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Antiphospholipid Antibody Testing: Which Are Most Useful for Diagnosis?

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The antiphospholipid syndrome (APS) is a systemic autoimmune disease clinically characterized by recurrent arterial or venous thrombosis or pregnancy complications including recurrent early miscarriages or fetal losses. The presence of antiphospholipid antibodies (aPL) is mandatory to make the laboratory diagnosis of APS. In clinical practice, the ''gold standard'' tests are those that detect anticardiolipin antibodies (aCL) by ELISA or the lupus anticoagulant (LA) by coagulation tests. Although other specificities for aPL have been described, their clinical utility has still to be established.

History of antiphospholipid antibodies

Wasserman [1] was the first to describe aPL, a complement-fixing antibody that reacted with extracts from bovine hearts, while carrying out his research into the development of the serologic test for syphilis. But only in 1941 was the relevant antigen identified as cardiolipin [2], becoming the basis for the Venereal Disease Research Laboratory (VDRL) test for syphilis. Blood screening for this disease led to the observation that many patients with systemic lupus erythematosus (SLE) had a positive VDRL test, without any other clinical or serologic evidence of syphilis [3].

The knowledge of the ''lupus anticoagulant phenomenon'' goes back to the 1950s when Conley and Hartmann [4] reported a prolongation of the prothrombin time in patients with SLE. In 1954, this ''circulating

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anticoagulant'' was associated with recurrent abortions [5], followed in 1957, by a report on its association with the biologically false-positive test for syphilis [6]. In 1963, the ''circulating anticoagulant'' was associated with thrombotic manifestations in SLE [7], but it was not until 1972, that Feinstein and Rapaport [8] introduced the term ''lupus anticoagulant'' and described it as an inhibitor directed against coagulation cascade phospholipids, particularly at the prothrombin conversion to thrombin step.

In 1983, Hughes [9] described in full clinical detail the APS (which he originally entitled ''anticardiolipin syndrome''). His clinical observations included the tendency to both arterial and venous thrombosis, the ''primary'' syndrome in the absence of SLE, the livedo, thrombocytopenia, recurrent pregnancy loss, and prominent neurologic involvement. This group set up a sensitive solid phase immunoassay for the detection of aPL.

Antiphospholipid syndrome: laboratory diagnosis

Laboratory diagnosis of APS relies on the demonstration of a positive aCL antibody test by an in-house or commercially available enzyme-linked immunosorbent assay (ELISA) or on the presence of LA by a coagulation-based test. aCL should be tested in a β-2 glycoprotein I (β2-GPI)-dependent manner and LA should be diagnosed according to the International Society on Thrombosis and Haemostasis criteria [10–12].

Anticardiolipin antibodies

The aCL test is positive in about 80% of patients with APS, the LA test is positive in about 20%, and both are positive in about 60% of cases [13]. It is important that both tests be performed in patients suspected of having APS.

Although a sensitive test, aCL can be positive in a variety of disorders, including connective tissue diseases and infectious disorders such as syphilis [14,15], Q fever [16], and AIDS [17]. However, in these conditions, the predominant isotype is usually of the IgM class, present in low titers, and generally not associated with thrombotic features.

aCL have been shown to be a risk factor for first deep venous thrombosis [18] and recurrent venous thrombosis [19]. In a large prospective study of 360 unselected patients with LA with or without aCL, Finazzi and colleagues [20] showed that aCL above 40 GPL in patients with previous thrombotic events were independent predictors of subsequent vascular thrombosis. Other studies also found GPL titers to be important in identifying a higher risk group of patients for subsequent thrombo-occlusive events [21,22], although other studies have disagreed [23,24]. Their predictive value for arterial thrombosis and pregnancy morbidity in the general population is still to be defined.

