

Thrombophilia: 2009 Update

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Current Treatment Options in Cardiovascular Medicine 2009, 11:114–128

Current Medicine Group LLC ISSN 1092-8464

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Opinion statement

As venous thrombosis is mostly caused by disturbances in the plasma coagulation system, abnormalities of coagulation factors are mostly risk factors for venous thromboembolism (VTE). Relatively little is known about thrombophilias that predispose to arterial thromboembolism. Although some abnormalities in the fibrinolytic pathway appear to predispose to arterial thrombosis, the associations are weak and often inconsistent between studies. At present, there is not enough consistent and clinically meaningful information to include fibrinolytic parameters in a clinical thrombophilia workup. Controversy exists as to which patients and family members to test for thrombophilia. Several testing guidelines exist. Routine screening for inherited thrombophilias is not indicated in patients with VTE provoked by immobility, surgery, and malignancy, or in those with arterial thrombosis with arteriosclerosis risk factors. Heterozygous factor V Leiden (FVL) and prothrombin 20210 mutations increase the risk for recurrent VTE only slightly once anticoagulation is stopped. Therefore, decisions regarding the length of anticoagulant therapy typically are not influenced by finding one of these heterozygous mutations. The main reason to perform thrombophilia testing in a patient is to detect a strong thrombophilia (ie, antithrombin deficiency, antiphospholipid antibody syndrome, homozygous FVL, double-heterozygous FVL plus prothrombin 20210 mutation, protein C deficiency, and maybe protein S deficiency). The finding of a strong thrombophilia has several clinical consequences: it decreases the threshold to recommend long-term anticoagulation in a patient with unprovoked VTE; facilitates discussion regarding whether anticoagulant or antiplatelet therapy is the preferred empiric treatment for a patient who had an unexplained arterial, nonarteriosclerotic thromboembolic event; and leads to the consideration of testing asymptomatic female family members for the identified thrombophilia(s) so they can be counseled on their risk of thromboembolism, the use of hormonal therapies, and the potential benefit of pre- and postpartum anticoagulant therapy.

Introduction

Thrombosis is the leading cause of death worldwide—from arterial (myocardial infarction or stroke) or venous thromboembolism (VTE; pulmonary embolism [PE]). Thrombus formation involves complex interactions among the subendothelium, platelets, pro- and anticoagulant proteins, endothelium, leukocytes, and microparticles of various origins, as well as several proteins in the fibrinolytic pathway [1,2•]. Arterial

thrombosis tends to occur at the site of atherosclerotic plaque rupture, where platelets are recruited to the site of vascular injury and activated [1,2•]. The activation of the coagulation cascade by tissue factor leads to the generation of a fibrin lattice that stabilizes the thrombus. Arterial thrombosis is caused by platelet-rich thrombi. The triggering events for the formation of venous thrombosis are less well understood. Venous stasis, endothelial

abnormalities, and thrombophilias—the components of Virchow’s triad—promote venous thrombosis. Clots typically are fibrin rich. VTE typically is a multifactorial disease, with transient—that is, environmental—risk factors, identifiable or not, plus inherited or acquired predispositions—that is, thrombophilias. Although it is increasingly recognized that endothelial cells, circulating leukocytes, and tissue factor-bearing microparticles contribute to the pathophysiology of thrombosis, little of clinical relevance is known about abnormalities in these pathways.

Several thrombophilias are known to predispose to thrombosis, mostly VTE. They may be inherited or acquired, or have components of both types. Although

thrombophilia testing of patients with venous and arterial thromboses became relatively popular after the discovery of factor V Leiden (FVL) in 1994 and the prothrombin 20210 mutation in 1996, the past few years have seen a decreasing enthusiasm for testing, as it has been recognized that the finding of a thrombophilia often does not fully explain why a thrombotic event happened, does not change treatment (either length of anticoagulant treatment or intensity of vitamin K antagonist [VKA] therapy), and has no significant impact on other affected family members. The following section discusses individual thrombophilias in detail.

