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Thrombosis and Antiphospholipid Antibodies

Introduction: The antiphospholipid syndrome (APS), also known as Hughes syndrome, is characterized by venous and arterial thrombosis, thrombocytopenia and recurrent miscarriages. The possibility of APS should be considered in the setting of a DVT or miscarriage in a pregnant woman. The condition may occur either secondarily in autoimmune disease (e.g. systemic lupus erythematosis) or as a primary event. In general, the clinical manifestations of APS are similar for primary and secondary forms of the disease. The diagnosis of APS requires specific clinical manifestations as well as the identification of specific antibodies in the serum. In 2002 the international consensus statement on preliminary criteria for the classification of the antiphospholipid syndrome proposed clinical and laboratory criteria (Table 1). Since that time several articles stress the importance of beta 2 glycoprotein 1 (B2GP1) antibodies in making the diagnosis.

Antiphospholipid (cardiolipin) antibodies: Antibodies to phospholipids (APL) are a heterogeneous group of autoantibodies directed against phospholipid-binding proteins. Some antiphospholipid antibodies will induce their target protein to absorb a small amount of phospholipid added to plasma during the prothrombin time (PT) and activated partial thromboplastin time (aPTT). These particular antibodies will prolong phospholipid dependent coagulation tests and are called lupus anticoagulants. Other phospholipid antibodies are directed against cardiolipin, a phospholipid component of mitochondria originally described in bovine cardiac cells. These antibodies are detected using microtiter plates with cardiolipin and beta 2 glycoprotein I as the combined substrate.[5]

It is important to note that a normal activated partial-thromboplastin (aPTT) time does not exclude the

Table 1

Clinical criteria*1

Vascular thrombosis

- One or more clinical episodes of arterial, venous or small-vessel thrombosis, occurring within a 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.

*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. No limits are placed on the interval between the clinical event and the positive laboratory findings.[1]

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presence of lupus anticoagulant antibodies (Table 2). A patient suspected of APS with a thrombotic event should be screened for anticardiolipin antibodies and with other assays that are sensitive to lupus anticoagulant antibodies. The diagnosis may be unsuspected in patients where the antiphospholipid syndrome results in a more indolent chronic process, leading to ischemic injury with slow, progressive loss of organ function.

Approximately five to 10 percent of the general population will have detectable APL in their blood. Only a minority will have persistent antibodies and have thrombotic or adverse pregnancy events. Several studies have investigated whether certain types of phospholipid antibodies are better predictors of venous thrombosis. Patients who test positive for APL on one occasion need to have a positive test on at least one more occasion,[6] weeks or more apart, before considering the diagnosis of APS. Antibody levels change over time and it is unclear if a transient elevation after an initial thrombotic event has the same risk for recurrence as a patient with a persistent antibody.[5]

Pathogenesis: Although there are several theories about the mechanism by which APL produce thrombosis, one stands out as being the most reasonable. APL modulate the proteins involved in the regulation of coagulation. Beta 2 glycoprotein I is thought to act as a natural anticoagulant and is bound to the endothelial surface of vessels. When antibodies to beta 2 glycoprotein I bind to antigen-endothelial cell complexes, there is loss of the natural anticoagulant properties of beta 2 glycoprotein I. Activation of the coagulation cascade is uninhibited and results in thrombosis.

Finally, thrombosis in the APS has similarities to heparin-induced thrombocytopenia. Both syndromes induce thrombosis in multiple arterial and venous beds and are associated with antibodies. In heparin-induced thrombocytopenia, the site of thrombosis is often determined by prior cardiovascular disease, whereas in the APS, there is a high rate of recurrence of similar thrombotic events. A "second hit" such as a vascular injury may be necessary for thrombosis to occur in both syndromes.

Laboratory tests to order: To support the clinical impression of antiphospholipid syndrome three laboratory tests are helpful:

1. Lupus anticoagulant antibodies is a test done at Rex Hospital Laboratory that utilizes a modification of the dilute Russell's Viper Venom Time test (dRVVT). This method is widely accepted as the method of choice to detect both IgM and IgG lupus anticoagulants. It is more sensitive than the APTT and has a low false negative rate. The test combines a screening and confirmatory step. Although lupus anticoagulants are frequently incidentally discovered by a prolonged aPTT, the aPTT does not have to be prolonged in patients with a lupus anticoagulant. The test is affected by anticoagulation therapy. A valid test requires the patient be off of anticoagulation for six weeks prior to testing.
2. Phospholipid (Cardiolipin) antibodies, IgG and IgM.
3. beta 2 glycoprotein I

