

# ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

Section Editor: Craig M. Kessler, MD

## Testing for Thrombophilias

Stephan Moll, MD  
Associate Professor  
Department of Medicine  
Division of Hematology-Oncology  
University of North Carolina at Chapel Hill  
School of Medicine  
Chapel Hill, NC

**H&O** What are the tests to consider when evaluating a patient for a thrombophilia?

**SM** A number of known thrombophilias predispose to thrombosis, mostly venous thromboembolism (VTE), less so arterial. They can be inherited or acquired, or have a component of both. As venous thrombosis is mostly caused by disturbances in the plasma coagulation system, with platelet participation playing a minor role, abnormalities of the coagulation factors (factor V Leiden; prothrombin 20210 mutation; or deficiencies of protein C, protein S and antithrombin) are mostly risk factors for venous thromboembolism. In arterial thrombosis, platelets play the predominant role, with only some participation of the coagulation proteins. Therefore, the aforementioned plasma coagulation factor abnormalities are typically not significant risk factors for arterial thrombosis. Very little is known about thrombophilias that predispose to arterial thrombosis.

Table 1 lists thrombophilia tests that I typically consider ordering when evaluating a patient suspected of having thrombophilia. I never test for the methylenetetrahydrofolate-reductase (MTHFR) polymorphisms C677T and A1298C, as they have not been shown to be risk factors for venous or arterial thrombosis or pregnancy complications, if the serum homocysteine level is normal. I reserve testing for rare thrombophilias, such as plasminogen deficiency (test: plasminogen activity), or dysfibrinogenemia (tests: thrombin clot time, fibrinogen activity, fibrinogen immunoelectrophoresis) for individuals with a strong family history of thrombosis. In individuals with unexplained intra-abdominal thrombosis (portal, splenic, mesenteric, or hepatic vein thrombosis) the suspicion for an underlying myeloproliferative disorder is

heightened and I closely look at complete blood count, consider JAK-2 (Janus-activating kinase-2) mutation testing, and order flow cytometry of peripheral blood for CD55/CD59 expression on white cells to assess for paroxysmal nocturnal hemoglobinuria.

**H&O** What are the caveats when interpreting thrombophilia test results?

**SM** When interpreting thrombophilia laboratory test results, it is important to be aware of the circumstances that lead to abnormal results without the patient having a true thrombophilia (Table 2). It is also important to know what influence acute thrombosis and therapy with heparin and vitamin K antagonists have on test results

**Table 1.** Thrombophilia Tests

### A. Arterial thromboembolism\*

- complete blood count (CBC)
- protein C activity
- protein S activity, free and total protein S antigen
- antithrombin activity
- anticardiolipin immunoglobulin (Ig)G and IgM antibodies
- anti- $\beta_2$ -glycoprotein-I IgG and IgM antibodies
- lupus anticoagulant
- homocysteine
- CBC
- fibrinogen and factor VIII activities<sup>†</sup>

### B. Venous thromboembolism\*

- complete blood count (CBC)
- 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- $\beta_2$ -glycoprotein-I IgG and IgM antibodies
- lupus anticoagulant
- homocysteine
- factor VIII, factor IX, and factor XI activities<sup>†</sup>

\*No indication for MTHFR testing in venous and arterial thromboembolism.

<sup>†</sup> Tests for established risk factors, but which the author does not routinely perform.

**Table 2.** Conditions Leading to Acquired Coagulation Factor Deficiencies

- Liver disease: decreased protein C, S, and antithrombin
- Vitamin K antagonist therapy: decreased protein C and S
- Estrogens (oral contraceptives, pregnancy, postpartum state, hormone replacement therapy): decreased protein S
- Inflammatory diseases: decreased protein S
- Acute thrombosis: decreased antithrombin and protein S
- Heparin therapy: decreased antithrombin

(Table 3). Unfortunately, quite a few patients are labeled as having a thrombophilia because their blood was tested at the wrong time or the test results were misinterpreted. Particularly in the patient who carries a diagnosis of deficiency of protein C, protein S or antithrombin, it is wise for the physician to question the diagnosis, until review of records and laboratory results has clarified that timing of the testing was correct and no confounding issues led to a transient decrease in these protein levels. Furthermore, repeat testing on a new blood sample at an appropriate time point is recommended. As lupus anticoagulant test result reporting can be quite confusing, it is also advisable practice to always question the diagnosis of antiphospholipid antibody (APLA) syndrome in a patient and to review what APLA tests were really done, whether they were just borderline positive (which may be clinically irrelevant) or moderately or strongly positive, and whether they were positive on at least two separate occasions, 3 months or more apart, as the revised Sapporo criteria of APLA syndrome require.

