

Recent Advances in the Diagnosis of Antiphospholipid Syndrome

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Abstract

Antiphospholipid antibodies are autoantibodies directed against anionic phospholipids or protein-phospholipid complexes measured in solid-phase immunoassays such as anticardiolipin (aCL) antibody or detected in phospholipid-dependent clotting tests as lupus anticoagulant (LA). The term “antiphospholipid syndrome (APS)” was first coined to denote the clinical association between antiphospholipid antibodies and a syndrome of episodes of thrombosis in arteries and/or veins, pregnancy loss, and thrombocytopenia. The diagnosis of APS is based on the finding of “moderate-to-high” aCL antibody titer and/or an LA test with any one of the characteristic clinical features presented. Recently, the diagnostic criteria of APS was revised and several newer assays that use phosphatidylserine, a mixture of negatively charged phospholipids or β 2-glycoprotein 1 (β 2-GP1) have been proposed for more specific measurements of antibodies present in APS. In this section, recent progress in the laboratory diagnosis of antiphospholipid syndrome will be discussed.

1. The Antiphospholipid Syndrome

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by a combination of arterial or venous thrombosis, or recurrent pregnancy loss and thrombocytopenia with presence or elevated titers of antiphospholipid (aPL) antibodies [1,2]. Antiphospholipid antibodies are autoantibodies directed against anionic phospholipids or protein-phospholipid complexes measured in solid-phase immunoassays as anticardiolipin antibody (aCL) or detected in phospholipids-dependent clotting tests as lupus anticoagulant (LA) [3-5]. Because thrombotic disease, miscarriage and transient antiphospholipid antibody positivity are all common events, persistence of the positive tests must be demonstrated and other causes and contributory factors considered [6,7]. An international consensus on classification criteria for definite APS was published in 1999 (Table 1) [1]. It can either occur as a free-standing syndrome (primary APS) or be associated with other autoimmune diseases, especially systemic lupus erythematosus (SLE), it is referred to as secondary APS [6,8]. The range of disease associations with antiphospholipid antibodies is

extremely broad [6].

2. Pathogenesis

Several hypothesis have been proposed to explain the cellular and molecular mechanisms by which antiphospholipid antibodies promote thrombosis [9].

1) Activation of endothelial cells [10]

Binding of antiphospholipid antibodies induces activation of endothelial cells, as assessed by upregulation of the expression of adhesion molecules, the secretion of cytokines, and the metabolism of prostacyclins

2) Oxidant-mediated injury of vascular endothelium [11]

Autoantibodies to oxidized low-density lipoprotein (LDL) in association with anticardiolipin antibodies, and some anticardiolipin antibodies cross-react with oxidized LDL

3) Antiphospholipid antibodies interfere with or modulate the function of phospholipid binding proteins involved in the regulation of coagulation, such as prothrombin, protein C, annexin V and tissue factor [12-19].

4) A hypothesis based on parallelism with heparin-induced thrombocytopenia [20,21].

Table 1.

International Consensus Statement of Preliminary Criteria for the Classification of the Antiphospholipid Syndrome.*

| | |
|--------------------------------|---|
| Clinical criteria | |
| Vascular thrombosis | One or more clinical episodes of arterial, venous, or small-vessel thrombosis, occurring within any tissue or organ |
| Complications of pregnancy | One or more unexplained deaths of morphologically normal fetuses at or after the 10th week of gestation; or One or more premature births of morphologically normal neonates at or before the 34th week of gestation; or Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation |
| Laboratory criteria | |
| Anticardiolipin antibodies | Anticardiolipin IgG or IgM antibodies present at moderate or high levels in the blood on two or more occasions at least six weeks apart |
| Lupus anticoagulant antibodies | Lupus anticoagulant antibodies detected in the blood on two or more occasions at least six weeks apart, according to the guidelines of the International Society on Thrombosis and Hemostasis ²³ |

*A diagnosis of definite antiphospholipid syndrome requires the presence of at least one of the clinical criteria and at least one of the laboratory criteria

3. Laboratory Criteria

The most commonly detected subgroups of antiphospholipid antibodies are lupus anticoagulant, aCL antibodies, and anti- β 2-glycoprotein I antibodies. Division into these subgroups is broadly based on the method of detection [9,22].