The latter two tests are both performed at Mayo Medical Laboratories, and are not affected by anticoagulation therapy. They are more specific than the lupus

Table 2.
Classification And Detection Of Antiphospholipid Antibodies 1

Antibody	Method Of Detection
Lupus anticoagulant Antibodies	The first step is prolongation of the aPTT assay with the use of platelet-poor plasma. The second step is a failure to correct the prolonged aPTT by mixing the patient's plasma with normal plasma. The third step is confirmation of the presence of lupus anticoagulant antibodies by shortening or correction of the aPTT after the addition of excess phospholipid 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 inhibitor is suspected.
Antiphospholipid antibodies	Solid-phase immunoassay (usually enzyme-linked immunosorbent assay) is performed on cardiolipin-coated plates, usually in the presence of bovine serum beta 2-glycoprotein I. Anticardiolipin antibodies from patients with APS are beta 2-glycoprotein I–dependent; antibodies from patients with infectious diseases are beta 2-glycoprotein I–independent.
Anti–beta 2 glycoprotein I antibodies	Solid-phase immunoassay (usually enzyme-linked immunosorbent assay) is performed on human beta 2-glycoprotein I–coated plates (usually gamma-irradiated polystyrene). Anti–beta 2-glycoprotein I antibody assays detect antibodies to human beta 2-glycoprotein 1, rather than bovine beta 2-glycoprotein I (as in anticardiolipin antibody assays).

anticoagulant but less sensitive. A negative test for beta 2 glycoprotein I does not rule out antiphospholipid syndrome.

Recently, Galli confirmed that antibodies directed against beta 2 glycoprotein I were associated with recurrent thrombosis and pregnancy loss.[4] APL alone are not a risk factor for thrombosis and are not associated with thrombi unless a lupus anticoagulant is also present. Antibodies directed against beta 2 glycoprotein are more specific in making the diagnosis of antiphospholipid syndrome. In a patient with a DVT and no laboratory abnormalities or symptoms consistent with APS other than anticardiolipin antibodies, there is little support to treat the patient any differently from a patient with just a DVT.

Monitoring anticoagulant therapy in antiphospholipid syndrome: Many patients with APS have a prolonged aPTT because of a lupus anticoagulant. This can complicate monitoring unfractionated heparin therapy. In these patients, an anti-factor Xa assay can be used instead of the aPTT. Low molecular weight heparin can also be substituted for unfractionated heparin and monitored if necessary with the anti-Xa assay. Also, a small number of APS patients appear to have a lupus anticoagulant that affects the prothrombin time (PT). This could potentially complicate monitoring coumadin therapy. Mixing studies may help determine the cause of the prolonged PT when done prior to starting oral anticoagulation.[2]

Catastrophic antiphospholipid syndrome: In most patients with APS, thrombotic events occur singly. Recurrences may occur months or years after the initial event. However, a minority of patients present with generalized arterial and venous thrombosis with a mortality rate of 50%. This is termed catastrophic antiphospholipid syndrome. The disease can mimic thrombotic thrombocytopenia purpura (TTP). Precipitating causes include infections, surgical procedures, withdrawn of anticoagulant therapy and use of drugs such as oral contraceptives. An aggressive therapeutic approach usual includes heparin, IV IgG, steroids, plasmapheresis and possible chemotherapy.[2]

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References:
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4. *Galli M, Borrelli G., Jacobsen E.M., et al. Clinical significance of different antiphospholipid antibodies in the WAPS (warfarin in the antiphospholipid syndrome) study. Blood. 2007;110:1178-1183.*
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Semen Analysis Changes

Beginning in April, *semen specimens for infertility* evaluation will be forwarded to Mayo Medical Laboratories (MML) for analysis. The change is prompted by a combination of changes in instrumentation and difficulty maintaining interpretive proficiency in a test which is ordered infrequently. MML will provide a complete chemical and morphologic (WHO morphology score) analysis. In order for adequate evaluation, the ejaculate specimen must be received by MML within 24 hours of collection. This means that specimens must be collected and delivered to Rex Laboratory only between the hours of 1000 – 1300, Monday through Thursday. (Specimens should NOT be collected prior to 1000 and may be collected on the premises.) The total ejaculate specimen should be kept at ambient temperature. For accurate results, patients should have two to seven days of sexual abstinence prior to collection. MML requires documentation of the number of days of sexual abstinence at the time of specimen submission.

The above comments do not apply to *postvasectomy semen specimens* obtained to evaluate the success of the surgical procedure. Those specimens will continue to be evaluated on site. Postvasectomy specimens should be delivered to the Laboratory between 0700- 1200, Monday – Friday, exclusive of holidays.