Individual thrombophilias

Factor V Leiden

- **General information:** Activated protein C (APC) is a potent inhibitor of the coagulation system, cleaving the activated forms of factors V and VIII (FVa and FVIIIa). The FVL mutation is a point mutation in the factor V gene, leading to a factor V molecule with an arginine-to-glutamine substitution at position 506. This abolishes a cleavage site of APC and makes FVa less susceptible to inactivation, referred to as “APC resistance.” FVL accounts for more than 90% of APC resistance. Other causes of APC resistance include less common genetic mutations of factor V (factor V Cambridge, factor V Liverpool) and acquired causes of APC resistance, including antiphospholipid antibodies (APLAs), solid and hematologic cancers, and pregnancy. FVL is inherited in an autosomal dominant fashion. The high prevalence of FVL in the general population suggests that it has led to evolutionary selection advantages, including less blood loss during delivery and improved survival during sepsis [3].
- **Prevalence:** FVL is the most common inherited thrombophilia known, with a prevalence of 3% to 8% in Caucasian populations and 1.2% in African Americans. It rarely is found in native African, Chinese, or Japanese populations [4]. Homozygous FVL occurs in 1 in 500 to 1 in 1600 Caucasians.
- **Testing:** The APC resistance test is a functional activated partial thromboplastin time (aPTT)-based coagulation assay using factor V–deficient plasma. Polymerase chain reaction (PCR) testing of the FVL gene is widely available.
- **Risk for thrombosis:** Heterozygosity for FVL is mildly thrombophilic, leading to a 2.7-fold increased risk of VTE; homozygosity is a stronger thrombophilia, with an 18-fold increased risk compared with individuals without the FVL mutation [5]. Additional VTE risk factors, such as age, smoking, obesity, and use of estrogens, increase the risk further. In a landmark population-based study, the lowest and highest absolute 10-year risks for VTE were 1% and 10% in heterozygotes, and 3% and 51% for homozygotes, depending on age, smoking status, and obesity [5]. The most recent meta-analysis demonstrated that FVL heterozygosity is associated only weakly with increased risk of VTE recurrence, with an increased odds ratio of 1.41 [6•]. Limited data are available on the risk of recurrence in individuals with homozygous FVL, but one study showed it to be 1.8-fold higher compared with heterozygous individuals [7]. For practical purposes, there is no clinically meaningful association between FVL and arterial thromboembolic events: a recent meta-analysis demonstrated only a 1.2-fold increased risk in FVL carriers compared with noncarriers (95% CI, 1.02–1.41) [8].

- **Treatment:** Because heterozygosity for FVL confers only a mildly increased risk of recurrence compared with individuals without FVL, its finding alone should not alter anticoagulation treatment decisions. Furthermore, asymptomatic family members of persons with FVL heterozygosity need not be tested unless they are considering estrogen therapy or pregnancy, in which case, testing may be considered. However, even in this situation, there is no strong evidence-based rationale for testing, as solid management decisions can typically be made without the knowledge of the mutational status. Finding the homozygote state in a patient with an unprovoked VTE may be an argument to treat the patient with longer-term anticoagulation.
- **New developments:** An ongoing extensive, federally funded literature review, “Outcomes From Testing for Factor V Leiden and Prothrombin G20210A” (<http://www.ahrq.gov/clinic/tp/fvltp.htm>), is expected to be completed by mid-2009.

Prothrombin 20210 mutation

- **General information:** The substitution of a guanine for adenine at nucleotide 20210 in the noncoding region of the prothrombin gene is the second most common inherited thrombophilic risk factor known. Presence of the mutation is associated with increased prothrombin plasma levels. It is inherited in an autosomal dominant fashion.
- **Prevalence:** The mutation is found most commonly in individuals of southern European ancestry, with a prevalence throughout Europe of 0.7% to 4%. In the United States, it occurs in 2% of the general population and in 0.5% of the African American population. The prothrombin 20210 mutation is very rare in non-Caucasian populations. Homozygous prothrombin 20210 mutation occurs in approximately 1 in 4000 individuals of Caucasian heritage.
- **Testing:** PCR methods are available to detect the prothrombin 20210 mutation with a high degree of specificity and sensitivity. Testing plasma factor II activity or antigen levels is not clinically useful in individual patients.
- **Risk for thrombosis:** Heterozygosity for the prothrombin 20210 mutation is mildly thrombophilic, conferring a threefold increased risk of VTE compared with the noncarrier state. Meta-analysis shows that heterozygosity for the prothrombin 20210 mutation is associated only slightly with an increased risk of VTE recurrence, with an increased odds ratio of 1.72 [6•]. Thus, treatment decisions on length of anticoagulant therapy typically are not based on the presence or absence of the prothrombin 20210 mutation. Meta-analysis has demonstrated only a minimal association between the prothrombin mutation and arterial thromboembolism: the risk for an arterial event is only 1.32-fold increased in carriers of the mutation compared with noncarriers (95% CI, 1.03–1.69) [8]. However, there is a somewhat stronger association between the prothrombin 20210 mutation and stroke and myocardial infarction in younger patients [9]. Population-based data regarding the risk of thrombosis for homozygotes for the prothrombin gene mutation are not available. A recent summary of the 70 cases of homozygous individuals published in the medical literature indicates a marked phenotypic heterogeneity [10]. The risk of VTE recurrence in homozygous individuals is not known.
- **Treatment:** Because heterozygosity for the prothrombin 20210 mutation confers only a modest risk of recurrence, its finding alone typically does not alter anticoagulation treatment decisions. Furthermore, similar to

the discussion about FVL, family members of persons who are heterozygous need not be tested unless they are considering estrogen therapy or pregnancy, in which case, testing may be considered.

