

ANTIPHOSPHOLIPID ANTIBODY SYNDROME

ANTIPHOSPHOLIPID ANTIBODIES (APLA)-DEFINITIONS AND HISTORY

APLA are antibodies directed against certain phospholipids. They are found in a variety of clinical situations. APLA are important to detect because in certain patients they are associated with a syndrome that includes a hypercoagulable state, thrombocytopenia, fetal loss, dementia, strokes, optic changes, Addison's disease, and skin rashes. APLA were first described by Conley in the 1950's when it was noted that patients with lupus often had prolonged activated partial thromboplastin times (APTT). Soon thereafter the association with false positive VDRLs was noted. Despite the elevation of the aPTT it was observed that these patients did not develop hemorrhagic complications unless they also had hypoprothrombinemia or thrombocytopenia. Bowie first described the association of APLA and thrombosis in 1964. Feinstein and Rapaport in a 1972 review called this phenomenon the "lupus anticoagulant". Harris, recognizing that cardiolipin is a major component of the VDRL test, developed and popularized the anticardiolipin (ACL) antibody test in the mid-1980's. In the 1980's it became well recognized that patients did not have to have SLE to have symptomatic disease from APLA.

One major difficulty in studying APLA is that no one really knows what the underlying pathogenic mechanism is that leads to the clinical syndrome. This review will side-step this issue and concentrate on clinical matters.

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The underlying mechanism leading to the clinical syndrome associated with APLA is still unknown. Perhaps the antibodies inhibit the function of proteins C or S, damage the endothelium, activate platelets or inhibit prostacyclin. Despite several decades of research, the etiology of the thrombotic tendency associated with APLA remains unknown.

SEMANTICS

APLA Syndrome: Patients with APLA and one "major clinical criterion" are said to have "APLA syndrome." The major clinical criteria include venous or arterial thrombosis (including neurological disease such as stroke), thrombocytopenia, or frequent miscarriages.

Secondary APLA syndrome is APLA plus another autoimmune disease, most commonly lupus.

Primary APLA syndrome is APLA syndrome occurring outside of the setting of lupus. In distinction to SLE-APLA patients, primary APLA patients are more often male and will have low titer ANA's but no other criteria for SLE.

Anticardiolipin Antibody is an APLA in which the antibody is detected by an ELISA assay.

Anti-beta₂glycoprotein (Anti-B₂GP) is a subgroup of APLA also detected by ELISA assay. Anti-B₂GP are thought to be more specific for APLA that cause thrombosis.

"Lupus anticoagulant" and "lupus inhibitor" are terms which are interchangeable. Lupus inhibitor is an APLA in which the antibody is detected by a coagulation test.

WHO GETS APLA?

Approximately 30-50% of patients with SLE will have APLA. The antibodies can also be found in patients with other autoimmune diseases. Patients without lupus or other autoimmune disease can have symptomatic APLA ("Primary APLA Syndrome"). Children will often develop transient non-thrombotic APLA after viral infections. This laboratory finding often comes to attention during pre-operative evaluation for tonsillectomy. Up to 30% of patients with HIV infection will also develop APLA. The infection-associated APLA are not associated with thrombosis and are usually anti-B₂GP negative. Medication may also induce APLA. Chlorpromazine is the most common cause, but APLA has also been associated with use of Dilantin and tumor necrosis factor inhibitors. In screening studies of blood donors and normal controls, up to 10-20%

of asymptomatic people have APLA. However, the APLA in these people are usually low-titer and most often occurs in young women.

APLA: CLINICAL ASSOCIATIONS

APLA are associated with a number of disease states. The best described conditions are venous thrombosis, arterial thrombosis, neurological disease, frequent miscarriages, and thrombocytopenia.

Venous Thrombosis. Venous thrombosis was the first described manifestation of APLA and is the one most clinically predominant. Overall, retrospective studies show that 31% of patient with APLA have venous thrombosis. Patients with lupus and APLA have a thrombosis rate of 42%; patients with infectious or drug-induced APLA have a thrombosis rate of less than five percent. Patients with APLA are over-represented in young patients with deep vein thrombosis. Prospective studies have demonstrated a relative risk for venous thrombosis of 5.3 for patients with IgG anticardiolipin antibodies. Patients with APLA-associated venous thrombosis may be difficult to treat. These patients have high rates of recurrent thrombosis (20-50%/year) if anticoagulation is stopped. Occasional patients may be refractory to warfarin/direct oral anticoagulants and will need to be on long-term heparin therapy.

