Thrombophilia Testing Panel Now Available

Bronson Laboratory offers a thrombophilia testing panel consisting of the following: factor VIII activity, antithrombin III activity, protein C activity, protein S antigen, factor V Leiden mutation assay, prothrombin 90210A mutation assay, and a lupus anticoagulant assay. Abnormal values for protein C, protein S, and antithrombin III will be automatically reflexed to confirmatory testing at the Mayo Clinic laboratories. Although these tests are orderable individually, there is little rationale to do so except as noted below.

The activity assays are clotting tests that require adequate amounts of functional protein to achieve a normal result. For reasons having to do with greater achievable precision, the free protein S antigen test is used in lieu of an activity assay, but it too depends on adequate amounts of circulating protein for a normal finding. Importantly, abnormal values for these tests may indicate either an acquired or inherited condition. The mutational assays, on the other hand, directly assess whether a patient has an inheritable deficiency of these factors, and whether the mutation is homozygous or heterozygous. As such, they need only be run once in a patient’s lifetime. The lupus anticoagulant test involves two clot-based assays, the aPTT and dRVVT, as screening tests to exclude antiphospholipid syndrome. Normal clotting times for both of these assays essentially excludes a lupus anticoagulant. A prolonged aPTT will reflex to additional testing that includes mixing studies to exclude a factor deficiency or drug effect as the cause of the prolongation. An abnormal dRVVT will prompt repeat testing with an excess of phospholipid reagent to adsorb any antiphospholipid antibody, which should lead to correction of the dRVVT if a lupus anticoagulant is present. Two positive lupus anticoagulant tests separated by at least 12 weeks in the proper clinical setting are necessary for a diagnosis of phospholipid syndrome. The syndrome may alternatively be diagnosed by demonstrating IgG or IgM antcardiolipin or beta 2-glycoprotein antibodies, which are tests sent out to a reference lab.

Integral to the effective use of the testing panel is an accurate assessment of the likelihood of a thrombophilic state in a given patient, and whether the presence of a thrombophilia will have any impact on the patient’s therapy. For instance, the vast majority of older patients with venous thromboses in the setting of a recognized inciting event will not have a preexisting thrombophilic condition, and accordingly testing is generally not recommended in this population. In regards to arterial thromboses, the overwhelming majority are a direct consequence of atherosclerosis, and the added presence of a thrombophilia in this setting will not affect patient management.

Importantly, most people with an inheritable thrombophilic mutation will never experience a thrombotic event, and most of those who do will never do so again. Because of this, screening the general population or even asymptomatic relatives of individuals with thrombophilic mutations is discouraged because of the potential for harmful overtreatment. By similar reasoning, some hematologists would recommend the same period of anticoagulation, often 3-6 months after (continued on page 4)

Clinical circumstances that suggest a laboratory evaluation for thrombophilia

- Patient with a confirmed acute VTE
- Asymptomatic patients with a family history of VTE
- Only selected patients from the above clinical circumstances should undergo a laboratory evaluation for thrombophilia
- Testing should be considered in the following situations:
  - Women who intend to become pregnant and/or use oral contraceptives and who have symptomatic first-degree relatives with AT, PC or PS deficiency, or FVL
- Physical exam and complete medical history to evaluate for presence of acquired risk factors for thrombosis (see text)

Thrombophilia – High Priority Tests

- Global coagulation tests: PT, PTT, TT
- Factor V Leiden mutation
- Prothrombin G20210A mutation (P20210)
- AT activity
- PC activity
- PS assay
- Factor VIII activity
- Antiphospholipid antibodies

Figure 1: Outline of the clinical circumstances that suggest a thrombophilia laboratory evaluation and the high priority screening tests. APS, antiphospholipid syndrome; AT, antithrombin; FVL, factor V Leiden; HRT, hormone replacement therapy; LA, lupus anticoagulant; PC, protein C; PS, protein S; PT, prothrombin time; PTT, partial thromboplastin time; TT, thrombin time; VTE, venous thromboembolism.
Thrombophilia Testing Panel Now Available (continued)

(continued from page 3) a single episode of venous thrombosis and lifetime anti-
coagulation after a second event, regardless of the presence or absence of an inherited thrombophilia. Although there remains some controversy regarding whom to test, the XXXVI College of American Pathologists Consensus Conference offers helpful guidance in this regard, and the reader is encouraged to consult this detailed but very readable document. Their recommendations, issued in 2002, but still widely used, are evidence-based and reflect broad expert consensus. Figure 1 summarizes their guidelines for thrombophilia testing. An important and guiding concept is that the risk of thrombosis increases incrementally with each inherited or acquired risk factor present, and that in most cases abnormal thrombosis is only triggered when at least two such factors coexist in the same patient. Thus for instance, if a woman plans to become pregnant or use oral contraceptives (1 thrombosis risk factor) and has a symptomatic first-degree relative with an inherited thrombophilia (strong possibility of a 2nd risk factor) then thrombophilia testing should be considered. Because of the principle of additive incremental risk, if the clinical data on a given patient suggest that it is beneficial to assay for the presence of one thrombophilic abnormality, then all inheritable and acquired possibilities should be assessed. This forms the rational basis for ordering a panel of tests when thrombophilia testing is desired.

If such testing is deemed appropriate in a particular patient, the next important consideration is when to perform the testing. In general, the evaluation is best performed after an acute thrombotic event has resolved and the patient has been off warfarin therapy for at least a month. The reason for this is that the coagulation and fibrinolytic factors are consumed during acute thrombosis, and low measured values will likely reflect this process rather than a genetic deficiency. In addition, both protein C and protein S synthesis are directly inhibited by warfarin. Finally, the early therapy for a thrombotic event will not be dictated by the presence or absence of a thrombophilia anyway. Normal results for these factors do exclude a deficiency, however, and the mutational assays for the much more common factor V Leiden and prothrombin mutations are unaffected by acute thrombosis or warfarin. One reasonable testing strategy might therefore be to order the factor V Leiden and prothrombin mutational assays in the hospital and defer the other tests until later. Less ideally, one could order a full thrombophilia panel during the initial hospitalization, and if the fibrinolytic factors are indeed abnormal and a genetic deficiency is a serious concern, then follow-up or confirmatory testing for these factors could be performed at a later date, again after warfarin discontinuation.

It is important to remember that there are many acquired causes of protein C and protein S deficiency besides warfarin, and that together these will greatly outnumber the genetic deficiencies. Liver dysfunction, vitamin K deficiency, L-asparaginase therapy, and DIC can all cause deficiencies of both proteins, as can the aforementioned acute thrombosis. In addition to these causes, protein S (but not C) deficiency may be seen with pregnancy, estrogen therapy, nephrotic syndrome, and the acute phase state. Acquired antithrombin III deficiency may be seen in the nephrotic syndrome, fulminant hepatic failure/end-stage liver disease, and DIC. L-asparaginase therapy and unfractionated heparin infusions may also produce a deficiency.

In summary, thrombophilia testing should be reserved for selected patients whose management plan hinges upon knowing whether they have an inheritable or acquired thrombophilic state. Established guidelines for testing and/or a hematology consult should ideally be sought before testing. Thoughtful interpretation of the results is then critical, and particular caution must be employed in attributing any apparent deficiency to a genetic, rather than an acquired, cause.

Please direct any questions you may have on this testing to Kevin Herzog, MD at 341-8997 or Amy Schmidt, MT(ASCP) at 341-8620.

— Kevin Herzog, MD

References:

