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Author manuscript

*Am J Hematol.* Author manuscript; available in PMC 2016 April 27.

Published in final edited form as:

*Am J Hematol.* 2012 May ; 87(Suppl 1): S75–S81. doi:10.1002/ajh.23196.

## Antiphospholipid Syndrome Laboratory Testing and Diagnostic Strategies

Thomas L. Ortel, M.D., Ph.D.

Professor of Medicine and Pathology, Division of Hematology, Duke University Medical Center

### Abstract

The Antiphospholipid Syndrome (APS) is diagnosed in patients with recurrent thromboembolic events and/or pregnancy loss in the presence of persistent laboratory evidence for antiphospholipid antibodies. Diagnostic tests for the detection of antiphospholipid antibodies include laboratory assays that detect anticardiolipin antibodies, lupus anticoagulants, and anti- $\beta_2$ -glycoprotein I antibodies. These assays have their origins beginning more than sixty years ago, with the identification of the biologic false positive test for syphilis, the observation of ‘circulating anticoagulants’ in certain patients with systemic lupus erythematosus, the identification of cardiolipin as a key component in the serologic test for syphilis, and the recognition and characterization of a ‘cofactor’ for antibody binding to phospholipids. Although these assays have been used clinically for many years, there are still problems with the accurate diagnosis of patients with this syndrome. For example, lupus anticoagulant testing can be difficult to interpret in patients receiving anticoagulant therapy, but most patients with a thromboembolic event will already be anticoagulated before the decision to perform the tests has been made. In addition to understanding limitations of the assays, clinicians also need to be aware of which patients should be tested and not obtain testing on patients unlikely to have APS. New tests and diagnostic strategies are in various stages of development and should help improve our ability to accurately diagnose this important clinical disorder.

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The Antiphospholipid Syndrome (APS) is a disorder characterized clinically by recurrent venous and/or arterial thromboembolic events, or pregnancy morbidity (1, 2)(Table 1). In addition to these clinical manifestations, the *sine qua non* for the syndrome is the persistent presence of a unique collection of autoantibodies that target specific phospholipid-binding proteins. Although the presence of these autoantibodies is what defines the syndrome, the actual mechanism(s) whereby these autoantibodies cause a hypercoagulable state remains elusive. Animal models confirm the prothrombotic tendency of the autoantibodies (3), and several recent findings have provided insights into possible prothrombotic pathways. To better understand the criteria that have been developed to determine which patients have the syndrome, it is helpful to review the development of the laboratory strategies that are now critical for making the diagnosis.

## Laboratory Testing for Antiphospholipid Antibodies

Recognition of the thrombotic disorder currently defined as the antiphospholipid syndrome began with several convergent observations originating from laboratory tests applied from the clinical laboratory. The Wasserman test for the diagnosis of syphilis was first introduced into clinical practice in 1907 (4). Over thirty years later, the U.S. Congress passed the National Venereal Disease Control Act in 1938, and healthcare providers gradually expanded screening procedures for syphilis using this serologic test to include a variety of patient populations. Subsequently, it was recognized that a subset of individuals had a biologic false positive test for syphilis, testing positive in the serologic assay without any clinical evidence for syphilis (5). Although many of these individuals were asymptomatic, several reports, beginning in the 1950's, described patients with biologic false positive tests for syphilis who sustained thrombotic events (6).

In 1952, Conley and Hartmann (7) described two patients with systemic lupus erythematosus (SLE) who had unusual coagulation test results in association with hemorrhagic symptoms. The whole blood clotting time and the prothrombin time were both prolonged due to a "circulating anticoagulant" that interfered with the conversion of prothrombin to thrombin. The thrombin times were normal, indicating that the anticoagulant effect was not due to heparin. One patient's "anticoagulant titer" decreased with corticosteroid therapy, suggesting the presence of an immune component to the disorder (7). Subsequent reports confirmed the presence of 'circulating anticoagulants' in some patients with SLE, often patients who also had a biologic false positive test for syphilis, but hemorrhagic complications were generally not observed in these patients (8).

