Dos and don'ts in diagnosing antiphospholipid syndrome

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Antiphospholipid syndrome (APS) is an acquired autoimmune thrombotic tendency that is identified by the presence of abnormal antiphospholipid laboratory tests in patients who have a history of vascular thrombosis and/or pregnancy complications including recurrent spontaneous miscarriages and a group of other complications due to placental insufficiency. Diagnostic testing for APS is often problematic because of many misconceptions regarding these empirically derived assays. This chapter is intended to provide hematology-oncology consultants with practical information about the uses and limitations of assays used to diagnose APS.

Introduction

Antiphospholipid (aPL) syndrome (APS) is an acquired autoimmune thrombotic tendency in which patients have circulating antibodies (Abs) against plasma proteins that bind to phospholipids. The concepts of this disorder evolved through the observations of astute clinicians and much of the approach to diagnosis is based on consensus rather than on solid evidence. In retrospect, the first suggestions of the existence of this disorder can be traced to the early 1950s when clinicians independently described 2 laboratory anomalies, an inhibitor that prolonged the activated partial thromboplastin time (aPTT) and the biologic "false-positive" syphilis test. The coagulation inhibitor came to be known as the "lupus anticoagulant (LA) phenomenon," a misnomer that has persisted even though most patients with this laboratory finding do not have systemic lupus erythematosus (SLE). The LA is now understood to reflect the presence of Abs that can interfere with phospholipid-dependent coagulation reactions in vitro. The serologic test for syphilis was refined into immunoassays that quantify Abs that bind to microtiter plates coated with cardiolipin (diphosphatidyl glycerol), which is the major antigenic target in the original test.[1,2] Approximately 25 years ago, clinical studies indicated that elevated levels of anticardiolipin (aCL) Abs were correlated with thrombotic manifestations and a new disease entity, the "anticardiolipin syndrome," subsequently renamed the "antiphospholipid syndrome,"[3] was described. It later became clear that these Abs did not primarily recognize phospholipid, but rather proteins that bound to the phospholipid, the most important of which was identified to be β_2-glycoprotein I (β2GPI)[4,5]; assays were then developed to measure to measure Abs that specifically recognize those cofactors. APS investigators at an international conference in Sydney, Australia, reached a consensus on clinical and laboratory criteria for the investigational diagnosis of APS, which are now referred to as the "Sydney Criteria."[6] The clinical criteria include documented vascular thrombosis and pregnancy complications that may be attributable to placental vascular insufficiency; the consensus-based laboratory assays are the LA, aCL IgG and IgM, and anti-β2GPI IgG and IgM. Only one of these tests needs to be abnormal for a diagnosis of APS, but the abnormality(ies) must be confirmed by repeat testing after a period of at least 12 weeks from the initial positive test(s).

Tip #1

The consensus criteria for APS were meant to identify patients who could be defined as having "definite APS" for research studies. In clinical practice, however, selected patients may be suspected to have the disorder without necessarily meeting the strict investigational criteria. Existing knowledge is continuing to evolve, and one should not hesitate to refer puzzling or difficult patients to a hematologist experienced in caring for patients with APS.

How do I decide which patients should be selected for laboratory testing?

In general, asymptomatic patients should not be screened for aPL test abnormalities with the hope of identifying those at risk for thrombosis, and pregnant women without histories of complications should not be screened for these tests to identify high-risk pregnancies. These assays carry significant rates of false positivity. The prevalence of positive immunoassays in the asymptomatic "normal" population has ranged from approximately 3% to nearly 20% in clinical studies. In one group of young women who served as healthy controls for a study, 18.2% had elevated levels of aCL Abs and 12.8% tested positive for LA.[7] Obtaining a positive aPL test result in an otherwise disease-free individual has the major downside of opening the door to possibility of unnecessary anticoagulant prophylaxis with the potential of hemorrhagic complications.

The Antiphospholipid Antibodies Subcommittee of the International Society of Thrombosis and Hemostasis has offered recommendations on selecting patients for LA testing, recommendations that can also be reasonably applied to deciding on aPL immunoassays. The subcommittee recommended that: (1) elderly patients with venous or arterial thromboembolism be included in a low-appropriateness group for testing, and (2) young patients with recurrent spontaneous early pregnancy loss and provoked venous thromboembolism and asymptomatic patients who are incidentally found to have a prolonged aPTT should be included in a moderate-appropriateness group. Included in the high-appropriateness group category are nonelderly patients with unprovoked and unexplained venous thromboembolism, arterial thrombosis in young patients ($<$ 50 years of age), thrombosis at unusual sites, late pregnancy loss, and any thrombosis or pregnancy morbidity in patients with autoimmune diseases (eg, SLE, rheumatoid arthritis, autoimmune thrombocytopenia, or autoimmune hemolytic anemia).[8]

Tip #2

Laboratory testing for aPL Abs should generally be limited to patients who present with the thrombotic and/or the pregnancy

manifestations of the disorder. Some experts would consider patients with SLE and perhaps other autoimmune disorders to be an exception to this rule because they are at increased risk for having aPL Abs and for experiencing thrombosis.