Neurological Disease. APLA have been associated with stroke, especially in younger patients. A variety of other neurological disorders have been associated with APLA. The underlying cause of these disorders appears to be thrombosis. Some patients have large vessel disease while more often patients have small vessel involvement. Patients with APLA often will have multiple MRI abnormalities consistent with small white matter infarcts. The neurological diseases include:

Stroke: APLA is found in 10-46% of young patients with stroke and in 10% of stroke patients overall. Stroke patients with APLA tend to be younger (42 years vs 62 years). These patients also have a recurrence rate of 6-30%/year and a mortality rate of 10%/year. Certain groups of patients appear to be at even higher risk of recurrence. These would include SLE patients with APLA and patients with Sneddon's syndrome (described below).

Early-Onset Dementia: This is becoming a well-recognized and feared feature of APLA. The dementia is multi-infarct in nature and occurs often without a history of major stroke episodes. APLA-related dementia on the average occurs a decade earlier (52 years) than non-APLA dementia. Sneddon's syndrome is a combination of livedo reticularis and cerebral ischemic events. Sneddon's is a form of APLA which often results in major morbidity and mortality. The skin involvement in Sneddon's may be severe enough to result in ulceration. Patients with Sneddon's syndrome seem also to have a high incidence of thrombocytopenia.

Ocular Events: Amaurosis fugax, retinal artery thrombosis, and retinal vein thrombosis have been reported as part of APLA syndrome in multiple case reports.

Other: APLA are found in as many as 50% of patients who get migraines. As will be discussed below, patients may have encephalopathy as part of severe APLA. Rare patients with chorea form movements with APLA have been reported where the movement disorder resolves with anticoagulation

Fetal Loss. Fetal loss is seen in 38% of SLE patients with APLA. The incidence of fetal loss in non-SLE APLA is controversial. When women who have recurrent fetal loss are screened the incidence of APLA is 30%. The pathophysiology is thought to be due to placental microthrombosis. The incidence of HELLP syndrome is higher in these patients and tends to occur earlier in the pregnancy. Fetal growth retardation is also common due to placental infarctions.

Thrombocytopenia. Certain APLA will react with activated platelets, leading to thrombocytopenia. Only activated platelets expose the proper phospholipid epitopes that the APLA react with. Therefore, it is the patients with the thrombotic manifestations of APLA who will also get the thrombocytopenia. The treatment of these patients is clinically challenging since the thrombocytopenia often occurs in patients who are anticoagulated for thrombosis. Danazol was used in the past and appeared to be uniquely effective for these patients but is now increasing use of rituximab to treat these patients.

Hypoprothrombinemia Patients with APLA (almost always those with lupus inhibitors) may have an elevated prothrombin time (INR) for two reasons. The APLA may be present in such high titers that they will also interfere with the PT/INR test. Alternatively, 10% of patients with lupus inhibitors will develop non-

neutralizing antibodies to prothrombin. This leads to increased clearance of prothrombin from the plasma and hypoprothrombinemia. Since patients with hypoprothrombinemia can present with hemorrhagic complications, it is important to check for this when faced with an APLA patient with an elevated PT/INR. The work-up includes a 50:50 mix on the PT and a measure of the plasma level of prothrombin. The 50:50 mix if the INR is due to the APLA it will not correct. If there is an antibody to prothrombin the 50:50 mix correct as the mechanism of action of the antibody is to speed degradation of prothrombin but not inhibitors its activity. Plasma infusions and steroids are effective in raising the prothrombin level in patients with prothrombin antibodies.

Other Associated Diseases: Patients with APLA may have an assortment of skin findings included livedo, Raynaud's phenomenon, ulcers, and superficial thrombophlebitis. Up to 26% of patients with SLE and APLA have cardiac valve vegetations and mitral regurgitation. Rarely patients have had valve destruction so extensive as to require valve replacement. Myocardial dysfunction is seen in 5% of SLE-APLA patients. Primary pulmonary hypertension has been associated with APLA. Ten percent of patients with chronic thromboembolic pulmonary hypertension have APLA. Adrenal insufficiency from microvascular thrombosis has also been seen in APLA patients.

CATASTROPHIC APLA (CAPS)

Rarely, patients with antiphospholipid antibody syndrome can present with fulminant multiorgan system failure. CAPS is caused by widespread microthrombi in multiple vascular fields. These patients will develop renal failure, encephalopathy, adult respiratory distress syndrome (often with pulmonary hemorrhage), heart failure, dramatic livedo reticularis, and worsening thrombocytopenia. Many of these patients have pre-existing autoimmune disorders and high titers of anticardiolipin antibodies.