3.1. Lupus Anticoagulant

Lupus anticoagulant activity in plasma is caused by anti- β 2-GPI and anti-prothrombin antibodies, either alone or in combination.

International guidelines for the detection of LA have been published [23] and the first step requires prolongation of coagulation in at least one phospholipid-dependent coagulation assay with the use of platelet-poor plasma.

The second step is a failure to correct the prolonged coagulation time by mixing the patient's plasma with normal plasma. The third step is confirmation of the presence of lupus anticoagulant by shortening or correction of the prolonged coagulation time after the addition of excess phospholipids or platelets that have been frozen and then thawed. The fourth step is ruling out other coagulopathies with the use of specific factor assays if the confirmatory test is negative or if a specific factor is suspected.

In principle, the laboratory tests should use a detection or screening stage and a confirmation stage (Table 2), the

Table 2.

Procedures Used for the Detection of LA [6].

| |
|--|
| Screening tests |
| APTT |
| DRVVT |
| KCT |
| TTI |
| Confirmation of the presence of LA |
| Mixing tests with normal plasma |
| Confirmation of phospholipid dependence |
| Platelet neutralization procedures |
| LA-insensitive reagents |
| Hexagonal phase lipids |
| High-concentration phospholipid |
| Comparison with a similar, insensitive test (e.g. Ecarin time) |

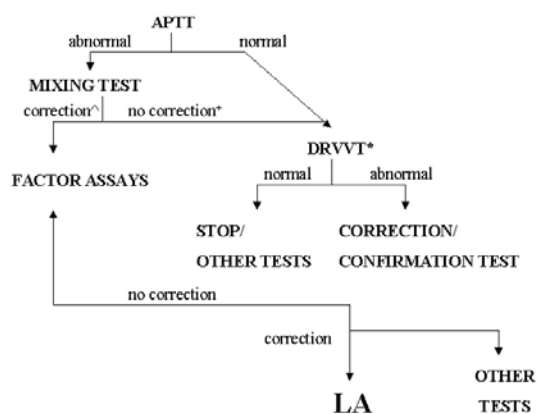


Figure 1. A suggested flow chart for LA testing. +, a prolonged APTT which fails to correct for confirmation of LA; ^, a weakly positive test may correct with normal plasma; *, alternatives to the DRVVT as the second screening test are other venom tests, the KCT or the TTI [6,23].

latter often performed using different reagents in the same type of test. No LA test consistently shows 100% specificity and sensitivity and, because of the heterogeneous nature of aPLs, more than one test system should be used for detection of LA. Sensitivity may be increased using different lupus anticoagulant tests, including the kaolin clotting time (KCT), which may be more sensitive for antiprothrombin antibodies, and the dilute Russell Viper Venom time (dRVVT), which may be more sensitive for the lupus anticoagulant subset of antibodies to β 2-GPI [22]. Summary of the recommended approach for the detection of LA was depicted in Figure 1.

3.2. Anticardiolipin (aCL) Antibody

International Consensus Statement on Preliminary Classification Criteria for Definite Antiphospholipid syndrome defined as aCL antibody of Ig G and/or IgM isotype in blood, present in medium or high titer, on 2

or more occasions, at least 6 weeks apart, measured by a standardized enzyme-linked immunosorbant assay for β 2-glycoprotein I (β 2-GPI) dependent anticardiolipin antibodies [1].