What constitutes a meaningful abnormality for the aPL immunoassays?

It is important to recognize that most patients with elevated aCL Abs encountered during the course of general screening studies do not have APS. Many patients have Ab levels that are elevated in response to infections not associated with thrombotic complications. Patients with syphilis, Lyme disease, HIV, and other infections can be erroneously diagnosed as having APS based on elevated aCL Ab levels when concurrent stroke or arterial thrombosis is present.

Weakly positive levels of aCL and anti-β2GPI Abs should not be considered significant. Most authorities require Ab levels to be at or above the 99th percentile to be considered clinically significant. High levels of aCL Abs are associated with increased risk of thrombosis. During a 10-year follow-up of patients with elevated levels of aCL Abs, approximately 50% of patients who presented with the Abs but without clinical manifestations of APS went on to develop APS.[9] In a systematic literature review, 15 of 28 studies showed significant associations between aCL Abs and thrombosis.[10] In all cases, there was a correlation between higher Ab titers and significantly elevated odds ratios for thrombosis. With respect to pregnancy losses, a meta-analysis of 25 studies on aPL Abs in women with recurrent fetal losses[11] showed significant correlation with the presence of an increased aCL IgG; however, the highest odds ratio was seen with LA positivity.

Anti-β2GPI immunoassays are considered to be more specific but less sensitive for APS than aCL Ab assays.[12] Although these Abs are usually present in conjunction with abnormal aCL, patients with APS can also present with only Abs to β2GPI.[13,14] Despite their higher specificity for APS (98%), anti-β2GPI Abs alone cannot be relied upon for the diagnosis because of their low sensitivity (40%-50%),[15,16] so concurrent testing for both Abs and for LA is advised.

Although positivity for anti-β2GPI IgG and IgM are included in the investigational criteria for APS, clinical studies on the significance of anti-β2GPI assays have yielded inconsistent results. In a systematic literature review, 34 of 60 studies demonstrated statistically significant associations between anti-β2GPI Abs and thrombosis.[10] Of 10 studies that included multivariate analysis, only 2 confirmed that IgG anti-β2GPI Abs were independent risk factors for venous thrombosis.

Tip #3

Laboratory results above the usual reference range of 2 SDs above the mean are not sufficient for diagnosing high-risk APS. Weak positive test results for aPL immunoassays are unlikely to have any clinical significance and, in general, do not warrant maintaining patients with vascular thrombosis on long-term anticoagulant therapy or anticoagulating women with pregnancy complications. In keeping with the uncertainty of diagnosing APS, there is significant controversy regarding the appropriate cutoff values for these assays that are clinically significant.

Which coagulation assay(s) should I use to detect LAs?

The Subcommittee on Antiphospholipid Antibodies of the International Society of Thrombosis and Hemostasis has also proposed specific criteria for standardizing the diagnosis of LAs.[8] There are several different forms of the LA test, all of which are meant to report aPL Ab-mediated inhibition of phospholipid-dependent coagulation enzyme reactions. For reasons that are not well understood, the LA tests are frequently negative by one method but positive by another. The committee therefore recommended ordering 2 different LA tests that are based on different assay principles whenever APS is suspected. The 2 assays that were preferred were the dilute Russell viper venom time (dRVVT) panel, which is widely used in clinical laboratories and is believed to be specific for detecting LA in those patients at high risk of thrombosis,[17] and an LA-insensitive aPTT.[18,19]

Tip #4

When it comes to LA assays, do not rely on a single negative test. Use at least 2 testing principles; for example, a dRVVT and an LA-insensitive aPTT.

What do I do about my APS patient with a prolonged aPTT who is reported by the laboratory to have multiple coagulation factor deficiencies?

This situation is usually encountered when an aPTT that was ordered for a preoperative screening panel is found to be prolonged. The surgeon is concerned about a coagulopathy and the hematology-oncology consultant request coagulation factor assays, usually factors VIII, IX, XI, and XII. The laboratory results return showing multiple factor deficiencies and the clinicians are concerned about the risk of bleeding.