Cardiolipin, a complex phospholipid isolated from bovine heart extracts in the early 1940's, was subsequently identified as a key antigen in Wasserman's serologic test for syphilis (9). Intriguingly, cardiolipin was also shown to block the anticoagulant effect observed in plasma samples from patients with SLE, suggesting an antibody response that was directed against a phospholipid (10). In 1963, Bowie and colleagues (11) described eight patients with SLE who had evidence for a 'circulating anticoagulant' based on the use of a plasma clotting time. One of these patients had significant bleeding manifestations, but four presented with thromboembolic events, linking these 'anticoagulants' with thrombosis, which was subsequently confirmed by other investigators (12, 13). The term 'lupus anticoagulant' (LA) was first coined by Feinstein and Rapaport in 1972, based on the initial observations that the disorder was first described in patients with SLE, and laboratory testing of plasma from these patients suggested an anticoagulant effect (14). Subsequent work would demonstrate that the term was inaccurate on both accounts.

Because of the relative non-specificity of the assays used to detect the LA at the time, as well as the insensitivity of the precipitation assays used to detect anticardiolipin antibodies in patients with a biological false positive test for syphilis, Harris and colleagues developed a radioimmunoassay for the detection of anticardiolipin antibodies in patient with SLE and thrombotic complications (15). Using this assay on serum samples from 65 patients (59 with SLE), they demonstrated strong correlations between anticardiolipin antibody levels and the LA, venous and arterial thrombosis, and thrombocytopenia (15). In contrast, there was no

association with anti-DNA antibodies. For ease of use, this assay was subsequently converted to an enzyme-linked immunoassay (ELISA), and quickly became a key analytical approach for the rapid detection of this clinically-important group of autoantibodies (16). Standardization of this assay was recognized as a problem from early in its development, however (17), and this issue remains a concern over twenty-five years later (18).

As with testing for anticardiolipin antibodies, it was quickly recognized that reproducible criteria for the identification of a LA needed to be clearly defined. The Lupus Anticoagulant/Antiphospholipid Antibody Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) reviewed the status of laboratory identification of LA and published in 1991 several recommendations for clinical laboratory testing and diagnosis (19). In this communication, the subcommittee recommended four criteria for the diagnosis of a LA, summarized in Table 2. The “screening” step uses a phospholipid-dependent assay, such as the dilute Russell's viper venom time (dRVVT), the activated partial thromboplastin time (aPTT), or the dilute prothrombin time (dPT), in which the amount of phospholipid in the assay is diluted to make the test more sensitive to the presence of a LA. The mixing test consists of mixing patient plasma with plasma from a pool of healthy donors (typically on a one-to-one ratio) to confirm the presence of an inhibitory substance in the patient's plasma (as opposed to a factor deficiency). Third, and most importantly, the subcommittee recommended that phospholipid-dependence be demonstrated by the relative correction of the abnormal clotting time following the addition of phospholipid or platelets (19). These three criteria were also recommended by the Lupus Anticoagulant Working Party on behalf of the British Committee for Standards in Haematology, Haemostasis and Thrombosis task force that same year (20). The SSC recommendations were updated in 1995 (21) and 2009 (22), but the basic strategy remains the same.

The presence of a possible ‘cofactor’ for the LA effect was first noted in 1959 (23). In the 1970's, it was reported that the LA effect in up to two-thirds of patient plasma samples was ‘augmented’ by the addition of normal plasma (24). In 1989, it was shown that anticardiolipin antibodies do not bind to immobilized cardiolipin if plasma is not used in the assay (25). All of these observations led investigators to search for a possible cofactor that was critical for autoantibody binding to anionic phospholipids. This search ultimately culminated in three separate groups identifying the plasma protein  $\beta_2$ -glycoprotein I as this essential cofactor (26-28).