Despite ongoing international efforts, interlaboratory agreement on aCL measurement is still low, mainly due to some methodologic and calibration

issues. However, the use of a semiquantitative measure (ie, ranges of positivity low, medium, or high) seems to be adequate in most clinical settings and is less subject to error [25,26]. The use of a reliable, validated aCL ELISA kit may offer better reproducibility [26]. For in-house assays, calibrators derived from monoclonal antibodies, HCAL and EY2C9 [27] have been introduced in an effort to optimize standardization.

The observation that many aCL are directed to an epitope on β2GPI led to the development of the anti-β2GPI antibody (anti-β2GPI) immunoassay [28]. Anti-β2GPI are strongly associated with thrombosis and other features of APS [29]. Initial clinical studies of anti-β2GPI ELISAs suggest that positivity in these assays is more closely associated with aPL-related clinical manifestations than positivity in conventional aCL ELISAs [29]. As β2GPI-independent aCL usually does not correlate with thrombotic events, this may explain why anti-β2GPI ELISA is a more specific assay for the diagnosis of APS than aCL detected by conventional ELISA [30].

Anti-β2GPI assays have also identified a small number of patients who have clinical manifestations of the APS but are negative in conventional aPL assays [31].

Lupus anticoagulant

LA is identified by coagulation assays, in which it prolongs clotting times. A number of features need to be demonstrated: prolongation of a phospholipid-dependent clotting time; evidence of inhibition shown by mixing studies; evidence of phospholipid dependence; and exclusion of specific inhibition of any one coagulation factor. As they are very heterogeneous antibodies, it is necessary to perform more than one coagulation test to reach the diagnosis according to the classification criteria [11]. In principle, the laboratory tests to detect the LA should use a sensitive screening test followed by a specific confirmation test [12]. Both activated partial thromboplastin time and dilute Russell's viper venom time are suitable for testing LA [32,33]. However, in some subjects receiving oral anticoagulation, accurate detection of the LA might not be possible. In these cases, LA testing might be postponed until the patient is off anticoagulation, which is not sensible in most cases. Instead, patient sample can be diluted 1:2 with normal plasma (if international normalized ratio <3.5) before performing the tests [12,34]. Testing the Taipan or Textarin times might also be useful in these cases [12].

To satisfy classification criteria, the presence of aCL or LA should be detected on at least two occasions, 8 to 12 weeks apart [10,34]. Persistence of the positive tests must be demonstrated and other causes and underlying factors considered.

In general, LA are more specific than aCL for APS, although less sensitive. In general, there is a high concordance between LA and aCL [35–37], but these antibodies are not identical [38]. A meta-analysis evaluating the

risk of venous thrombosis in SLE [39] showed that LA-positive subjects were six times more likely to have a thrombotic event than patients who were LA negative. Patients with aCL had a twofold increase in the risk of having a thrombotic event when compared with patients without aCL. These data were confirmed by a subsequent meta-analysis of aPL and venous thrombosis in patients without underlying immunologic disease that concluded that LA was a more specific predictor of thrombosis than aCL [40].

It has been demonstrated that patients with SLE are at a substantial risk of venous thrombosis over time. Both the presence of LA and polyclonal aCL are associated with the risk of venous thrombosis, although LA seems to be a better predictor of risk than aCL [41]. This has been confirmed in a recent systematic review of the literature where the LA was shown to be a risk factor for thrombosis, independent of the site (venous or arterial) and the type of the event (first or recurrence). In this analysis, aCL were not such strong risk factors, unless the IgG isotype and medium or high titers were considered [42].

Other antiphospholipid antibodies

The clinical utility of aPL antibody assays to phospholipids other than cardiolipin and to phospholipid-binding proteins other than β2GPI remains unclear [43]; the precise serologic "fingerprint" of the patients most at risk of thrombosis remains elusive [44].

