Thrombophilia Testing, Recurrent Thrombosis, and Women’s Health

Gregory Piazza, MD, MS

Case Presentation 1: A 33-year-old previously healthy man presented with sudden-onset dyspnea and sharp right-sided chest pain. He had noted right leg edema and calf discomfort a week earlier. He denied recent trauma, surgery, or immobility. His mother had a history of postpartum deep vein thrombosis (DVT). On physical examination, he was tachycardic with a heart rate of 114 bpm, normotensive with a blood pressure of 102/76 mm Hg, and hypoxic to 88% on room air. Contrast-enhanced chest computed tomogram demonstrated bilateral segmental pulmonary embolism. Right lower-extremity venous ultrasound documented femoral and popliteal DVT.

Case Presentation 2: A 78-year-old woman with hypertension and obesity developed acute left leg edema and pain 2 days after open reduction and internal fixation of a right hip fracture. On physical examination, the patient had severe edema and tenderness of the left lower leg and thigh. Left lower-extremity venous ultrasound documented left common femoral, distal femoral, and popliteal DVT.

Overview

Thrombophilias describe inherited and acquired hypercoagulable states that increase the risk of venous and, in some cases, arterial thrombosis. The prevalence of thrombophilias varies according to the population studied. In the general population, thrombophilias are less frequent than “traditional” venous thromboembolism (VTE) risk factors such as cancer, immobility, and obesity. However, in patients who have experienced an initial episode of VTE or have a family history of VTE, the prevalence of thrombophilia increases.

In a European registry of 21,367 consecutive patients with symptomatic VTE, thrombophilia testing was performed in 21%. Thrombophilia was detected in 32% of those in whom testing was performed. The most frequently detected thrombophilias were factor V Leiden (26%), antithrombin deficiency (20%), and prothrombin gene mutation (18%). The rate of thrombophilia detection was similar in patients with idiopathic (unprovoked) and provoked VTE.

Thrombophilias may be classified according to their diagnostic yield. High-yield thrombophilia testing includes evaluation for factor V Leiden, prothrombin gene mutation, and antiphospholipid antibodies. Lower-yield thrombophilia testing focuses on deficiency of protein C, protein S, or antithrombin; homocysteine levels, methylenetetrahydrofolate reductase gene mutations; plasminogen activator inhibitor-1 levels; plasminogen activator inhibitor-1 gene mutation; and levels of factors VIII, IX, XI, and fibrinogen. Importantly, the yield of particular thrombophilia tests may vary according to patient demographics.

Thrombophilias may also be categorized on the basis of whether the hypercoagulable condition is inherited or acquired and according to the risk of initial thrombosis (Table 1). High-risk thrombophilias include deficiency of protein C, protein S, or antithrombin; homozygosity for factor V Leiden or the prothrombin gene mutation; compound heterozygosity for factor V Leiden and the prothrombin gene mutation; severe hyperhomocysteinemia (>100 μmol/L); and elevated antithrombin and antiphospholipid antibodies. Although quite rare (0.2%), severe hyperhomocysteinemia is a high-risk thrombophilia associated with recurrent thrombosis in 42% of cases.
Major Thrombophilias

Factor V Leiden

The factor V Leiden mutation describes a guanine-to-adenine substitution at nucleotide 1691 that results in a glutamine instead of arginine at amino acid residue 506. Factor V becomes resistant to cleavage by activated protein C because of this mutation. The prevalence of factor V Leiden is greatest among white patients (5%), especially those of Northern European descent. Factor V Leiden results in an almost 3-fold increase in the risk of a first episode of VTE. Although factor V Leiden increases the frequency of VTE at any age, the mutation results in the greatest increase in risk among patients ≥70 years of age. Factor V Leiden testing usually begins with a screening assay for activated protein C resistance, followed by direct DNA-based genotyping for positive screens.

Prothrombin Gene Mutation

The prothrombin gene mutation most commonly describes a guanine-to-adenine substitution at nucleotide 20210 in the 3' untranslated region of the prothrombin gene. However, other polymorphisms of the prothrombin gene have been reported. Heterozygotes for the prothrombin gene mutation have 30% higher plasma prothrombin levels than normal. Heterozygosity for the prothrombin gene mutation confers a 4-fold increased risk of VTE. Testing for prothrombin gene mutation requires direct DNA-based genotyping.

Antiphospholipid Antibodies

Antiphospholipid antibodies are a class of autoantibodies directed against epitopes on plasma proteins that are exposed when these proteins bind phospholipids on plasma membranes. Antiphospholipid antibodies may increase the risk of arterial and venous thrombosis through a variety of proposed mechanisms, resulting in endothelial injury and coagulation activation. DVT, pulmonary embolism, and stroke are the most common complications of antiphospholipid antibodies, although any segment of the arterial or venous circulation may be affected. A small subset of patients will develop catastrophic antiphospholipid antibody syndrome, characterized by thrombosis in multiple vascular beds culminating in multisystem organ failure. Testing for antiphospholipid antibodies includes lupus anticoagulant assays to detect autoantibodies that prolong in vitro clotting times and enzyme-linked immunosorbent assay for anti-cardiolipin antibodies, anti-β2-glycoprotein I antibodies, and anti-prothrombin antibodies. Because antiphospholipid antibodies may be transiently present in the setting of an acute thrombotic event or infection, testing should be repeated at least 12 weeks after an initial positive test to document the persistence of the antiphospholipid antibody.