First purified and characterized in 1961 (29),  $\beta_2$ -glycoprotein I has been extensively studied and characterized. The protein sequence was published in 1984 (30), revealing a pattern of internal homology characterizing five separate domains, or segments, each ~60 amino acids in length. The crystal structure of  $\beta_2$ -glycoprotein I revealed an elongated “J”-shaped molecule with the five domains arranged like beads on a chain, with four complement control protein modules and a distinctly folding fifth domain (31). Multiple functions have been attributed to this plasma glycoprotein, including enhancing tissue plasminogen activator-mediated plasminogen activation (32), interfering with von Willebrand factor-dependent platelet adhesion and aggregation (33), and contributing to the clearance of

apoptotic cells (34). However, patients with a deficiency of  $\beta_2$ -glycoprotein I do not appear to have an increased risk for thrombosis (35, 36).

Although the specific function of  $\beta_2$ -glycoprotein I remains unclear, patients with autoantibodies directed against this plasma protein do exhibit an increased risk for thrombotic complications (37, 38) as well as pregnancy morbidity (39, 40). Clinically relevant anti- $\beta_2$ -glycoprotein I antibodies predominantly target the first domain of the protein (41), although other regions of the molecule have also been reported to be autoantibody epitopes in patients with APS (42). Anti- $\beta_2$ -glycoprotein I antibodies that bind specifically to domain 1 have been shown to cause LA activity and are more strongly associated with thrombosis than anti- $\beta_2$ -glycoprotein I antibodies that target other regions of the molecule (43).

In addition to  $\beta_2$ -glycoprotein I, prothrombin has been identified as a second phospholipid-binding protein against which many APS patients have been shown to exhibit autoantibodies associated with an increased risk for prothrombotic events (44). The data supporting the use of anti-prothrombin antibodies is limited, however, and these antibodies are not included in the diagnostic criteria for APS (Table 1). Antibodies directed against other anionic phospholipids (e.g., phosphatidylserine, phosphatidic acid, etc), against other phospholipid-binding plasma proteins (e.g., protein C, protein S)(45), and IgA isotype-specific antibodies against cardiolipin and  $\beta_2$ -glycoprotein I have also been identified in certain patients with APS. Testing for these autoantibodies is not recommended for patients clinically suspected of having APS who are negative for the recommended laboratory diagnostic criteria (Table 1).

These clinical laboratory tests, the anticardiolipin IgG and IgM antibodies, the LA, and the anti- $\beta_2$ -glycoprotein I IgG and IgM antibodies, are the key diagnostic tests used in the evaluation of patients for APS (Table 1). However, the prothrombotic nature of these different groups of autoantibodies, which are overlapping yet not identical, has not been intuitively obvious. Furthermore, the processes whereby an individual patient may exhibit different clinical manifestations associated with the syndrome, specifically thromboembolic events vs. pregnancy morbidity, remains unknown. As discussed further below, we anticipate the development of new diagnostic tests based on new insights into the pathophysiology of APS will improve our diagnostic accuracy for patients with the most severe clinical aspects associated with these autoantibodies.

## Definition of the Syndrome

As summarized above, various clinical manifestations were noted as being associated with the presence of antiphospholipid antibodies as early as the 1960's, most notably thromboembolic events and recurrent pregnancy loss (11). Although proposals for diagnostic criteria for the syndrome first appeared in the early 1990's (46), most authorities consider the classification criteria for the antiphospholipid syndrome that were proposed from a post-conference workshop held in 1998 in Sapporo, Japan, following the Eighth International Symposium on Antiphospholipid Antibodies (47), and updated in 2006 (1) to provide the most comprehensive diagnostic approach for APS (Table 1).

Clinical criteria for the syndrome include vascular thrombotic events as well as pregnancy morbidity (Table 1). Vascular events include venous thromboembolism, arterial thromboembolism, particularly in younger individuals, and microvascular thrombosis. As for any venous thromboembolic event, appropriate imaging studies supporting the diagnosis are essential. Microvascular thrombosis needs to be confirmed by histopathology, and should be present without significant evidence for inflammation involving the vessel wall. Superficial venous thrombosis alone is not included in the clinical criteria for APS. Coexisting inherited or acquired risk factors for thrombosis do not exclude the diagnosis of APS, but should be considered when developing therapeutic management strategies for the individual patient. Pregnancy-related complications are clearly defined, including the use of strict definitions of eclampsia and severe pre-eclampsia (48), as well as placental insufficiency (1).