The consultant should be aware that LA can interfere with the factor assays and induce underestimations of contact activation pathway factors. However, it is critical to recognize that occasional patients will coincidentally have both types of anticoagulants, LA and specific coagulation factor inhibitors. This problem can be resolved in most cases by requesting that the laboratory also perform the coagulation factor assays with an aPTT reagent that is insensitive to LA. Some clinical laboratories might not be aware of these issues and might not have access to LA-insensitive reagents. It is therefore advisable for hematologists to work with the clinical laboratory directors to check on the sensitivity of their laboratory reagents and their utilities. As described in the next section, it becomes even more important to be able to identify a specific coagulation factor inhibitor in the LA patient who presents with bleeding.

Tip #5

When you are confronted with an APS patient who has a prolonged aPTT, multiple coagulation factor deficiencies, and no clinical evidence for a bleeding tendency, the deficiencies are likely to represent laboratory artifacts induced by the LA effect. Nevertheless, the possibility of a true coagulation factor deficiency must be ruled out.

What are the causes of bleeding in APS patients?

The typical patient with a positive LA test is *not* at increased risk for bleeding. However, aPL patients can also have concurrent abnormalities that place them at increased risk for bleeding, the most common being complications of anticoagulant therapy. The following are among the other abnormalities that may be expected in APS patients

who exhibit bleeding tendencies in the absence of concurrent immune thrombocytopenic purpura.

Prothrombin deficiency

Patients with LA or APS can also have a true deficiency of prothrombin (factor II).[20,21] These can usually be suspected by the presence of a significantly prolonged prothrombin time (PT) and aPTT. These patients also usually have markedly elevated antiprothrombin Abs demonstrated by ELISA. These Abs are generally nonneutralizing and, in patients who do not have a positive LA test result, mixing incubation studies to screen for an inhibitor and specific inhibitor assays are usually negative.

Concurrent acquired coagulation factor inhibitor

Rare patients will concurrently test positive for a true autoantibody inhibitor along with the LA. The most common of these are acquired inhibitors of factor VIII, and the second most common are acquired inhibitors of factor XI. Distinguishing these inhibitors from the LA effect requires that the coagulation assays be performed with LA-insensitive aPTT reagents.

AVWS

Acquired von Willebrand syndrome (AVWS) can occur in patients with underlying autoimmune conditions such as SLE and APS. The disorder is generally suspected when patients develop a hemostatic disorder that has the characteristics of VWD later in life. AVWS is marked by the absence of a family history for bleeding or VWD and abnormalities of laboratory assays for VWF that can mimic type 1 VWD, but more often mimic the type 2A variant of the disorder. The pathophysiologic mechanism for the VWF abnormality, although presumed to have an autoimmune basis, has not been definitively established, because most of these patients do not have evidence for Abs against VWF or for an inhibitor of VWF.[22]

Acquired platelet function abnormality

Acquired platelet function abnormality should be suspected when a patient with a positive LA or APS test has a pattern of superficial and mucosal bleeding despite a normal platelet count. This condition has been termed "acquired storage pool disease" and can be evaluated with the appropriate platelet aggregation and release studies.

Acquired factor XIII inhibitor

In the relatively rare condition known as acquired factor XIII inhibitor, patients usually present with profound and persistent bleeding, often delayed, in the face of normal conventional screening studies. They are usually identified by abnormal factor XIII deficiency screening tests (ie, clot lysis screen performed with 5M urea or acetic acid) that are not corrected with mixing incubation. If the disorder is suspected but the screening tests are negative, the clinician is advised to consider arranging for quantitative assays of factor XIII enzymatic activity.

Tip #6

When encountering bleeding in APS patients with a normal platelet count, the differential diagnosis should include prothrombin deficiency, an inhibitor against a specific coagulation factor, AVWS, an acquired thrombocytopathy, or acquired inhibitor to factor XIII.

How specific are positive aPL tests for APS?

Testing positive for aCL Abs does not necessarily mean that a patient has APS. The positive test may be triggered by a preceding infection, the most common of which are syphilis and Lyme disease. In addition, aCL positivity can be triggered by EBV, CMV, HIV, and hepatitis C virus. In general, these patients do not have LA or elevated Abs against β2GPI. Although the bulk of these Abs are not associated with the APS disease process, occasional patients with HIV and hepatitis C virus may develop the autoimmune thrombotic manifestations of APS.

Tip #7

Always rule out infection as the potential cause of positive aCL immunoassays.

Are there laboratory predictors for a high risk of thrombosis?

