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Al Borg Medical Laboratories 5th Newsletter

DIAGNOSING A COMPLEX CONDITION

Antiphospholipid syndrome (APS) is an autoimmune disease with serious consequence including venous/arterial thrombosis, and pregnancy complications such as recurrent fetal loss and severe preeclampsia. Sera taken from patients with APS often contain antibodies to cardiolipin, an acidic phospholipid - hence its original name “antiphospholipid syndrome”. To this day, APS remains a complex illness that is inherently difficult to diagnose.

APS is a major women’s health issue, as 75–90% affected are women. It is estimated that APS is the cause of 15–20% of all thrombotic events, 10–25% of recurrent miscarriages, and one-third of the strokes in patients under the age of 50. The syndrome is also thought to be present in 40–50% of patients with systemic lupus erythematosus (SLE).

Pathogenesis of APS

Thrombosis has a key role in the clinical manifestations of APS. Several mechanisms have been proposed for APS-related thrombosis; however, it is most probably multifactorial in etiology. The thrombotic tendency may be caused by antiphospholid antibodies through the following mechanisms:

- Inhibition of the factors of the anticoagulant system, affecting thrombin formation and antithrombin activity.
- Impairment of fibrinolytic activity.
- Interference with coagulation factors and complement, particularly the intrinsic and protein C pathway.
- Direct effect of antiphospholipid antibodies on cell function, such as platelets, endothelial cells and vascular cells.

There is growing evidence that phospholipid antibodies are present in patients months to years before the onset of any clinical symptoms of APS. Many individuals with APS may never experience a thrombotic event or pregnancy loss. Secondary risk factors, co-morbidities, and other factors influence whether a patient actually experiences the clinical manifestations of APS. Thrombosis may be initiated by trigger events, such as surgery, pregnancy, or the use of oral contraceptives.Thrombotic events can affect arteries and veins of all sizes, including the microvascular system. The thrombosis can also be recurrent.The most frequent thrombotic events are deep-vein thrombosis (DVT) and ischemic stroke. An accelerated form of this syndrome that results in multiple organ failure is a very rare variant.

Diagnosis

It was proposed that a patient must have at least one clinical criterion and one positive laboratory criterion to be diagnosed with APS.

Clinical criteria

- Vascular thrombosis:
  - At least one confirmed clinical episode in an arterial, venous, or small vessel in any tissue or organ that has been confirmed.
- Pregnancy morbidity:
  - One or more unexplained deaths of a normal fetus at or beyond the 10^{th} week of gestation.
  - Three or more unexplained consecutive spontaneous abortions before the 10thweek of gestation.
  - One or more premature births of a normal neonate delivered prematurely before the 34thweek of gestation due to severe pre-eclampsia, eclampsia, or severe placental insufficiency (where other causes are excluded).

Laboratory criteria

- Positive results on two or more occasions at least 12 weeks apart for at least one of the following tests:
  - Lupus anticoagulant (LA);
  - Cardiolipin antibodies (aCL) of IgG and/or IgM isotype in medium or high titre, > 40 units;
  - ß2-glycoprotein I (ß2GPI) antibodies of IgG and/or IgM isotype.
- The elapsed time of 12 weeks between the initial and confirmatory test is to increase the probability of excluding temporary infection-associated antibodies.
- If there are fewer than 12 weeks, or more than 5 years between a positive APS test and the clinical manifestations, the classification criteria should not be used.

Although the pathophysiology of APS is now relatively well understood, difficulties still persist with making a definitive diagnosis based solely on clinical criteria. The prevalence of the clinical symptoms is high and the differentials for vascular thrombosis and pregnancy morbidity are relatively broad. Laboratory testing can therefore be very helpful in providing definitive diagnosis.

APS laboratory testing

Laboratory testing for APS includes both functional coagulation assays and immunology testing.

1. Lupus anticoagulant (LAC) testing a functional assay detecting the effect of phospholipid antibodies on the coagulation cascade. Phospholipid antibody tests are a direct measure of the presence or absence of the antibodies themselves.
2. There are two main classes of antiphospholipid antibodies assays available

- Cardiolipin antibodies detect antibodies binding to ß2-glycoprotein within context of a protein/phospholipid complex,
- ß2-glycoprotein I antibodies detect antibodies binding to ß2-glycoprotein in absence of phospholipids.