Protein C and S deficiencies

- **General information:** Proteins C and S are vitamin K–dependent natural anticoagulant proteins. At the site of endothelial injury, thrombin binds high-affinity thrombomodulin. Circulating protein C is bound by the endothelial cell protein C receptor and presented to the thrombin–thrombomodulin complex for activation. APC requires free protein S as a cofactor to inhibit activated factors V and VIII. Both qualitative and quantitative deficiencies in proteins C and S have been recognized since the mid-1980s as being thrombophilic. Detailed reviews of all aspects of protein C and S deficiency have been published recently [11•,12•].
- **Prevalence:** Protein C deficiency has been estimated to occur in 1 in 200 to 500 persons [11•]. More than 160 different genetic abnormalities have been identified [11•]. Individuals with homozygous or double-heterozygous protein C mutations who have protein C levels less than 1% are at risk for neonatal purpura fulminans [13]. Protein S deficiency occurs in approximately 1 in 500 persons [12•]. Almost 200 different mutations resulting in protein S deficiency have been identified [12•]. Similar to protein C deficiency, homozygotes and double-heterozygotes carry a risk of neonatal purpura fulminans, although these extreme thrombophilic states are rare. Both protein C and S deficiencies are inherited in an autosomal dominant fashion. Acquired cases of protein C and S deficiency are associated with sepsis, disseminated intravascular coagulopathy (DIC), liver disease, vitamin K deficiency, and acute thrombosis. Estrogen exposure, including pregnancy and oral contraception, decreases the level of protein S.
- **Testing:** Functional assays are the preferred laboratory test for protein C and S deficiencies because they can identify both quantitative and qualitative defects. Appropriate timing of laboratory testing is essential because acute thrombosis and VKA therapy can decrease levels of both proteins. Patients should be at least 3 weeks removed from warfarin therapy before testing is obtained. In general, it is true that the lower the activity level, the higher the risk for thrombosis.
- **Risk for thrombosis:** Because of the genetic diversity of mutations associated with protein C and S deficiency, rates of thrombosis vary widely among individuals and families with known defects. Estimates of the relative risk of venous thrombosis with protein C or S deficiency range from 2 to 11 times the normal population's risk [14,15]. However, in some families, nearly 75% of those with a protein deficiency will develop thrombosis [16]. Rates of recurrent thromboses are mildly increased with protein C and S deficiency, with hazard ratios between 1.4 and 1.8 compared with those with VTE without a known thrombophilia [17]. Although some studies have not shown an association of protein C or S deficiency with increased risk of myocardial infarction or stroke, a recent large family cohort study showed an association of protein S and C deficiency, but not antithrombin deficiency, with arterial thromboembolism in individuals less than 55 years old [18]. Rates of fetal loss after 28 weeks' gestation are increased in patients with protein C or S deficiency [19].
- **Treatment:** Treatment of protein C and S deficiency with anticoagulation requires special consideration because warfarin-induced skin necrosis may occur. This transient hypercoagulable state is related to abrupt

declines in protein C activity after the initiation of VKA. Any patient with acute VTE who is started on VKA needs concurrent anticoagulation with a parenteral anticoagulant for at least 5 days and until the international normalized ratio (INR) is greater than 2, but this is particularly important in those with known protein C or S deficiency. Because of the increased association with recurrent thrombosis and protein C and S deficiency, consideration of long-term anticoagulation after a first unprovoked thrombotic event is appropriate in these patients. Newborns with homozygous or double-heterozygous protein C deficiency should be strongly considered for treatment with protein C concentrate [11•]. No protein S concentrate for clinical use exists.

- **New developments:** Since 2007, a human plasma-derived protein C concentrate (Ceprotrin; Baxter Healthcare, Deerfield, IL) has been US Food and Drug Administration (FDA) approved and clinically available for treating and preventing venous thrombosis and purpura fulminans in patients with severe congenital protein C deficiency.