In addition to the clinical manifestations included in Table 1, patients with APS may also have a variety of other characteristic clinical findings that do not represent diagnostic criteria for the syndrome. In fact, the presence of some of these manifestations may alert the clinician to suspect the diagnosis of APS in a patient who presents with a thromboembolic event and/or pregnancy morbidity. These manifestations include heart valve disease, livedo reticularis, thrombocytopenia, nephropathy, and various neurological manifestations (1). Heart valve lesions, such as vegetations, valve thickening, and valvular dysfunction, are common in patients with APS, independent of SLE (2). A common dermatologic manifestation of antiphospholipid antibodies is livedo reticularis, but the presence of this finding does not predict thrombosis risk, with the exception of patients with Sneddon's syndrome (1). Non-criteria renal manifestations include small artery vasculopathy and chronic renal ischemia (49, 50). While the presence of these findings in a patient with vascular thrombosis and/or recurrent pregnancy morbidity should alert the clinician to suspect the presence of APS, the identification of these "non-criteria" findings alone do not identify a patient as having the syndrome.

## Difficulties with the Diagnosis of APS

In general, thrombotic complications are not uncommon, and indiscriminate application of laboratory testing for antiphospholipid antibodies can lead to overdiagnosis of the syndrome. For example, Dunn and colleagues (51) applied the Sapporo criteria (Table 1) to 103 patients with APS being treated at three University-based anticoagulation clinics. Only 10 patients met criteria for definite APS; sixteen patients had a possible diagnosis of APS, and 71 of the patients did not meet criteria (51). Seventy patients had abnormal anticardiolipin antibody results, but more than half (38 of 70) had low-positive antibody levels, and repeat testing was performed in only 49 cases. Although the international consensus criteria are primarily intended to provide guidelines for patients with APS to be included in clinical trials, the available data would suggest that the diagnosis is frequently inappropriately applied in the clinical setting.

### Who should be tested?

Pengo and colleagues (22) recommended that testing for LA (and, by extension, anticardiolipin antibodies and anti- $\beta_2$ -glycoprotein I antibodies) should be limited to patients who have a significant probability of having APS. Patients in whom testing would be most appropriate would include individuals with unprovoked venous thromboembolism and (unexplained) arterial thrombosis in young patients (less than 50 years of age), thrombosis at unusual sites, late pregnancy loss, and any thrombotic events or pregnancy morbidity in patients with other autoimmune disorders (e.g., SLE). Patients in whom testing is reasonable include individuals with recurrent spontaneous early pregnancy loss and provoked venous thromboembolism in young patients. Individuals in whom testing for antiphospholipid antibodies is unlikely to be helpful include elderly patients with venous or arterial thromboembolism. Although not being used to diagnose the syndrome, strictly speaking, testing for a LA can be useful when evaluating asymptomatic individuals who have an unexplained prolonged aPTT identified during the course of routine laboratory testing. Generalized searches performed on asymptomatic individuals or categories of patients other than those described above are not recommended in order to avoid obtaining false-positive results that are relatively common on account of the limited specificity of the assays.

### When should testing be performed?

Optimally, testing should be performed when a patient is clinically stable and not during an acute event. Interpretation of the results of laboratory tests performed near the time of an acute thromboembolic event can be difficult, as acute phase reactants such as factor VIII and fibrinogen may be markedly increased during acute events, altering coagulation test results. In addition, acute events may also trigger the appearance of anticardiolipin antibodies which are transient (52). Consequently, it is recommended that all positive results should be confirmed by repeat testing, at least twelve weeks apart, to distinguish patients with persistent antiphospholipid antibodies from those with transient antibodies (Table 1).

### Can testing for a LA be performed while a patient is on anticoagulant therapy?

Interpretation of laboratory results for a LA can be difficult in patients who are receiving anticoagulant therapy. Different antithrombotic therapies have different effects on the laboratory testing, and are reviewed individually below and summarized in Table 3. In general, anticoagulant therapy does not interfere with testing for anticardiolipin or anti- $\beta_2$ -glycoprotein I antibody testing.