1.Lupus anticoagulants (LAC)

The term “lupus anticoagulant” was first used in 1972 to describe an inhibitor directed against the coagulation cascade phospholipids.The name is a misnomer as most people testing positive for “lupus anticoagulant” (LAC) do not have SLE and it has a procoagulant effect in vivo, however the name persists despite attempts to modify.The LAC test detects various APA’s on the basis of their interference with the phospholipid dependent steps in the coagulation cascade. Testing for the presence of LAC is performed following strict guidelines set up by International Society of Thrombosis and Haemostasis (ISTH) which include the preparation of platelet-poor plasma, and utilizing screening, mixing and confirmation tests.The ISTH guidelines state that laboratories are to perform two different screening tests.They recommend to perform (a) the dilute Russell’s Viper Venom Time (dRVVT) and (b) the activated partialthromboplastin time (aPTT) using a reagent with low phospholipid content.If either test is positive, the results are to be confirmed using a bilayer or hexagonal-phase phospholipid based reagent.

Interpreting LAC test results

- It has been shown that LAC is more consistent with the clinical manifestations of APS than phospholipid antibodies.
- Testing should not be performed while patients are on anticoagulant therapy. Heparin and vitamin K antagonist treatments may impair the detection of the lupus anticoagulant.

2.Phospholipid antibodies

The challenge with the laboratory tests for phospholipid antibodies (APA) is that there is no diagnostic “gold standard”. The presence of APA is a necessary inclusion criterion to make the diagnosis, but is not diagnostic of APS. The antibodies may also be found in children with viral infection, other autoimmune disorders, patients with infections, malignancy and even in healthy individuals.

a.Cardiolipin (aCL) antibodies

The real limitation of the aCL assay is inter-assay variability. The results for different assays on the market particularly differ in the lower measuring range for the test.

Interpreting aCL antibodies test results

- Only medium and high levels, > 40 units of aCL antibodies (IgG or IgM) are included in the diagnostic criteria which improve the specificity.
- At lower concentrations, aCL-IgM tends to give false-positive results for APS, particularly in the presence of rheumatoid factor or cryoglobulins.

- aCL IgA is of little diagnostic value; it has more use in classifying patients into diagnostic subgroups for risk since IgA aCL may also be associated with thrombocytopenia, skin ulcers and vasculitis.

b.ß2-glycoprotein I (ß2GPI) antibodies

ß2GPI antibodies have been incorporated into the 2006 updated criteria and are considered to be the most clinically significant antibodies. The assay shows higher specificity than the aCL assay and can be the only positive test in 3–10% of the APS patients.

Interpreting ß2GPI antibodies test results

- High titres of ß2GPI antibodies are associated with a high risk of thrombosis.
- Interferences by cryoglobulins or rheumatoid factor may cause a false positive in interpretation of IgM ß2GPI antibodies.

Predicting future APS-associated events

Several studies have been done on the use of these laboratory tests in identifying patients at risk for thrombotic events or severe pregnancy complications:

- Positive antiphospholipid lab tests, in the absence of clinical criteria, should only be considered to be risk factors rather than diagnostic criteria for APS. Patients should be assessed for additional thromboembolic risk factors (e.g., smoking, obesity, hypertension) for risk reduction.
- Clinical studies have shown that testing for the antibody profile is more useful in identifying thrombotic risk than the result of any individual test.
- The presence of both aCL–IgG antibodies and ß2GPI antibodies is believed to identify patients at higher risk for APS.
- The presence of LAC has been observed more frequently in patients without clinical events, and may be false positive in the elderly.
- ß2GPI antibodies have been correlated with thrombosis, pre-eclampsia, and eclampsia as well as having a role in young women with ischemic stroke.

Tests to Order in Cases of Suspected APS

Patients presenting with clinical symptoms suggestive of APS should have the following tests ordered, particularly those without other common risk factors:

- Lupus anticoagulant
- Cardiolipin antibodies, IgG and/or IgM
- ß2GPI glycoprotein I antibodies, IgG and/or IgM

Results of these tests will be adjunctive to the clinical findings and should be not be considered diagnostic of, but rather as risk factors for thrombosis, pregnancy loss and clinical manifestations of APS.If positive, confirmatory tests should be repeated within 6 to 12 weeks.

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Test	Sample Collection	Specimen preparation	Storage/transport temprature	Perfomed (Run Days)	reported
Lupus Anticoagulant	Light blue-capped tube (Na citrate)	1 ml frozen platelet poor plasma in plain plastic Wassermann tube with no additives	Critical Frozen (separate tube	Saturdays & Tuesdays	Same day
Anti-Cardiolipin Antibodies (IgG & IgM)	Red-capped tube (Plain)	1 ml refrigerated serum	refrigerated		Same day
Anti-Beta 2 Glycoprotein 1 Antibodies (IgG & IgM)	Red-capped tube (Plain)	1 ml refrigerated serum	refrigerated		Same day