Antithrombin deficiency

- **General information:** Antithrombin (AT) is an enzyme that interrupts the coagulation process by inhibiting thrombin and activated factors X, IX, and XI. If bound to heparin, the AT-mediated inhibition of thrombin is increased 4000-fold. Qualitative and quantitative defects of AT encompass AT deficiency. These include genetic mutations that alter the heparin- or thrombin-binding sites of AT. A detailed review of all aspects of AT deficiency, with particular emphasis on clinical management, was published recently [20•].
- **Prevalence:** AT deficiency occurs in 1 in 2000 to 5000 people. More than 130 different genetic mutations are known. Deficiencies are typically heterozygous, as homozygous deficiencies are almost always incompatible with life. Acquired cases of AT deficiency are associated with sepsis, DIC, liver disease, the nephrotic syndrome, and asparaginase chemotherapy.
- **Testing:** Testing for AT deficiency should be performed using a functional assay to detect quantitative and qualitative defects. Heparin use may decrease AT levels by 30%. Testing is best performed a few weeks after the initial thrombotic event. An abnormal result should lead to repeat testing on a new blood sample, and a patient should not be diagnosed as having AT deficiency on the basis of a single abnormal test result.
- **Risk for thrombosis:** Mutations affecting the thrombin-binding domain are strongly thrombophilic, causing VTE in nearly 50% of affected family members [20•]. The prevalence of VTE in individuals with a defect in the heparin-binding site is much lower—only about 6% of such individuals will develop VTE [20•]. The risk of recurrent VTE once anticoagulation is stopped is high, between 10% and 17% per year [21]. Although some cases of arterial thromboembolism in AT-deficient individuals have been reported, this association is weak, if present at all [18]. The risk for fetal loss is slightly increased in women with AT deficiency [19].
- **Treatment:** Asymptomatic individuals with AT deficiency typically are not started on long-term anticoagulation [21]. However, they need to receive thorough deep vein thrombosis (DVT) prophylaxis in risk situations. There are no guidelines or consensus statements regarding when to use AT concentrates. Because the risk of recurrent VTE is high, once anticoagulation has been discontinued, it is typically recommended that a patient with AT deficiency who had a VTE event be considered for long-term anticoagulation. It is not known whether the

same recommendation should apply to patients with AT deficiency due to a defect in the heparin-binding site. AT deficiency occasionally confers resistance to anticoagulation with heparin. Large doses of unfractionated heparin may be required to achieve appropriate prolongation of aPTT. In cases of severe thrombosis and inadequate anticoagulation, AT concentrate may be given, but there is no consensus on who should receive it, at what dosage, and for how long.

- **New developments:** Human plasma–derived AT concentrate is available in Europe and the United States. Recombinant AT concentrate (ATryn; GTC Biotherapeutics, Framingham, MA) is available in Europe and is presently under FDA review for use in the United States. The FDA’s decision is expected by February 2009.

Antiphospholipid antibody syndrome

- **General information:** APLAs are acquired autoantibodies targeted against phospholipids and phospholipid-binding proteins, such as β_2 -glycoprotein-I and prothrombin. They are associated with arterial thromboembolism and VTE, as well as with pregnancy loss. A variety of different mechanisms leading to thrombosis have been described, including effects of the antibodies on platelets, endothelial cells, monocytes, and trophoblasts, and interference with complement activation, the protein C pathway, and fibrinolysis [22,23]. Clinical classification of the APLA syndrome requires venous or arterial thrombosis or unexplained recurrent early or one or more late pregnancy losses, together with persistent laboratory evidence of APLA, at least 12 weeks apart [24•]. Criteria for definite APLA syndrome have been described as the so-called Sapporo criteria [24•].
- **Prevalence:** The prevalence of APLA syndrome is not known, but APLAs are found in nearly 50% of patients with systemic lupus erythematosus (SLE) and 1% to 5% of the general population. Nearly 40% of patients with SLE meet diagnostic criteria for the APLA syndrome.
- **Testing:** The Sapporo criteria recognize the following antibodies as criteria for APLA syndrome: IgG and IgM anti- β_2 -glycoprotein-I antibodies, IgG and IgM anticardiolipin antibodies, and lupus anticoagulant [24•]. Lupus anticoagulants are detected by various functional coagulation assays, because the APLAs react with the phospholipids needed for the ex vivo coagulation process, thus prolonging clotting times [25]. False-positive lupus anticoagulant tests are not uncommon, occurring more frequently in patients who are on oral anticoagulants, those who are older, and those who have mildly positive lupus anticoagulant test results [26]. Because APLAs may be transient, the Sapporo criteria require repeatedly positive tests at least 12 weeks apart to confirm a diagnosis of APLA syndrome [24•]. Several other APLA tests are not part of the Sapporo criteria because their association with thrombosis or pregnancy loss has not been established; these include IgA anti- β_2 -glycoprotein-I and anticardiolipin antibodies, as well as antibodies against phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol. Presently, there is no indication for testing for these additional APLAs.
- **Risk for thrombosis:** APLAs are highly thrombophilic and are associated with both arterial and venous thrombosis. Positivity for all three APLA tests—lupus anticoagulant, anticardiolipin, and anti- β_2 -glycoprotein-I antibody—is associated with the highest risk of thrombosis and pregnancy loss [27]. APLA syndrome is also implicated in recurrent VTE. Patients with the APLA syndrome who discontinue anticoagulation have a 29% chance of recurrent thrombosis in the first 6 months and a 50%

chance of recurrence within 2 years [28], although patients in this study did not conform to the Sapporo criteria for APLA syndrome.