Impact on Recurrent VTE

Thrombophilia testing is often performed to assess the risk of recurrent VTE in a patient with an initial event. However, only a subset of thrombophilias have been documented to significantly increase the risk of VTE recurrence. Although antiphospholipid antibodies and deficiency of protein C, protein S, or antithrombin consistently increase the risk of recurrent VTE, more commonly diagnosed thrombophilias such as factor V Leiden and the prothrombin gene mutation do not appear to increase the risk of recurrence.

In the Leiden Thrombophilia Study, 474 patients with an initial VTE event underwent extensive thrombophilia testing, including evaluation for factor V Leiden and levels of homocysteine, fibrinogen, factor VIII, factor IX, factor XI, protein C, protein S, and antithrombin. The cumulative rate of recurrent VTE was similar in patients with and without thrombophilia (adjusted hazard ratio, 1.4; 95% confidence interval, 0.9–2.2). With the exception of patients with hyperfibrinogenemia, none of the tested thrombophilias were associated with an increased risk of recurrent VTE.

A subsequent study evaluated the impact of factor V Leiden and the prothrombin gene mutation on VTE recurrence. Heterozygosity for either factor V Leiden or the prothrombin gene mutation was not associated with an increased risk of VTE recurrence. Furthermore, compound heterozygosity and homozygosity for either factor V Leiden or prothrombin gene mutation did not increase the risk of recurrent VTE.

Determining whether a VTE event was provoked or unprovoked (idiopathic) appears to have greater implications for the prevention of VTE recurrence than the results of thrombophilia testing. In an analysis of 1626 patients with an initial VTE, unprovoked VTE (adjusted hazard ratio, 2.3; 95% confidence interval, 1.82–2.9) was a more powerful predictor of recurrent VTE than thrombophilia status (adjusted hazard ratio, 1.44; 95% confidence interval, 1.03–2.03) and increasing age (adjusted hazard ratio, 1.14; 0.9–1.4).
95% confidence interval, 1.06–1.12). Extending anticoagulation with either warfarin or a direct oral anticoagulant reduces the risk of recurrent VTE by 60% to 90% over placebo in patients with unprovoked VTE who have completed limited-duration anticoagulation. Low-dose aspirin in patients with unprovoked VTE who have completed limited-duration anticoagulation also reduces the risk of recurrent VTE. Persistently elevated D-dimer levels at 1 month after the completion of limited-duration anticoagulation in patients with an initial unprovoked VTE may identify some patients at increased risk for VTE recurrence.

Impact on Women’s Health
Thrombophilia has important implications for women’s health, particularly contraceptive therapy, fertility, and pregnancy.

Hormonal Contraceptive/Replacement Therapy
Use of combination oral contraceptive pills, especially those containing third-generation progestins, has been associated with at least a 3-fold increased risk of VTE. Use of combination oral contraceptive pills in patients with thrombophilia such as factor V Leiden heterozygosity is associated with at least a 30-fold increase in risk of VTE. The increased risk of VTE appears to be highest around the time of oral contraceptive pill initiation and within the first 6 months.

Infertility
In a subset of women, thrombophilia results in infertility, which may manifest as difficulty with conception, recurrent pregnancy loss, or both. The mechanism by which thrombophilia causes infertility does not appear to be limited to a hypercoagulable state but may also include abnormalities of trophoblast differentiation and placentation. Thrombophilias are associated with both early and late pregnancy loss.

Thrombophilia and Pregnancy
Thrombophilias also increase the risk of pregnancy-related complications, including VTE. For example, the relative risk increase for VTE ranges from 9-fold in women with heterozygosity for factor V Leiden to 34-fold in those with homozygosity for the mutation. However, the absolute risk increase in pregnant women with factor V Leiden is 0.2%. Therefore, although the relative risk of VTE resulting from thrombophilia in pregnancy is high, the absolute risk is low.

The risk of other pregnancy-related complications such as preeclampsia and placental abruption is also increased in the presence of thrombophilia.

Thrombophilia Testing
Thrombophilia testing is often considered in patients with VTE at a young age, recurrent VTE, thrombosis in unusual sites, a strong family history of VTE, and recurrent pregnancy loss. Rationales for performing thrombophilia evaluations include selecting the optimal agent and duration of anticoagulation, predicting the risk of VTE recurrence, determining the optimal intensity of thromboprophylaxis, assessing VTE risk with pregnancy or hormonal contraceptive or replacement therapy, and identifying family members at risk for thrombosis. Patients seeking an explanation for an arterial or venous thrombosis, especially if unprovoked or expected, will frequently request thrombophilia testing.