### H&O Which patients should be tested for thrombophilias and what guidelines exist?

**SM** Controversy exists regarding which patients and family members to test for thrombophilias. Individual physicians, institutions, and countries have different practice patterns and opinions, and there is no universally valid and accepted consensus. I am aware of four sets of guidelines that have been promulgated, and they vary greatly as to whom to test. These guidelines have been published by the American College of Medical Geneticists (2001), the British Committee for Standards in Haematology (2001), the European Genetics Foundation (2005), and the Thrombosis Interest Group of Canada (2006). The most conservative guidelines, in terms of which patients to test, are the British guidelines from 2001, and the most liberal, promoting the most widespread testing, are the European guidelines from 2005.

There has not been an effort to harmonize the guidelines and produce consensus. Earlier in 2008 an important “Evidence-based Practice Center” grant was awarded from the Agency for Healthcare Research and Quality and the Centers for Disease Control to study “Outcomes from Testing for Factor V Leiden and Prothrombin G20210A” (<http://www.ahrq.gov/clinic/tp/fvltpt.htm>). Results are expected by mid-2009. I expect that they will lead to a rekindling of activities trying to create some meaningful, evidence-based guidelines as to who should be tested for thrombophilia and who not. It may then be the time for the National Institutes of Health (NIH) to convene an expert panel to develop a consensus, similar to the NIH’s recent panel and consensus on “The Diagnosis, Evaluation and Management of von Willebrand Disease” ([http://www.nhlbi.nih.gov/guidelines/vwd/1\\_frontmatter.htm](http://www.nhlbi.nih.gov/guidelines/vwd/1_frontmatter.htm)). After all, the factor V Leiden genetic test is one of the most commonly performed genetic test in the United States. In many cases, I believe, factor V Leiden and other thrombophilia testing is not necessary. Furthermore, as the MTHFR polymorphism should not, based on meta-analyses of clinical trials, be considered anymore to be a risk factor for venous or arterial thrombosis or for pregnancy complications, new consensus guidelines might also prevent unnecessary testing for this polymorphism. Finally, there is certainly a significant healthcare expenditure associated with these tests, and, for that reason, a consensus statement would also be of benefit.

### H&O What is your personal approach to thrombophilia testing?

**SM** The practice of many hematologists who make thrombophilia testing decisions in their patients has changed in the last several years; mine have as well. Initially, after the discovery of factor V Leiden in 1994 and the prothrombin 20210 mutation in 1996, many physicians were thrilled about the ability to test for underlying hypercoagulable states and widespread testing became commonplace. However, in the last several years, as well designed studies have shown that the presence of a mild thrombophilia, such as heterozygous factor V Leiden or heterozygous prothrombin 20210 mutation, does not have any clinically meaningful impact on the risk of recurrent VTE once anticoagulation is stopped, the logic behind widespread testing has been appropriately questioned.

The main reason why I test patients for thrombophilia is to detect a strong thrombophilia (antithrombin deficiency, antiphospholipid antibody syndrome, homozygous factor V Leiden, double heterozygous factor V Leiden plus prothrombin 20210 mutation, protein C deficiency, and possibly protein S deficiency), because finding of a strong thrombophilia has a number of consequences in my practice: a) it decreases my threshold