Organ affected include:

- Cardiac disease: cardiomyopathy
- Pulmonary disease: pulmonary hemorrhage, ARDS
- Neurologic disease: seizures, coma, encephalopathy
- Renal disease: renal failure due to thrombosis
- Skin disease: livedo reticularis, skin necrosis
- Bone: necrosis
- Severe thrombocytopenia

It appears that the best therapy for these patients is aggressive immunosuppression with plasmapheresis then (perhaps) IV cyclophosphamide monthly or rituximab. Early recognition of this syndrome can lead to rapid therapy and resolution of the multiorgan system failure.

TESTS FOR ANTIPHOSPHOLIPID ANTIBODIES (APLA)

APLA's are important to detect because in certain patients they are associated with a syndrome that includes a hypercoagulable state, thrombocytopenia, fetal loss, dementia, strokes, Addison's disease, and skin rashes. There are two main tests for APLA's: testing for presence of antibodies to cardiolipin and the coagulation-based tests for APLA.

Coagulation-Based Tests: As noted above, APLA reacts with phospholipid. The phospholipids provide a surface where the coagulation reactions take place. The basis for all the coagulation-based tests is that antibodies on the phospholipid will prolong the coagulation reactions and thus the test time. Once an elevated aPTT is found, one must verify it by showing it does not fully correct with a 50:50 mix. To prove the inhibitor is dependent on phospholipids one then adds phospholipid derived from platelets or hexagonal phase phospholipids. APLA reacts strongly with these phospholipids, and addition of these will correct the coagulation tests by absorbing out the APLA.

To summarize, one screens for APLA with coagulation-based tests to see if any clotting times are prolonged. If a test result is elevated, then a 50:50 mix is done to ensure the elevation is not due to a specific factor deficiency. Then one uses a phospholipid source to correct the clotting time and verify the presence of APLA.

Specific Assays

aPTT: The routine aPTT only detects 30% of patients with APLA and are inadequate as a single test for APLA screening. One can increase sensitivity by using different aPTT reagents.

Kaolin clotting time: This test uses no added phospholipid and is a sensitive test to detect APLA. However, it is technically demanding to do properly and is now rarely performed.

Platelet Neutralization Test: This test takes a coagulation reaction that is prolonged by plasma and does not correct with a 50:50 mix. Extracts of platelet phospholipids are added to the plasma and an aPTT is performed. The platelet phospholipid is very avid for APLA and "soaks up" the antiphospholipid antibody and corrects the aPTT. If the aPTT corrects with addition of platelets this is diagnostic for APLA.

Hexagonal Phospholipid Neutralization: This test is based on the same principle as the platelet neutralization test but it uses hexagonal phospholipid which is more specific for antiphospholipid antibodies. Current test kits that use hexagonal phospholipids also have added plasma and inhibitors of heparin. The additional reagents allow this assay for lupus inhibitors to be performed on anticoagulated patients.

Silica Clotting Time: This test uses silica to activate coagulation and a reagent with very low levels of phospholipids. If the test is prolonged, then it's repeated with a high phospholipid reagent. If the PTT prolongation was due to antiphospholipid then the addition of high phospholipid reactant will correct the prolongation. This test is reported as a ratio between the PTT with and without added phospholipids.

Dilute Russell Viper Venom Time (dRVVT): This test is very sensitive to any interference with phospholipids and is very sensitive to APLA. It is performed by initiating the coagulation cascade with Russell Viper venom which directly activates factor X and is very sensitive to phospholipids. dRVVT will also be raised in warfarin patients and with deficiencies of X, V, or II so there is also a phospholipid neutralization step use to derive a dRVVT ratio (without/with phospholipids) to find antiphospholipid antibodies.

Anticardiolipin Antibodies (ACLA): This is an ELISA test for antibodies to cardiolipin. Therefore, it can be performed on plasma that has been anticoagulated. Tests results are reported in arbitrary units. Tests are also reported as specific isotype (IgG, IgA, and IgM). It is still debated whether specific isotypes indicate specific diseases but most "secondary" (i.e. associated with infection) ACLA tend to be IgM subtypes. Only high titers (>40 units) are associated with APLA.

Anti-beta2 glycoprotein antibodies: Although "APLA" are named for antibodies to phospholipids, the real targets are phospholipid-protein combinations. For anticardiolipin antibodies the protein target is beta₂-glycoprotein (B₂GP). It appears that anti-B₂GP antibodies may be more specific for pathogenic APLA, as they are usually negative in infection-associated APLA and other APLAs not associated with thrombosis. Currently there is research to see if anti- beta₂-glycoprotein antibodies directed against certain parts of beta₂-glycoprotein are more predictive of thrombosis. Only levels > 99th percentile are associated with APLA.

DIAGNOSTIC APPROACH TO APLA

Unfortunately no single test can screen a patient for APLA. One must perform the entire panel on patients suspected of having APLA. The panel would include

- Anticardiolipin antibodies
 - Anti- beta₂-glycoprotein
- Plus at least two coagulation based tests such as:
- dRVVT
 - "Lupus Inhibitor Screen" (different aPTT reagents).