Strategies for Thrombophilia Testing and Cost Implications
Various strategies for thrombophilia testing have been proposed. The “kitchen sink” approach runs all available tests. A selective strategy performs the highest-yield tests first and focuses on those tests that will affect therapy or for which there is an intellectual curiosity, for example, if a patient has a compelling family history. Finally, a “no testing” approach is to defer all testing because the results are not expected to affect disease management.

Selection of an approach to thrombophilia testing must take into account cost implications. A kitchen sink approach, if applied broadly, would result in a considerable cost to healthcare systems with limited yield, given the low prevalence of thrombophilia in the general population. Although saving on laboratory costs, a no testing approach would fail to identify patients with high-risk thrombophilias who would benefit from extended-duration anticoagulation and may incur the expense of potentially preventable recurrent VTE.

The cost-effectiveness of thrombophilia testing for patients with an initial VTE has been the focus of a number of decision analysis studies. In a Markov model, strategies of thrombophilia testing or no testing followed by anticoagulation for 6 to 36 months were compared in a cohort of patients with idiopathic DVT. Thrombophilia testing followed by 24 months of anticoagulation in patients with a hypercoagulable state proved to be more cost-effective than 6 months of anticoagulation without testing.

A subsequent modeling study assessed the cost-effectiveness of changing standard 3-month warfarin-based anticoagulation for acute VTE to 10-year, 20-year, or lifelong therapy on the basis of the results of thrombophilia testing. Thrombophilia testing in patients with acute pulmonary embolism was cost-effective regardless of sex or age. For patients with acute DVT, thrombophilia testing was cost-effective in men <70 years of age and women <50 years of age.

A Stepwise Approach to Thrombophilia Testing
A stepwise strategy for thrombophilia testing considers the clinical scenario (when to test), the implications of testing (why to test), and then the overall approach to testing (how to test). A selective strategy begins with an initial thrombophilia evaluation focused on the highest-yield testing, factor V Leiden, prothrombin gene mutation, and antiphospholipid antibodies (Figure). Although antiphospholipid antibodies require confirmation 12 weeks after an initial positive, polymorphisms detected on genetic testing...
for factor V Leiden or prothrombin gene mutation represent true positives, regardless of when the testing is performed. A secondary evaluation for less common thrombophilias such as deficiencies of protein C, protein S, and antithrombin may be performed after completion of anticoagulation if a high clinical suspicion for thrombophilia exists and the initial evaluation is negative. Because low levels of protein C, protein S, and antithrombin may be observed in the setting of acute thrombosis and anticoagulation and do not necessarily indicate true thrombophilia, testing for these thrombophilias should be deferred for the short term (Table 2).

Although the desire to perform thrombophilia testing during the patient’s hospitalization for VTE is frequently high, a strong case can be made to defer evaluation to the follow-up outpatient visit. Clinicians often have very limited time and resources during the hectic hospitalization for a thrombotic event to explain the implications of a thrombophilia diagnosis on management and to answer questions. Furthermore, the psychological shock of suffering thrombosis may hinder a patient’s ability to absorb the implications of a discussion on thrombophilia testing and its ramifications.

**Case Presentation 1:** Given the patient’s youth, family history of VTE, and unprovoked event, thrombophilia testing was performed after discharge from the hospital. A lupus anticoagulant was detected and subsequently confirmed on a second test 6 weeks later. Because of a high risk of VTE recurrence in the setting of a lupus anticoagulant and an unprovoked event, the patient was maintained indefinitely on warfarin anticoagulation with an international normalized ratio of 2 to 3. At the 1-year follow-up, he had recovered fully and had not experienced another pulmonary embolism or DVT.

**Case Presentation 2:** Given the patient’s age and the provoked nature of her DVT, thrombophilia testing was not performed. She was treated with 6 months of anticoagulation with rivaroxaban. At the 1-year follow-up, she had recovered fully and had not suffered a VTE recurrence.

**Table 2. Tips for Thrombophilia Testing**

Follow a stepwise strategy for thrombophilia testing that considers the clinical scenario (when to test), the implications of testing (why to test), and then the overall approach to testing (how to test).

Use a selective strategy that focuses on the highest-yield thrombophilia testing first.

Defer testing for deficiencies of protein C, protein S, and antithrombin because low levels do not necessarily indicate true thrombophilia in the setting of acute thrombosis and anticoagulation.

Remind patients that a negative thrombophilia evaluation does not exclude thrombophilia because there are many hypercoagulable conditions that have yet to be identified and for which testing does not exist.

Consider deferring thrombophilia testing to the follow-up outpatient visit when there will be more time for discussion and when the patient will have recovered psychologically from the acute thrombotic event.

**Disclosures**

None.

**References**