**Table 3.** Influence of Acute Thrombosis, Heparin, and Vitamin K Antagonists on Thrombophilia Test Results

Test	Acute thrombosis	Unfractionated heparin	Low-molecular weight heparin	Vitamin K antagonists
Factor V Leiden genetic test	reliable	reliable	reliable	reliable
APC resistance assay	reliable*	n/a*	n/a <sup>†</sup>	reliable*
Prothrombin 20210 genetic test	reliable	reliable	reliable	reliable
Protein C activity or antigen	n/a <sup>‡</sup>	reliable	reliable	low
Protein S activity or antigen	may be low	reliable	reliable	low
Antithrombin activity	may be low	may be low	may be low	reliable
Lupus anticoagulant	reliable <sup>§</sup>	n/a <sup>¶</sup>	n/a <sup>¶</sup>	n/a <sup>¶</sup>
Anticardiolipin antibodies	reliable <sup>§</sup>	reliable	reliable	reliable
Anti- $\beta_2$ -glycoprotein-I antibodies	reliable <sup>§</sup>	reliable	reliable	reliable
Homocysteine	reliable	reliable	reliable	reliable

APC=activated protein C resistance; n/a=not applicable.

\*Reliable if the assay is performed with factor V depleted plasma; thus, clinicians need to inquire how the individual laboratory performs the assay.

<sup>†</sup>Depending on the way the assay is performed results may be unreliable; health care provider needs to contact the laboratory and ask how the specific test performs on heparin.

<sup>‡</sup>Probably reliable but limited data in literature.

<sup>§</sup>Test often positive or elevated at time of acute thrombosis, but subsequently negative.

<sup>¶</sup>Though many test kits used for lupus anticoagulant testing contain a heparin neutralizer making these tests reliable on unfractionated heparin (UF) and possibly low-molecular weight heparin (LMWH), clinicians need to inquire with their laboratory how their individual test kit performs in samples with UF and LMWH.

to recommend long-term anticoagulation in a patient who has had an episode of spontaneous VTE; b) it leads me to discuss with the patient who had an unexplained arterial, nonarteriosclerotic thromboembolic event whether anticoagulant or antiplatelet therapy might be preferred treatment to prevent recurrent events; and c) it prompts me to recommend testing for the identified thrombophilia(s) in asymptomatic female family members and advise them against the use of estrogen-based birth control methods and for anticoagulation prophylaxis during the postpartum, and possibly the antepartum, period. I realize and discuss with the patient, that there is, at present, a paucity of clinical outcome and intervention studies regarding these strong thrombophilias, and that management decisions have a strong empiric component.

Discovery of heterozygous factor V Leiden alone or heterozygous prothrombin 20210 mutation alone typically has no impact on the management of the patient with VTE or arterial thrombosis and does not need to lead to the recommendation that other family members be tested for the mutation. Thus, my goal of testing for factor V Leiden and the prothrombin mutation is not to detect the heterozygous state, but the homozygous, double heterozygous, compound thrombophilic states.

Another contribution to the decision to test a patient

for thrombophilia is that knowledge of the presence or absence of thrombophilia helps explain why a patient developed a thrombosis and, thus, fills the intellectual need wishing to understand the etiology of a thrombotic event. Patients often want to know whether they have a thrombophilia because they: a) want to understand what caused their thrombotic event; b) feel that knowledge of the presence of a thrombophilic abnormality may encourage them to make lifestyle changes that may decrease the risk of recurrent thrombosis; c) think they may receive more diligent thrombosis prophylaxis if they are able to tell their healthcare provider that they have a thrombophilia; and d) want to advise their asymptomatic family member to get tested for the thrombophilia they have been found to have, so that they can be proactive in lifestyle changes and prophylactic measures to decrease their future risk for thrombosis. However, most of the times thrombophilia testing because of these reasons does not affect clinical management, and appropriate lifestyle changes should be made by anybody with risk factors for VTE. Nevertheless, physicians need to be aware of these patient-perceived benefits of thrombophilia testing and need to address them in their discussion with the patient. However, all patients, no matter whether a person has a detectable thrombophilia or not, should a) know the risk factors for and symptoms of VTE; b) know his/her indi-

**Table 4.** When to Consider Thrombophilia Testing

- Unexplained VTE at a younger age (<50 years)
- Recurrent spontaneous VTE
- Unexplained VTE at an unusual site (portal, mesenteric, splenic, hepatic veins, sinus or cerebral veins, 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 (<50–60 years) who has no significant arteriosclerosis risk factors and no atrial fibrillation/flutter or cardioembolic source, including patent foramen ovale.