**Unfractionated heparin**—Heparin interferes with the hemostatic pathway by accelerating antithrombin-mediated inhibition of thrombin, factor Xa, and other serine proteinases in the pathway. Characteristically, the aPTT will be prolonged and will not correct with mix. In some patients, the PT may be modestly prolonged as well, in the presence of therapeutic heparin. A useful test to identify the presence of heparin is the thrombin clot time (TCT), which measures the time required for a clot to form in a plasma sample to which a measured amount of thrombin has been added. Heparin will characteristically prolong this assay by inhibiting the thrombin, whereas a LA should not prolong the TCT. Certain assay kits

contain heparin neutralizers that can enable detection of a LA in the presence of heparin up to a level exceeding the usual therapeutic range (for example, up to 1 U/mL) (53).

**Low-molecular weight heparins**—Low molecular weight heparins (e.g., enoxaparin, dalteparin) are also antithrombin-dependent anticoagulants that are prepared by depolymerization of unfractionated heparin by chemical methods and/or enzymes, followed by fractionation of the mixture. The inhibitory effect is primarily directed against factor Xa, with variable degrees of inhibition of thrombin and the other serine proteinases. Following subcutaneous administration, the aPTT will be variably prolonged at peak levels, depending on the sensitivity of the individual reagents used in the clinical laboratory (54). The prolongation of the aPTT will also vary in relationship to when the last dose of the drug was administered. Dalteparin has been shown to not interfere with LA testing by the dRVVT (55). Regardless of the agent in use, it would be optimal to obtain tests for LA from patients on low-molecular weight heparins at an expected trough in therapy (i.e., immediately prior to a scheduled dose) to minimize the anticoagulant effect on the laboratory analyses.

**Fondaparinux**—Fondaparinux is a synthetic pentasaccharide that represents the minimal antithrombin-binding sequence from heparin. Fondaparinux does not prolong the aPTT in an *in vitro* spiking study (56), and it also has been shown to have no effect on the dRVVT (57) (Table 3). Fondaparinux would also be expected to have no effect on the TCT, since it has essentially no inhibitory activity towards thrombin. On the other hand, the presence of fondaparinux can be detected with an anti-factor Xa assay, which would similarly detect the presence of unfractionated heparin as well as low-molecular weight heparins, but not a LA (Table 3). As with the low-molecular weight heparins, testing would be optimally performed at an expected trough in the level.

**Warfarin and other vitamin K antagonists**—The vitamin K antagonists mediate an anticoagulant effect by interfering with vitamin K epoxide reductase, resulting in decreased functional levels of the vitamin K-dependent factors II, VII, IX, and X. Other vitamin K-dependent proteins, such as the natural anticoagulants protein C and protein S, are also decreased by the use of warfarin, but these changes do not contribute to the anticoagulant effect of this class of anticoagulants (in fact, they have the potential to contribute to rare prothrombotic manifestations that may be associated with the use of warfarin). Inhibition of the vitamin K epoxide reductase will result in the prolongation of the PT, the dRVVT, and, to a lesser extent the aPTT (Table 3). Mixing studies should correct the prolonged screening studies if they are due to the anticoagulant effect alone, but a confirmatory assay performed only with the addition of excess phospholipid should not. Several studies have shown that the dRVVT with confirmatory testing can be used to detect LA in patients on warfarin therapy (58, 59).