- **Treatment:** Because of the high rate of recurrent thrombosis, patients with APLA syndrome should be maintained on anticoagulation indefinitely. Randomized trials have shown that an INR target range of 2 to 3 is as effective in preventing recurrent thrombosis as a target range of 3 to 4 [29]. This probably holds true as long as the INR is reliable and indicates the true level of anticoagulation. However, because APLA can prolong both aPTT and prothrombin time clotting assays, test results may underestimate the therapeutic degree of anticoagulation. If the aPTT is prolonged at baseline because of a lupus anticoagulant, then anti-Xa levels must be used to monitor unfractionated heparin therapy. Every patient with APLA syndrome on VKA therapy should have the validity of his/her INR checked by comparing the INR to factor II activity or a chromogenic factor Xa assay. It can then be determined whether the INR is a reliable measure of that patient's anticoagulation and can be used in future monitoring. Point-of-care instruments are particularly prone to unreliably high INR readings in patients with APLA syndrome [30]. It is not known whether patients with arterial thrombosis and APLA syndrome are treated more effectively with antiplatelet or warfarin anticoagulation therapy. In the absence of prospective randomized trial data, no consensus exists on this topic.
- **New developments:** Rituximab has been shown to decrease APLA titers in some patients, but whether lowering or disappearance of APLA titers leads to a decreased thrombosis risk has not been studied. A clinical trial is investigating the effect of rituximab on APLA titers [31].

Elevated level of factor VIII

- **General information:** Elevated plasma levels of factor VIII are an independent and dose-dependent risk factor for VTE [32,33]. Elevations in factor VIII have a familial-inherited component, as documented by studies in families with thrombophilia and in monozygotic twins. No genetic polymorphism has been identified to explain elevations in factor VIII.
- **Prevalence:** Prevalence of factor VIII elevation is poorly understood. Factor VIII is an acute phase reactant, and its baseline levels vary considerably. Twenty-five percent of patients referred for thrombophilia evaluation will have elevations in factor VIII without elevations in C-reactive protein, suggesting that elevated levels are common in this population [33]. Factor VIII elevations commonly are seen in patients of African ancestry with venous thrombosis.
- **Testing:** Functional factor VIII clotting assays (also referred to as factor VIII activity or FVIIIc) are available, as are factor VIII antigen assays. Factor VIII activity assays are the preferred test.
- **Risk for thrombosis:** Population-based studies have demonstrated that elevations in factor VIII activity above 150% confer an increased risk of 4.8 for first-episode venous thrombosis compared with levels less than 100% [32]. It is not clear whether elevated factor VIII activity is also a risk factor for recurrent VTE, as data in the literature have been discrepant [34].
- **Treatment:** Because the role of elevated factor VIII levels and recurrent VTE is controversial, decisions regarding duration and intensity of anticoagulation therapy should be made independent of factor VIII levels. This also implies that there is no role for routine clinical testing for factor VIII levels as part of a thrombophilia workup.
- **New developments:** None identified.

Hyperhomocysteinemia

- Elevated levels of plasma homocysteine have been shown to be associated with an increased risk of venous and arterial thrombosis [35].
- The methylene-tetrahydrofolate-reductase (MTHFR) enzyme is a regulator of homocysteine metabolism. The C677T “thermolabile” mutation of MTHFR is common in North American populations, with nearly 12% of the population being homozygotes, and may be associated with higher plasma homocysteine levels. Meta-analyses have demonstrated that MTHFR polymorphisms in North America, where food is supplemented with folic acid, are not a risk factor for venous and arterial thrombosis or for pregnancy complications [36–38].
- Several prospective studies have demonstrated that lowering homocysteine levels does not decrease the risk of primary venous and arterial thromboembolic events [39,40] or of recurrent venous and arterial thrombosis [41,42]. This also applies to patients with elevated homocysteine levels secondary to chronic renal disease [43,44]. This implies that hyperhomocysteinemia may not have a causal effect on thrombosis, but instead may act as a marker for disease.
- Because the presence of MTHFR polymorphisms is not a thrombophilic state, there is no indication to screen for these mutations. Because lowering homocysteine levels has no demonstrated clinical benefit, there is no indication for treatment of elevated homocysteine levels with B vitamin or folic acid supplementation. Finally, because elevated homocysteine levels have no clinical consequences, there is no rationale to test for homocysteine levels in thrombophilia evaluations.
- **New developments:** A recent study may explain why elevated homocysteine levels are a marker of an increased risk for thrombosis, yet why lowering elevated levels does not lead to decreased thromboembolic events [45]. The study showed that hyperhomocysteinemia is associated with elevated factor VIII levels, which in themselves are a known risk factor for thrombosis. After adjusting for the elevated factor VIII levels, hyperhomocysteinemia was not associated with an increased risk for arterial and venous thrombosis [45].

Myeloproliferative disorders

- Essential thrombocytosis (ET) and polycythemia vera (PV) are myeloproliferative neoplasms that carry a substantial risk for thrombosis, arterial more commonly than venous [46]. A gain-of-function mutation of the Janus kinase-2 (JAK2) enzyme, referred to as JAK2V61F, is found in nearly 100% of patients with PV and 50% of those with ET. In patients with ET but not PV, the mutation has been shown to be associated with a higher risk for cardiovascular events [47], but further investigation is needed to determine whether the presence of the JAK2V61F mutation modifies the risk for thrombosis.
- The JAK2 mutation is commonly found in patients with splanchnic vein thrombosis (Budd-Chiari syndrome; portal, mesenteric, and splenic vein thrombosis), occurring in about a third of such patients [48–51]. Only about half of these JAK2-positive patients have an overt myeloproliferative disorder at the time of diagnosis. Some of the patients with splanchnic vein thrombosis will develop a myeloproliferative disorder during follow-up, and it appears that this happens more frequently in those who are JAK2 positive [48,51].

- One study showed that 5% of patients with cerebral vein thrombosis were positive for the JAK2 mutation [50]. The clinical significance of this finding is unclear and requires further investigation.
- A recent retrospective review of 664 consecutive patients with nonsplanchnic thrombosis (500 of whom had VTE) showed the prevalence of JAK2 mutation to be less than 1%. Presence of a JAK2 mutation without symptoms of a myeloproliferative disorder was not associated with increased risk of recurrent venous thrombosis or progression to a myeloproliferative disorder over a 4-year follow-up period [52]. This argues against the utility of screening patients with nonsplanchnic vein thrombosis for the JAK2 mutation as part of a thrombophilia workup [52].
- In summary, the authors' conclusions on testing for JAK2 are as follows:
 - Testing may be considered in patients with splanchnic vein thrombosis but typically does not change management.
 - No meaningful recommendations can be given regarding screening in patients with cerebral vein thrombosis.
 - Testing in evaluation of patients with nonsplanchnic vein thrombosis and non-cerebral vein thrombosis is not recommended.

Paroxysmal nocturnal hemoglobinuria

- Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal bone marrow disorder resulting from an acquired mutation of the PIG-A gene in a hematopoietic stem cell, leading to absent or decreased cell surface expression of glycoprotein I-anchored proteins on the surface of blood cells. It is associated with increased venous thrombosis, which occurs most often in the intra-abdominal veins, particularly the hepatic veins. Cerebral vein and peripheral vein thrombosis also occurs, but less commonly.
- The pathophysiology of thrombosis is not entirely understood, and no consistent abnormalities have been found. Several etiologies have been hypothesized [53].
- Until recently, prevention and treatment of PNH-associated thrombosis was limited to anticoagulation. Long-term treatment with the complement inhibitor eculizumab (Soliris; Alexion Pharmaceuticals, Cheshire, CT), FDA approved in March 2007, has been demonstrated to reduce the risk of clinical thromboembolism in patients with PNH [54].
- Screening for PNH by peripheral blood flow cytometry for CD55 and CD59 is warranted in thrombophilia evaluations with venous thrombosis and unexplained hemolysis or with peripheral blood cytopenias.

Abnormalities in fibrinolysis

- Multiple parameters of fibrinolysis have been investigated as potential causes of thrombophilia. A good comprehensive review of the topic was published recently [55•]. Investigation of these parameters has been challenging, in view of the fact that coagulation assays do not reliably reflect fibrinolysis of formed thrombus. Studies have yielded conflicting or indecisive results regarding an association of antigen levels, enzyme activity, or certain polymorphisms with the risk for arterial or venous thrombosis.
- Given the variability of data associating impaired fibrinolysis to arterial and venous thrombosis, workup for abnormalities in the fibrinolytic pathway (ie, testing for plasminogen, tissue plasminogen activator [tPA], plasminogen activator inhibitor-1 [PAI-1], and thrombin-activatable fibrinolysis inhibitor [TAFI]) is not meaningful. Results neither explain the

etiology of a thrombotic event in an individual patient nor influence decision making regarding the length of anticoagulant therapy.