VTE=venous thromboembolism.

vidual risk factors, and, thus, risk level for VTE; c) make an attempt to normalize body weight and stop smoking; d) get appropriate VTE prophylaxis in the classical risk situations (major surgery, significant immobility, etc.).

Table 4 lists the type of patients in whom I consider, but not necessarily universally recommend, thrombophilia testing. Individual decisions, typically in discussion with the patient, need to be made when deciding whom to test and how extensively to test.

### H&O What are the drawbacks of testing for thrombophilias?

**SM** There are a number of potential downsides of testing and reasons to decide against thrombophilia testing, including: a) the lack of therapeutic consequences even if a thrombophilia is detected; b) the risk of misinterpretation of a test result by the healthcare provider and of inappropriate medical advice; c) the risk of having to pay higher life insurance premiums or being denied life insurance if a genetic thrombophilia is detected; d) the possibility that paternity may have to be questioned if the inheritance pattern is inconsistent with test results of other family members; and e) an unacceptable cost of the tests to the individual patient, as well as to the healthcare system as a whole, as costs can easily exceed \$1,000. If the results of the test have no clinical implications, it can be difficult to justify this expense.

### H&O What effect does recent federal legislation have on thrombophilia patients?

**SM** The Genetic Information Nondiscrimination Act (GINA) was passed into law in May 2008. It prohibits employment and health-insurance discrimination on the basis of genetic information. Although this development, is positive patients and physicians considering thrombophilia testing need to be aware that the law does not prevent life insurance companies from using the genetic test results to request higher premiums or deny insurance coverage based on factor V Leiden and prothrombin 20210 mutation status.

### H&O Could you discuss patient education regarding thrombophilias?

**SM** Patient education is important when a patient has been diagnosed with thrombophilia, to clarify its effect on the patient's health as well as on other family members. The onus is on the testing physician to discuss the implications of the test results with the patient, or refer the patient to a genetic counselor and/or a hematologist. Online educational resources on a variety of thrombophilias and on the genetic aspects of family testing exists ([www.stoptheclot.org](http://www.stoptheclot.org), [www.fvleiden.org](http://www.fvleiden.org)). The nonprofit organization NATT (National Alliance for Thrombosis and Thrombophilia) has assembled peer- and consumer-reviewed patient education material on its website ([http://stoptheclot.org/learn\\_more/learn\\_more.htm](http://stoptheclot.org/learn_more/learn_more.htm)). Patients can download and print these educational materials, as can healthcare providers operating in thrombosis clinics, anticoagulation clinics, or offices at which patients with thrombosis and thrombophilia are seen.

### Acknowledgment

Dr. Stephan Moll receives grant support from the CDC: grant # 1U01DD000292-0 and a cooperative agreement (U27DD00326).

### Suggested Readings

Moll S. Thrombophilias: practical implications and testing caveats. *J Thromb Thrombolysis*. 2006;21:7-15.

Grody WW, Griffin JH, Taylor AK, Korf BR, Heit JA; ACMG Factor V. Leiden Working Group. American College of Medical Genetics consensus statement on factor V Leiden mutation testing. *Genet Med*. 2001; 3:139-148.

Haemostasis and Thrombosis Task Force, British Committee for Standards in Haematology. Investigation and management of heritable thrombophilia. *Br J Haematol*. 2001;114:512-528.

The European Genetics Foundation; The Cardiovascular Disease Educational and Research Trust; The International Union of Angiology; The Mediterranean League on Thromboembolism, Nicolaides AN, Breddin HK, Carpenter P, et al. Thrombophilia and venous thromboembolism. International consensus statement. Guidelines according to scientific evidence. *Int Angiol*. 2005;24:1-26

Mant M. Hypercoagulable/thrombophilic states. 2006. Available online: <http://www.tigc.org/eguidelines/hypercoagstates.htm>. Accessed September 2, 2008.