Several studies have indicated that strong positivity for more than one of the aPL Ab criteria assays is correlated with increased risk for developing clinical events. One study showed that multiple positivity for aPL Abs, but not single positivity, was associated with antenatal and postnatal deep vein thrombosis.[23] A study of pregnant women with APS reported that patients with triple aPL Ab-positivity (ie, positivity for LA, aCL, and anti-β_2GPI Abs) and/or previous thromboembolism had an increased likelihood of poor neonatal outcomes than patients with double or single aPL Ab positivity and no thrombosis history.[24] However, another study, named PROMISSE ((Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus), which prospectively examined the correlation of aPL tests with adverse pregnancy outcomes, determined that the LA was the only test that was significantly correlated with clinical events.[25]

A retrospective analysis of 162 APS patients who were triple positive for the aPL tests reported a high risk of recurrent thromboembolic events in this group, with a cumulative incidence of events of 44.2% after 10 years.[26] This finding was confirmed in a recent prospective analysis of 104 triple-positive patients who did not have prior histories of thrombosis or pregnancy complications and were observed over a mean duration of 4.5 years.[27] In that study, the cumulative incidence for developing a first thrombotic event after 10 years was 37.1%.

Tip #8

Patients with triple -positive aPL tests appear to be at high risk for a first thrombotic event and for recurrence.

What about the patient who is suspected to have APS on clinical grounds but tests negative for the standard criteria tests?

Clinicians will occasionally encounter a patient who appears to have the clinical manifestations of APS but who tests negative for the standard tests. In some patients, the picture may be clarified by testing for one or more of the "noncriteria" aPL tests discussed in the next sections.

IgA Abs to cardiolipin and β2GPI

From a practical perspective, IgA Abs to cardiolipin and β2GPI are the easiest of the alternative assays to obtain. Although Abs with the IgA isotype are not included in the consensus criteria, clinicians will occasional encounter patients with isolated IgA Abs who exhibit the clinical manifestations of APS. A retrospective case-control study of 56 patients with isolated anti-β2GPI IgA found that patients with this marker had significantly more thromboembolic events than

controls. Elevated anti-β2GPI IgA was reported to be associated with an increased risk of thromboembolic events in patients with SLE.[28] aPL Abs of the IgA isotype (either aCL or anti-β2GPI) were not included in the international consensus statement on the criteria for APS classification; however, testing was recommended in patients in whom APS is suspected but the IgG and IgM tests are negative.[29]

Antiphosphatidylserine Ab assay

Because cardiolipin is normally present in mitochondrial membranes and not on the cytoplasmic membranes of cells, it was hypothesized that immunoassays for these Abs may be more relevant to the APS disease process. In arterial thrombosis, antiphosphatidylserine Abs were reported to be better correlated with APS than aCL Abs.[30,31]

Other noncriteria assays

Several other noncriteria assays may also be helpful for APS patients. The antiprothrombin Ab assay is based on the idea that prothrombin is the second major cofactor for aPL Abs after β2GPI. In a systematic literature review, 17 of 46 studies showed significant associations between antiprothrombin Abs and thrombosis.[10] Of the 8 studies that included multivariate analysis, 2 confirmed that antiprothrombin Abs were independent risk factors for thrombosis, and 3 other studies showed that antiprothrombin Abs added to the risk borne by LA or aCL Abs. Overall, no association has been found between antiprothrombin Abs and risk of thrombosis, and this test is not generally considered to be useful. An assay that measures Abs against the phosphatidylserine-prothrombin complex was reported to be correlated with APS and LA.[32] IgG Abs against the phosphatidylserine-prothrombin complex were reported to be highly prevalent in APS patients compared with patients with other diseases, with an odds ratio of 12.8.[33]

Several additional assays that are currently available on a limited research basis may ultimately prove useful for the future evaluation of this category of patients. The antidomain I β2GPI assay identifies IgG Abs against a specific epitope on β2GPI. A recent multicenter study of 442 patients who tested positive for anti-β2GPI Abs reported that the detection of specific antidomain I IgG Abs was more strongly associated with thrombosis and obstetric complications than anti-β2GPI Abs detected using the standard anti-β2GPI Ab assays.[34]

It has also been reported that approximately half of patients with clinical manifestations of APS but lacking positivity for the standard aPL Abs (ie, patients with seronegative APS, aka SNAPS) had serologic evidence for antivimentin/cardiolipin Abs.[35] Vimentin is a cytoskeletal intermediate filament that was shown to be a target for aPL Abs.

Tip #9

When a patient has the clinical appearance of APS but negative standard aPL assay results, think about the possibility of seronegative APS. Noncriteria tests such as aCL and anti-β2GPI IgA Abs and antiphosphatidylserine Abs may help to clarify the picture.

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