Plasminogen

Although initially plasminogen deficiency was believed to be a risk factor for thrombosis, more recent and cumulative data indicate that a deficiency does not lead to an increased risk for arterial or venous thrombosis [55•,56]. Thus, at present, plasminogen should not be considered a hypercoagulable state. Accordingly, there is no role for including plasminogen antigen or activity determination in a thrombophilia workup.

Tissue plasminogen activator

Increased tPA antigen levels have been found to increase the risk for arterial thrombosis, even though not all studies have found such an association. No relationship with venous thrombosis has been detected [55•]. The observation that elevated tPA levels are associated with arterial thrombosis appears paradoxical. However, it has been speculated that this association may reflect an association of high PAI-1 levels and arterial thrombosis [55•], which is the principal inhibitor of tPA. Surprisingly, however, the association between PAI-1 levels and arterial thrombosis has been conflicting and unconvincing. Several polymorphisms in the tPA gene have been described, but no clear association has been found between these changes and arterial thromboembolism or VTE.

Plasminogen activator inhibitor-1

Although there are some inconsistent study findings regarding the association of elevated levels of PAI-1, the principal inhibitor of tPA, and the risk for VTE, overall it appears that increased levels are not a risk factor for VTE [55•]. The 4G/5G I/D polymorphism at position -675 is the most frequently studied polymorphism of PAI-1 and has been shown to be associated with elevated PAI-1 antigen levels. Data on the association of the polymorphism with VTE have been inconsistent [55•]. Results of studies on the relationship between PAI-1 and arterial thrombosis have been summarized as being conflicting and unconvincing [55•]. At this point, there is no clinical utility in obtaining PAI-1 activity or antigen levels or looking for PAI-1 polymorphisms when performing a thrombophilia workup in routine clinical practice, because the association of increased levels with the presence of polymorphisms is too feeble or inconsistent and the clinical relevance of finding an abnormality is unclear.

Thrombin-activatable fibrinolysis inhibitor

TAFI suppresses fibrinolysis by preventing binding of tPA to plasminogen and, therefore, the activation of plasminogen to plasmin. Although several studies have shown elevated TAFI levels to be associated with first or recurrent VTE and with arterial thrombosis, not all studies have shown consistent results [55•,57]. A large study of thrombophilic families recently showed no increased risk for arterial or venous thrombosis from high TAFI levels. In their report, the investigators discuss the results of the various previous inconsistent studies and critically review possible reasons for these inconsistencies, such as the difference in applied TAFI assays, the intrinsic instability of TAFI, and polymorphisms affecting TAFI levels [57].

Whom to test

Consensus guidelines

- There is no general consensus as to which patients and family members should be tested for thrombophilias. We are aware of the existence of

five guidelines or consensus statements, created by the American College of Medical Geneticists [58], the College of American Pathologists [59], the British Committee for Standards in Haematology [21], the European Genetics Foundation [60], and the Thrombosis Interest Group of Canada, which are presently under review [61]. These guidelines vary markedly in their recommendations as to who should and should not be tested, suggesting very limited testing [21], very liberal testing [60], or some intermediate level [58,59,61]. The 2008 antithrombotic and thrombolytic therapy guidelines from the American College of Chest Physicians do not use presence of hereditary thrombophilia as a major factor to guide length of anticoagulation for VTE, because, as the authors state, “evidence from prospective studies suggests that these factors are not major determinants of risk of recurrence” [62]. Presence of a thrombophilia should not lead to a higher target INR when VKA treatment is given, as the recurrence rate of VTE on standard-intensity VKA with a target INR of 2 to 3 is not increased in patients with thrombophilia compared with those without it [63].

Authors' approach

- The main reason we test patients is to detect a strong thrombophilia (ie, APLA syndrome, AT deficiency, homozygous FVL, double-heterozygous FVL plus prothrombin 20210 mutation, protein C deficiency, and maybe protein S deficiency). The finding of a strong thrombophilia has several consequences in our practice; it:
 - Decreases our threshold to recommend long-term anticoagulation in a patient who has had an episode of spontaneous VTE.
 - Leads to a discussion with the patient who has an unexplained arterial, nonarteriosclerotic thromboembolic event regarding whether anticoagulant or antiplatelet therapy is the preferred treatment for secondary prevention.
 - Prompts recommendation for testing of the identified thrombophilia(s) in asymptomatic female family members and advice against the use of estrogen birth control methods and for anticoagulation prophylaxis during the postpartum and possibly the antepartum period.
- We do not test for parameters of fibrinolysis or for the MTHFR polymorphisms, and recently stopped testing for homocysteine levels. Table 1 lists the thrombophilia tests we order when we are evaluating a patient for thrombophilia. Table 2 lists the type of patients for whom we consider thrombophilia testing. However, individual decisions, often in discussion with the patient, must be made when deciding whom and how extensively to test.