**Direct thrombin inhibitors**—Direct thrombin inhibitors include four parenteral agents (lepirudin, argatroban, bivalirudin, and desirudin) and the oral agent dabigatran etexilate, which was recently approved for the prevention of stroke and arterial thromboembolism in patients with atrial fibrillation. Lepirudin and argatroban are primarily used for the treatment of patients with heparin-induced thrombocytopenia. These agents will have variable effects

on the PT, but all will prolong the aPTT (Table 3). Because these drugs are inhibitors, mixing studies will typically not correct the prolonged screening assays. Similar to unfractionated heparin, all of these agents will cause a marked prolongation of the TCT (Table 3). Lepirudin, argatroban, and bivalirudin have all been shown to prolong the dRVVT (57), and argatroban has been shown in an *in vitro* analysis to potentially cause false-negative test results for LA (60). In contrast, lepirudin can potentially cause false-positive test results for LA (60). Data on the effect of dabigatran on the dRVVT are currently not available, but, given the mechanism of action of the agent, it would be expected to prolong the screening assay and potentially confound interpretation of the confirmatory step.

**Direct factor Xa inhibitors**—Direct factor Xa inhibitors include the oral agents rivaroxaban, apixaban, and edoxaban. Rivaroxaban has been approved in the United States for thromboprophylaxis following orthopedic surgery procedures and for the prevention of stroke and peripheral embolism in patients with atrial fibrillation. These agents exhibit a variable effect on the aPTT, which varies depending on the time from the last oral dose (61, 62). Although data are not available, it is expected that mixing studies would incompletely correct the prolonged aPTT, depending on the concentration of the drug at the time the plasma sample was drawn. The TCT is not affected by these agents, but the presence of an anticoagulant effect can be detected with an anti-factor Xa assay (Table 3). Rivaroxaban has been reported to cause false-positive test results for lupus anticoagulants, particularly with the dRVVT (63). As an alternative strategy, the taipan and ecarin clotting times, which contain direct activators of prothrombin, have been shown to be useful in the detection of LA in the presence of rivaroxaban (64).

### **How frequently should testing be repeated in patients who test positive for an antiphospholipid antibody?**

The transient presence of antiphospholipid antibodies, not infrequent in clinical practice, may lead to the incorrect identification of a patient as having APS (65). Consequently, it has been recommended that a positive test result should be confirmed at least twelve weeks after an initial positive result, to confirm autoantibody persistence as a criterion for the syndrome (1). Once the diagnosis has been confirmed, however, repeat testing is unnecessary, although re-evaluation with any changes in clinical manifestations is reasonable. For patients with APS in whom the autoantibody appears to resolve, it is unclear whether resolution of the abnormal laboratory test results indicates that the hypercoagulable state has resolved as well.

### **The Future of Diagnostic Testing for APS**

Although the diagnostic criteria for antiphospholipid antibodies and APS have improved with our understanding of the autoantibodies and the clinical manifestations of the syndrome, laboratory assays for anticardiolipin and anti- $\beta_2$ -glycoprotein I antibodies, and LA, remain the cornerstones of diagnosis. These assays have been associated with the clinical manifestations of the syndrome in numerous studies, but none of the assays has been uniformly specific for patients with APS. Consequently, several strategies that differ in their approaches are currently being explored as potential alternatives to improve the sensitivity and specificity of diagnostic laboratory testing for antiphospholipid antibodies.



### High-risk antiphospholipid antibody profiles

Several studies have demonstrated a strong association between positivity on multiple assays (specifically, LA, anticardiolipin antibodies, and anti- $\beta_2$ -glycoprotein I antibodies) and clinical manifestations of APS (66, 67). Pengo and colleagues (68) reported that the cumulative incidence of thromboembolic events in 104 subjects with a high-risk profile for antiphospholipid antibodies (triple positivity for LA, anticardiolipin antibodies, and anti- $\beta_2$ -glycoprotein I antibodies) and no prior thromboembolism was 37.1% by ten years (95% confidence interval, 19.9%-54.3%). These observations have led to the recommendation that testing for antiphospholipid antibodies in patients clinically suspected of having the syndrome should include all three assays, and that "triple-positive" patients would be considered to have the highest risk for complications of the syndrome. The clinical significance of patients who test positive for only one or two tests remains to be determined.

### Domain I-specific anti- $\beta_2$ -glycoprotein I antibodies

Two studies have been reported that investigated the clinical significance of autoantibodies specific for domain I of  $\beta_2$ -glycoprotein I. The first study demonstrated that domain I-specific anti- $\beta_2$ -glycoprotein I antibodies were associated more with thrombosis compared with other anti- $\beta_2$ -glycoprotein I antibodies (43). More recently, a double-blinded multicenter study investigated 442 patients, all with anti- $\beta_2$ -glycoprotein I antibodies (69). Slightly more than half the patients (243 of 442, or 55%) had anti-domain I antibodies, and these patients had an odds ratio of 3.5 (95% confidence interval, 2.3-5.4) for thrombosis compared to those patients without anti-domain I antibodies. Further investigations into the potential clinical utility of this assay have been recommended by a task force formed at the 13<sup>th</sup> International Congress on Antiphospholipid Antibodies held in Galveston, Texas, in April 2010 (70).

### Endogenous thrombin potential

Existing laboratory tests do not exploit potential pathophysiologic processes associated with antiphospholipid antibodies. Recently, several investigators have used the endogenous thrombin potential as a strategy to identify patients with antiphospholipid antibodies associated with thrombotic complications (71, 72). Devreese and colleagues (73) used this approach, supplemented with measurement of selected hypercoagulable markers (soluble P-selectin and factor VII), to develop a layered strategy with high sensitivity and specificity (~90% range) for thrombotic complications in patients with antiphospholipid antibodies.

### Annexin A5-resistance test

Annexin A5 binds with high affinity to anionic phospholipids and has been shown to form a protective 'shield' over membrane surfaces (74, 75). The presence of an intact shield interferes with the availability of anionic phospholipids for critical coagulation reactions (76). Several studies have shown that antiphospholipid antibodies are capable of disrupting this shield (77), resulting in the exposure of negatively charged surfaces that can support prothrombotic reactions. Using a coagulation-based assay developed from these observations, resistance to the anticoagulant effect of annexin A5 has been detected in blood samples from patients with APS (78). This approach has been used to distinguish patients

with APS from asymptomatic individuals with antiphospholipid antibodies as well as patients with venous thromboembolism but no evidence for antiphospholipid antibodies (79). More recently, resistance to annexin A5 has been shown to correlate with domain 1-specific anti- $\beta_2$ -glycoprotein I antibodies, linking two testing strategies for high-risk antiphospholipid antibodies (80).

Each of these new testing strategies seeks to identify patient populations at high-risk for clinical manifestations of APS. Prospective studies will need to be conducted that compare these approaches with assays currently in use to determine how to optimally integrate the newer analytical assays into our diagnostic approach to patients clinically suspected of having APS. In addition, laboratory strategies to identify patients at risk for recurrent thromboembolic events while on anticoagulant therapy would be extremely useful in this patient population. The coming years will be quite exciting as new laboratory tests and diagnostic strategies are developed, evaluated, and implemented for patients with APS.

## ACKNOWLEDGEMENTS

TLO is supported by research grants from the Centers for Disease Control and Prevention (DD000014) and the National Institutes of Health (HL072289, HL087229).

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**Table 1**

International criteria for the classification of Antiphospholipid Syndrome (from references (1, 47))

Clinical Criteria

1. Vascular thrombosis, defined as one or more clinical episodes of arterial, venous, or small vessel thrombosis, involving any organ and confirmed by appropriate imaging and/or histopathologic analyses.
2. Pregnancy morbidity.
  - a. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10<sup>th</sup> week of gestation, with normal fetal morphology.
  - b. One or more premature births of a morphologically normal neonate at or before the 10<sup>th</sup> week of gestation because of eclampsia or severe pre-eclampsia, or placental insufficiency.
  - c. Three or more unexplained consecutive spontaneous abortions before the 10<sup>th</sup> week of gestation, with maternal anatomic or hormonal abnormalities, and maternal or paternal chromosomal abnormalities excluded.

