial or venous thromboses. Such studies are presently under way, and their results are anxiously awaited.

ELEVATED FACTOR VIII LEVELS

Elevated levels of another protein involved in coagulation, factor VIII, have also been associated with thrombosis. In multivariate analysis, Koster et al[64] found that when compared with subjects with factor VIII levels of 1000 IU/L or less, those with levels greater than 1500 IU/L possessed an almost 5-fold increased risk for thrombosis. Of patients with a first episode of DVT, 25% were found to have levels above 1500 IU/L compared with 11% of healthy matched controls. In addition, in patients with unexplained thromboembolism referred for thrombophilia screening, elevated factor VIII levels were the most common abnormality detected.[65] Some have suggested that a C-reactive protein level should be measured to exclude elevated factor VIII levels as an acute-phase reactant, since levels can increase during acute illness.[66] At this time, little is known as to how elevated factor VIII levels affect the treatment of VTE.

ELEVATED FACTOR XI LEVELS

Increased levels of a component of the intrinsic factor pathway, factor XI, have recently been implicated as a risk factor for venous thrombosis.[67] Investigators measured the factor XI antigen levels of patients enrolled in the Leiden Thrombophilia Study and found that the relative risk of thrombosis for patients with levels above the 90th percentile was 2.2 compared with those with levels at or below that value. A continuous dose-response relation was noted; the higher the factor XI level, the higher the risk of thrombosis. Furthermore, this increased risk was independent of other known risk factors for thrombosis, such as oral contraceptive use, older age, and elevated levels of factor VIII and homocysteine. When patients with known genetic causes of thrombophilia, such as the prothrombin 20210A or factor V Leiden mutations, or deficiencies of antithrombin or proteins C or S were excluded from analysis, the odds ratio did not change. This suggests that the increased risk seen in patients

with elevated factor XI levels is not due to an association with these known causes of thrombophilia. The authors determined that in the general population up to 11% of all cases of thrombosis might be attributable to high levels of factor XI. Studies are under way to determine whether increased levels of this factor are genetically determined.[67]

WHOM TO EVALUATE

Since these risks for thrombosis are recently described, there are no large, well-done studies to assess an optimal, cost-effective approach with regard to screening and case finding for these disorders. It would be reasonable to pursue a workup for those with any of the following: thrombosis at an early age (younger than 45 to 50 years), a family history of thrombosis, or recurrent thrombosis. Some clinicians might also order these tests if the patient developed a thrombosis at an unusual site (eg, mesenteric, cerebral, or hepatic vein thromboses) or for a patient who presented with a life-threatening event.

For patients who develop thrombosis before the age of 45 to 50 years and in those with a family history of thrombosis, it would be reasonable to order tests to evaluate whether the patient has 1 of the following disorders: deficiencies of anti–thrombin III, protein C, and protein S or the presence of antiphospholipid antibody syndrome, factor V Leiden, prothrombin 20210A mutation, and hyperhomocysteinemia. For those who develop their first episode of thrombosis after the age of 45 to 50 years and without a family history of thrombosis, it would be reasonable to omit tests looking for deficiencies of anti–thrombin III, protein C, or protein S, since these disorders would be much less likely.

Clinicians should be aware that levels of protein C, protein S, and antithrombin III are decreased in acute thrombosis, and therefore they should not check these levels during an acute event. Furthermore, antithrombin III levels are decreased by the administration of heparin sodium, and therefore should not be measured when the patient is receiving this anticoagulant. Since proteins C and S are vitamin K dependent, their levels are reduced during therapy with warfarin sodium; levels should be measured after the patient has been free of warfarin for at least 2 weeks. Currently, the role of screening for elevated factor VIII and XI levels is not clear.

CONCLUSIONS

Venous thrombosis continues to be a major cause of morbidity and mortality. Recent scientific advances have led to an improved understanding of factors that may explain causes for most patients that experience thrombosis. Future studies should be directed at determining the optimal cost-effective workup for hypercoagulability and whether long-term anticoagulation will prove beneficial after an initial episode of thrombosis for those with these abnormalities. These and other studies are anxiously awaited.

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