The current aCL test is sensitive and is positive in more than 80~90% of patients with APS and the IgG isotype is more prevalent in APS patients, but it also occurs with elevated IgM antibodies and infrequently with IgA antibodies [7,24]. There are reports of isolated IgM aCL, and occasionally isolated IgA aCL antibodies, associated with clinical manifestations of APS [25-27]. aCL antibody isotype distributions may vary in different ethnic groups [24,28-30]. Many individuals have aCL antibodies that are elevated in response to microbial infections and are not associated with risk for thrombotic complications. Patients with syphilis, Lyme disease, kala-azar, leptospirosis, cytomegalovirus, hepatitis C, Q fever, tuberculosis, parvovirus B19 infections, AIDS and other infections who have coincident thrombosis could be misdiagnosed with the APS syndrome on the basis of elevated aCL antibodies alone [31-37]. Antibodies induced by infection generally recognize phospholipids directly and not via protein cofactors such as β 2-GPI [6,7,15].

Important methodological considerations are the quality of the cardiolipin used and the technique for coating the microtitre plates. The binding of most aCL requires the presence of the protein cofactor β 2-GPI, which is essential for satisfactory and reproducible aCL results. This is provided by fetal calf serum or adult bovine serum in the blocking agent and/or sample diluent [6]. It is recommended that the aCL test is standardized by the use of affinity-purified standards or secondary standards derived from these [38]. These allow the calculation of aCL results in IgG or IgM antiphospholipid unit related to a given concentration of affinity-purified aCL immunoglobulin. Interlaboratory variation has plagued the study of APS and now it is established that interlaboratory agreement is best when positive aCL results are reported in semiquantitative terms such as low-positive, medium-positive, or high-positive. Even then, reasonable reproducibility and acceptable clinical correlation require standardization of the test. There has been considerable effort directed toward standardizing aPL test procedures and defining antibody levels [38-41] and international organizations are contributing to the standardization of the aCL test: the College of American Pathologists and NEQAS in the UK enrolls certified laboratories in quality control surveys for aCL testing and requires participation in the program for accreditation purposes [6,7]. In addition, they attempt to improve agreement between laboratories by distributing aCL survey samples and evaluating results.

Alternatively newer assays that use phosphatidylserine [42], a mixture of negatively charged phospholipids (APhL ELISA Kit; Louisville APL Diagnostics, Doraville, GA) have been proposed for more specific measurements of antibodies present in APS [43-44]. Both aCL and antiphosphatidylserine (aPS) show low sensitivity for thrombosis as they also detect infectious anti-

Table 3.

Sensitivities and Specificities of Different Antiphospholipid Assays.

| Assay | Sensitivity (%) | Specificity (%) |
|--------------------------------|-----------------|-----------------|
| Standard Anticardiolipin ELISA | 100 | 55 |
| APhL ELISA Kit | 100 | 100 |
| QUANTA LITE β 2GP1 | 90 | 90 |
| QUANTA LITE ACA IgG | 100 | 37 |
| EL-ACA screening | 100 | 55 |
| EL-ACA isotype specific | 100 | 55 |
| aCL/aPS FACS Kit | 90 | 82 |

Data are taken from the Anticardiolipin Wet Workshop at the 7th International Symposium on Antiphospholipid Antibodies [43]. APhL ELISA Kit and aCL/aPS FACS Kit from Louisville APL Diagnostics. EL-ACA screening and EL-ACA isotype specific from Incstar. QUANTA LITE β 2GP1 and QUANTA LITE ACA IgG from INOVA Diagnostics.

phospholipid antibodies not associated with thrombosis [45]. In 1996, a seventh anticardiolipin workshop was conducted and evaluated the results of standard aCL ELISA, two commercial aCL ELISA kits (INOVA Diagnostics, San Diego, CA and Incstar, Sillwater, MN), the APhL ELISA kit, an anti- β 2GPI kit and a flow cytometric technique in which IgG and IgM antibodies to cardiolipin and to phosphatidylserine can be determined simultaneously [43]. All techniques tested were 90~100% sensitive, correctly identifying almost all sera from patients with APS (Table 3), but none of the aCL assays proved to be 100% specific with variable results.