Laboratory criteria \*

1. Anticardiolipin antibody of IgG and/or IgM isotype in blood, present in medium or high titer, defined as >40 GPL or MPL, or >99<sup>th</sup> percentile.
  2. Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart and detected according to the guidelines of the ISTH Scientific Subcommittee on Lupus Anticoagulants/Phospholipid-Dependent Antibodies (Table 2).
  3. Anti- $\beta_2$ -glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma, in a titer >99<sup>th</sup> percentile.
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\* A positive laboratory test needs to be positive on at least two occasions, separated by twelve weeks, to be considered diagnostic for antiphospholipid syndrome.

**Table 2**

ISTH Criteria for lupus anticoagulant detection (compiled from references (19, 21, 22)).

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- Prolongation of a phospholipid-dependent clotting assay. Two tests based on different assay principles should be used (e.g., the dRVVT and aPTT).
  - Evidence of inhibitory activity on mixing tests with normal pooled plasma. A 1:1 proportion of patient and normal pooled plasma, without preincubation, should be used.
  - Evidence that inhibitory activity is phospholipid-dependent. Confirmatory testing should be performed by increasing the concentration of phospholipid in the screening test(s) that was abnormal.
  - Differentiation from other coagulopathies that may give similar laboratory results or may occur concomitantly with lupus anticoagulants. Specific factor assays may be necessary to accomplish this step.
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Table 3

Effect of Lupus Anticoagulants and Anticoagulant Therapies on Commonly Used Clinical Laboratory Assays (adapted from reference (81))<sup>\*</sup>

Laboratory Test	Lupus Anticoagulant	Therapeutic UFH	Therapeutic LMWH	Therapeutic Fondaparinux	Vitamin K Antagonists	Direct thrombin inhibitors <sup>†</sup>	Direct Factor Xa inhibitors <sup>‡</sup>
PT	Normal or prolonged	Normal to mild prolongation	Normal	Normal	Prolonged	Variable prolongation	Variable prolongation
aPTT	Typically prolonged	Prolonged	Mild prolongation	Normal to minimal prolongation	Normal to mild prolongation	Prolonged	Variable prolongation
TCT	Normal	Prolonged	Variable prolongation	Normal	Normal	Prolonged	Normal
dRVVT (screening step)	Typically prolonged	Prolonged <sup>^</sup>	Variable effect	Normal	Prolonged	Prolonged <sup>**</sup>	Prolonged <sup>††</sup>
Mixing study of prolonged screening test	No correction	No correction	No correction	Typically not applicable	Correction	No correction	No correction
Anti-factor Xa assay <sup>‡‡</sup>	Normal	Elevated	Elevated	Elevated	Normal	Normal	Elevated

<sup>\*</sup> The column for lupus anticoagulant test results represents what would be expected for a plasma sample with no anticoagulant therapy, and the columns for the anticoagulant therapies represent what would be expected for plasma samples with each of the classes of anticoagulants in the absence of a lupus anticoagulant. A plasma sample from a patient with a lupus anticoagulant and one (or more) of the anticoagulant agents would have test results reflecting the combined presence of more than one variable. Abbreviations used include: PT; prothrombin time; aPTT, activated partial thromboplastin time; TCT, thrombin clotting time; dRVVT, dilute Russell's viper venom time; UFH, unfractionated heparin; LMWH, low-molecular weight heparin.

<sup>†</sup> Direct thrombin inhibitors include the parenteral agents lepirudin, argatroban, bivalirudin, and desirudin, and the oral agent dabigatran.

<sup>‡</sup> Direct factor Xa inhibitors include rivaroxaban, apixaban, and edoxaban. Relatively limited data are available on the latter two agents.

<sup>^</sup> Although unfractionated heparin will typically prolong the dRVVT, most commercially available dRVVT reagents contain a heparin neutralizer that neutralizes up to or slightly greater than 0.8 to 1.0 IU/mL UFH present in patient plasma.

<sup>\*\*</sup> Results are for lepirudin, argatroban, and bivalirudin; no data are available for desirudin or dabigatran etexilate.

<sup>††</sup> Results are for rivaroxaban only.

<sup>‡‡</sup> A 'normal' result indicates no evidence for anti-factor Xa activity in the plasma sample being tested, whereas an 'elevated' result indicates the presence of anti-factor Xa activity (antithrombin-dependent or independent).