Insurance discrimination based on genetic thrombophilia test results

- In the United States, health insurance and employment discrimination based on a person's genetic testing results is illegal, as signed into law in May 2008 under the Genetic Information Nondiscrimination Act (GINA). However, life insurance discrimination—in the form of insurance denial or higher premiums—based on genetic results is not covered under GINA and therefore may occur. Thus, critical consideration should be given to which individuals to test for genetic thrombophilias. The decision should be made together with the patient or the asymptomatic family member who is being considered for testing.

Table 1. Thrombophilia tests

Arterial thromboembolism
Complete blood count
Protein C activity
Protein S activity, free and total protein S antigen
Antithrombin activity
Anticardiolipin IgG and IgM antibodies
Anti- β_2 -glycoprotein-I IgG and IgM antibodies
Lupus anticoagulant
Fibrinogen and factor VIII activities*
Venous thromboembolism
Complete blood count
Factor V Leiden
Prothrombin 20210 mutation
Protein C activity
Protein S activity, free and total protein S antigen
Antithrombin activity
Anticardiolipin IgG and IgM antibodies
Anti- β_2 -glycoprotein-I IgG and IgM antibodies
Lupus anticoagulant
Factor VIII, factor IX, and factor XI activities*

*Although an established risk factor, test is not typically ordered by the authors.

Table 2. Indications for thrombophilia testing

Unexplained venous thromboembolism (VTE) at a younger age (< 50 years)
Recurrent spontaneous VTE
Unexplained VTE at an unusual site (portal, mesenteric, splenic, hepatic, sinus/cerebral, or renal veins)
Unusually extensive spontaneous VTE
Family history of spontaneous VTE
Asymptomatic individual with family history of known stronger thrombophilia
Antithrombin deficiency
Protein C deficiency
Protein S deficiency
Homozygous factor V Leiden
Homozygous prothrombin mutation
Compound thrombophilias
Recurrence of VTE while adequately anticoagulated
Unexplained arterial thromboembolism in a younger patient who has no significant arteriosclerosis risk factors and no cardioembolic source
≥ 3 unexplained pregnancy losses before week 10, or ≥ 1 after week 10

Test interpretation, patient education, awareness, and patient advocacy

Interpreting test results

- When interpreting thrombophilia laboratory test results, it is important to be aware of the circumstances that lead to abnormal results without a true thrombophilia being present, as well as the influence of acute thrombosis and therapy with heparin and VKA on test results.

Patient education

- When a thrombophilia has been found, educating the patient and his or her family members is important. Referral to a health care provider knowledgeable about thrombophilias or to a genetic counselor should be considered. There are online educational resources on a variety of thrombophilias and the genetic aspects of family testing (eg, www.stoptheclot.org, www.fvleiden.org). The nonprofit organization NATT (National Alliance for Thrombosis and Thrombophilia; www.stoptheclot.org) has assembled peer- and consumer-reviewed patient education material on its website [64•]. Patients can download these educational materials, as can health care providers operating in thrombosis clinics, anticoagulation clinics, or offices where patients with thrombosis and thrombophilia are seen.

The Surgeon General's *Call to Action*

- In September 2008, the US Surgeon General issued a *Call to Action* to prevent DVT and PE [65], calling on individuals, families, and communities to understand DVT and PE, be familiar with the risk factors and symptoms of DVT and PE, understand how to reduce the risks, and ask for and get appropriate VTE prophylaxis when hospitalized or in other VTE risk situations. The national goal is to reduce morbidity and mortality from VTE.

Patient advocacy

- Patient advocacy can be a powerful tool to promote public awareness, better health care delivery, and more research [66]. Therefore, patients with thrombosis and thrombophilia should get involved in patient advocacy, and health care providers should make their patients aware of opportunities to get involved, such as NATT (www.stoptheclot.org).

Acknowledgment

Dr. Stephan Moll receives grant support from a Centers for Disease Control and Prevention (CDC) grant (#1U01DD000292-0) and a cooperative agreement with the CDC (U27DD00326).

Disclosures

Dr. Moll has been a consultant for GTC Biotherapeutics, Talecris, and Ovation. No other potential conflicts of interest relevant to this article were reported.

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