3.3. Anti- β 2-Glycoprotein I (anti- β 2GPI) Antibodies

Human β 2-glycoprotein I is a plasma glycoprotein that can act as a physiological anticoagulant, in vitro at least, inhibiting the clotting cascade and platelet aggregation [46]. β 2-GPI is required as a cofactor for the binding of aCL to cardiolipin in APS, but not in infection. It was originally thought that aPL were directed against negatively charged phospholipids, but it is now clear that the antibodies are directed not only against phospholipids but also against a complex of a phospholipid with one of a group of phospholipid-binding plasma proteins (cofactors), such as β 2-GPI and prothrombin [3-5]. Antibodies directed against these cofactors can be detected by ELISA in the absence of phospholipids [47].

A variety of specific assays for β 2-GPI antibodies have been developed and several commercial kits are available. Solid phase immunoassay is performed on human β 2-GPI-coated plates, usually in the presence of

bovine serum β 2-glycoprotein I [6]. Although their presence is not currently included in the criteria for the APS [1], β 2-GP1 antibody assays show higher precision and better correlation with the thromboembolic complications in APS and SLE than assays for aCL and are less likely to show transient positive results in association with infection [46,47]. However, the problem of standardization remain. Gamma-irradiated polystyrene plates or other plates with a high density of surface electrostatic charge must be used to ensure efficient β 2-GP1 binding [6]. Recently importance of IgA anti-beta 2-glycoprotein I assays has claimed in many of aCL negative patients suspected of having APS. The combined use of both assays enhance positive testing results in up to 75% of patients with APS at any stage of illness [48].

3.4. Autoantibodies to Other Phospholipid-binding Proteins

Although β 2-GP1 is accepted antigen of aPL antibodies, aPL antibodies may also recognize different complexes of anionic phospholipids and a variety of plasma proteins, including prothrombin, annexin V, protein C, and protein S [49].

Antiprothrombin (aPT) antibodies generally exhibit poor specificity for venous thrombosis and recurrent fetal loss and may be found in patients with infection. Their precise clinical significance is not yet clear [6,45,49,50]. One report has claimed an association with the presence of anti- β 2-GP1 and the specificity of aPT and antiannexin antibodies for the diagnosis of APS [51].

Annexin V is found in placenta and vascular endothelium and is necessary for the maintenance of placental integrity. This protein has potent anticoagulant properties that are based on its high affinity for anionic phospholipids and its capacity to displace coagulation factors from phospholipid surfaces. Levels of Annexin V are markedly reduced on placental villi in patients with APS and it may be an important mechanism of thrombosis and pregnancy loss in the APS [52,53]. The role of annexin V antibodies in recurrent miscarriage has yet to be established. There are several reports that anti-annexin V antibodies have been shown to be correlated with thrombosis and intrauterine fetal loss in patients with SLE [54,55]. Another report presented high incidence of anti-annexin antibodies in APS and associated thrombotic tendency with systemic autoimmune diseases [50].

4. Conclusions

Our understanding of the pathogenesis and clinical features of primary or secondary antiphospholipid syndrome is increasing rapidly. APS is a multifactorial and complex condition which is clinically demanding with respect to both diagnosis and management. Despite an active international effort to improve diagnosis and treatment of the antiphospholipid syndrome, there remain

problems of lack of standardization and lack of prospective and multivariate epidemiologic analysis which restrict the diagnostic and predictive ability of commercially available tests. Based on the knowledge and experience accumulated to date, I propose a stepwise approach to the laboratory diagnosis of APS. Lupus anticoagulant and aCL autoantibody testing should be performed simultaneously as first-line tests. If these results are negative or equivocal in clinically suspected patients, repeat the tests after 6 weeks and more specific test, such as anti- β 2GP1 should be performed. Other tests, such as ELISA for prothrombin antibodies and annexin V antibodies, are still undergoing development and will require standardization and extensive evaluation. There is a clear need for the development of more specific laboratory techniques to identify those patients at particular risk of thrombosis and miscarriage.

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