Data on the clinical value of antibodies directed to prothrombin (another phospholipid binding protein) are contradictory. Antiprothrombin antibodies are heterogeneous, and can be directed to prothrombin coated onto irradiated plates (aPT) or to phosphatidylserine–prothrombin complex (aPS-PT) [45]. A recent systematic review showed no association between the presence of antiprothrombin antibodies and thrombosis, irrespective of isotype, site, and type of event and the presence of SLE [46]. In our experience, antiprothrombin antibodies are frequently found in SLE patients, and their presence is associated with APS [45]. Most significantly, some patients with aPL-related clinical features, who are negative for aCL, LA, and anti-β2GPI had antiprothrombin antibodies either by the aPT or the aPS-PT assays, suggesting that testing for these antibodies could be of clinical benefit in patients who are negative for the routine testing [45,47].

A number of other autoantibodies have been reported in patients with APS, including antibodies to annexin V [48,49], high and low molecular weight kininogens or, less frequently, prekallikrein and Factor XI [50,51]; to vascular heparan sulfate proteoglycan [52] heparin [53], factor XII [54–56], and thrombin [57]. Some data suggest that autoantibodies could be directed against components of protein C pathway [58], which includes protein C [59], protein S [60,61], and thrombomodulin [62]. The association of such antibodies with APS and their clinical significance is far from being known amid that these tests are far from standardized. Their application should be restricted only to research rather than to routine diagnostic use.

Which test should be used for the recognition of the antiphospholipid syndrome?

In 1998, a group of experts agreed by consensus that the two tests used in the recognition of APS should be the standardized β2-dependent aCL assay and the LA detected following the guidelines of the International Society for Thrombosis and Haemostasis [10]. Laboratory diagnosis of APS is based on a positive aCL antibody or LA test.

Although it cannot still be considered a replacement to aCL testing, a committee evaluating the new clinical, laboratory, and experimental insights since the 1999 publication of the Sapporo criteria considered to include IgG and IgM anti-β2GPI testing as a helpful diagnostic tool for the APS [34], particularly when aCL and LA are negative and APS is strongly suspected. However, due to the lack of standardization, their routine application still remains questionable. Laboratories around the world are being encouraged to solve these problems by standardizing the methodology applied and validating their measurements; the goal still has not been achieved.

A new assay that uses a mixture of negatively charged phospholipids has been proposed for more specific measurements of aPL [63]. The AphL phospholipid mixture was developed by testing aCL positive sera from a large number of patients with and without APS. A mixture able to discriminate APS from non-APS sera was identified [64]. A study examining this antigen suggests that the APhL ELISA kit may be a sensitive and relatively specific in identifying patients with APS [65].

Although new techniques for the detection of aPL, such as that detecting anti-β2GPI or antiprothrombin antibodies, have shown to be more specific than the aCL or LA [29,66], these tests are far from standardized. Moreover, one of the most important points to take into account is the lack of a universal positive or reference control. In these settings, the lack of agreement between laboratories (ie, source of the protein, type of ELISA plate, and so on) could highly influence the results obtained, making the application of these tests better restricted to research rather than to routine diagnostic use.

Seronegative antiphospholipid syndrome

This term was coined to characterize a group of patients with clinical manifestations of the APS, who are thought to have the syndrome despite negative results in conventional aPL testing (aCL or LA) [67,68]. Although it is universally recognized that the routine screening tests (aCL or LA) might miss some cases, careful differential diagnosis and repeat testing are mandatory before the diagnosis of "seronegative APS" can be made. This concept is important and certainly leaves room for further developments in testing for those autoantibodies that are thought to be associated with APS but not detected in conventional aPL assays.

Summary

Laboratory diagnosis of APS relies on the demonstration of a positive test for aPL. In clinical practice, the gold standard tests are those that detect β2GPI-dependent aCL or LA. The question on the use of anti-β2GPI as a routine diagnostic test remains unanswered, and testing for these antibodies should be only performed in very selected cases and not as an alternative to aCL or LA testing. Clinical utility and standardization are still lacking for other aPL specificities; therefore, their application as routine diagnostic tools is not recommended.

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