There are many confounding factors when testing for APLA. One is that there is a high rate of false positives with acute thrombosis - especially lupus inhibitors. Most patients who have only an isolated positive lupus inhibitor at the time of diagnosis will on repeat testing will have negative testing. Second, many patients will have low titers tests, especially anticardiolipin assays so only titers > 99th percentile or anticardiolipin antibody titers > 40 units are significant. Also the direct oral anticoagulants (DOACs) interfere with all coagulation based testing.

Currently the Sydney criteria are used to diagnoses APLA syndrome - this requires both clinic and laboratory findings

* **Clinical:** one or more episode of arterial, venous, or small vessel thrombosis (other than superficial thrombophlebitis) or pregnancy complications - either 3 or more miscarriages before 10 weeks, one or more fetal death after 10 weeks, premature birth before 34 weeks due to pre-eclampsia.

* **Laboratory:** one or more positive tests repeatedly positive when tested at least 12 weeks apart:

* Lupus inhibitor

* Anticardiolipin antibody - greater than either 99th percentile or greater than

MPL or GPL units

* Antibeta2glycoprotein - greater than 99th percentile

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Triple Positive patients are those with all three tests positive. These patients appear to be at high risk for thrombosis and are at higher risk of "breaking through" warfarin.

Occasional patients are seen who consistently have negative laboratory testing for APLA but have many of the clinical features of APLA such as thrombocytopenia, thrombosis and miscarriages. It is probable that these patients do have "APLA-negative APLA syndrome" and they should be treated as such.

THERAPY

Although there are few prospective trials of therapy in APLA, several lessons may be learned from retrospective. While APLA does appear to be an autoimmune disease, immunosuppression does not prevent recurrent thrombosis, fetal loss, or neurological syndromes. Therefore, immunosuppression should not play a role in the therapy of thrombotic APLA. The only exception to this is "catastrophic APLA" where plasmapheresis and immunosuppression plays a crucial role.

It used to be thought that anticoagulation with warfarin to an INR of 3.0-3.5 was effective in patients with APLA. However, randomized trials demonstrated that an INR range of 2.0-3.0 is just as effective as the higher INR range – at least for venous disease. As mentioned above, some patients will fail warfarin and will require more aggressive anticoagulation.

Controversial remains about treatment for arterial disease – some recommend INR 3.0-3.5 while there is data showing INR 2-3 plus aspirin may be effective.

The role of DOAC is unclear. A clinical trial show DOAC were inferior to warfarin in "triple positive" patients and are contraindicated. For select patients with single positive APLA DOAC may be a consideration if there are challenges with warfarin.

Thrombocytopenia. Thrombocytopenia in patients with APLA occurs in those patients prone to thrombosis due to activated platelets expressing the epitopes for APLA. The low platelet counts makes anticoagulation hazardous. In addition, patients with APLA are often high surgical risks. Thrombocytopenia may respond to steroids, immunoglobulin and IV-anti-D. Danazol 200 mg po QID is effective in many patients with APLA-related thrombocytopenia as well as rituximab.

Pregnancy and APLA: The approach is based on previous history. If there is a history of thrombosis, LMW heparin in therapeutic doses is used throughout the pregnancy. For frequent miscarriages, prophylactic doses of LMWH are used along with 81 mg of aspirin.

Difficulties in monitoring anticoagulation

Since APLA react with phospholipids, both the aPTT and the PT can be affected. If standard heparin is used to anticoagulate patients with APLA, treatment must be followed by monitoring heparin levels (target range 0.35 - 0.70 anti-Xa). The predictable dosing and anticoagulant effect is one advantage of using LMW heparin acutely for thrombosis in APLA patients. One should measure LMW heparin levels in patients with APLA on long-term therapy (0.7 - 1.1 anti-Xa units four hours after injection) or in those patients with renal failure.

Often patients with APLA will have minor elevations of PT/INR. Those few patients with elevated INR due to a lupus inhibitor can be very difficult to manage with warfarin. One option is to measure levels of factor X via a chromogenic assay (not affected by APLA). The therapeutic range of 15-30% has been shown to best correlate with therapeutic warfarin effect. Pairing factor X levels with INR one can determine an INR range that is effective.

One difficult issue is that of the patient with APLA with no thrombotic manifestations. Although some of these patients are at risk, especially those with SLE, many will never develop thrombosis. The current recommendation would be to search thoroughly for thrombosis. The work-up would include a brain MRI in patients with SLE or in patients with any neurological symptoms. Some would recommend for patients with triple positive disease or with autoimmune disease aspirin prophylaxis

Suggested Reading

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