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D-Xylose Test Discontinued

Effective immediately, the d-xylose test will no longer be offered. The rationale for this test has declined over the years with the advent of more sophisticated tests, including endoscopy. [1-3] As such we have witnessed a profound decline in the number of orders for this test. The vendor for the d-xylose will no longer sell the product to us in small enough lot sizes to match our test volume, thus we have elected to discontinue the test.

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References

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2. *Schwarz R.P. & Benson J.D. Gut Check #9 – Breath testing for GI disease. Rex Healthcare Laboratory Bulletin. Issue 134. January 2008.*
3. *Schwarz R.P. Personal communication. 2/22/08.*

Throat Swabs Revisited

Throat swabs received in the Rex Laboratory with a request for Strep Screen Culture or a written request for "throat culture" are both processed in the same manner. The swab is plated on a blood agar plate and incubated. The microbiology technologists examine the plates at 24 hours (and again at 36- 48 hours, if negative at 24) looking for beta-hemolytic colonies. Immunologic grouping tests are performed and results are reported as follows:

> "Streptococcus pyogenes (Group A) isolated. Group A remains universally susceptible to Penicillin." or…
>
> "Beta-hemolytic streptococci, Group C isolated." or…
>
> "Beta-hemolytic streptococcus not Group A or Group C"… or "No beta-hemolytic streptococci isolated"

The only cause of typical pharyngitis necessary to treat is Group A streptococcus (GAS) (Streptococcus pyogenes). Treatment with antibiotics alleviates the pharyngeal symptoms and prevents serious sequelae including acute glomerulonephritis and acute rheumatic fever. Because of the singular importance of GAS, the most common test for a throat swab is the Group A strep direct antigen test. Physicians caring for children may occasionally follow up negative antigen tests for GAS with culture, but in a recent observational study at Rex, culture in addition to rapid antigen testing did not significantly increase the sensitivity.[1]

Identification of other bacteria from the pharynx does not distinguish between infection and carrier state. Furthermore few microorganisms, other than GAS, require antimicrobial therapy. Because of this, culture of throat swabs is limited to blood agar plates looking for beta hemolytic streptococci. Other organisms, including Streptococcus pneumonia, Staphylococcus species, Haemophilus influenzae, and Moraxella catarrhalis are not sought or reported. Reporting these organisms can lead to inappropriate therapy and an increase in antibiotic resistance, both potentially harmful to the patient and the public. When another specific pathogen is sought, based on the clinical history and findings, the appropriate specific test should be ordered (not "throat culture"). These include specific tests for Bordetella pertussis, Corynebacterium diphtheriae, Neisseria gonorrheae, or viruses. Occasionally a clinician may be interested in a patient's carrier status of some pathogens. Nasopharyngeal swabs can be done with requests for evaluation of Staphylococcus aureus or Neisseria meningitidis. Lastly, throat swabs in patients with cystic fibrosis require more extensive work up and this history should be conveyed on the requisition or with a phone call.

To summarize, there is no specific "throat culture" order at Rex (this began in 1999[2]). When a culture is requested from swabs from the throat or if throat culture is written in on an order sheet, a Strep Screen Culture will be performed. This is the recommendation of the American Society of Microbiology[3] and the routine practice at the University of North Carolina at Chapel Hill.[4] If a physician would like more information on a specific culture please contact the author at (919) 784-3056 or microbiology at (919)784-6000.

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References

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2. *Kleeman, K.T. Respiratory Infection- Diagnosing Upper. Rex Laboratory Bulletin. July 1999, Issue Number 39, p. 2-3.*
3. *Isenberg, H.D. Clinical Microbiology Procedures Handbook. ASM Press. 2004, Section 3.11.1.*
4. *Gilligan, P. Personal Communication. March 2008.*

Blood Bank Armbands Discontinued

Effective March 5, 2008, specific Blood Bank armbands are no longer required for blood transfusion. This applies for both inpatients and outpatients. For inpatients, the regular patient identification bracelet will fulfill the same function as the blood bank armband did previously. Patients having blood collected during a preadmission process must keep their hospital identification armband on from the time the blood bank specimen is collected until the need for transfusion has passed. If the armband is removed, a new blood bank specimen will be required prior to transfusion.

For Outreach specimens collected in physician offices, all patients will be identified solely by their formal full name (first, middle/middle initial, & last) as it appears on their driver's license and date of birth. (Some patients "go by" names other than their formal name. If the name on the specimen does not match the name on the patient's identification bracelet applied during hospital registration, a blood bank specimen will have to be collected prior to transfusion. When filling out the laboratory requisition, please use the patient's full name (first, middle/middle initial, & last), sex, date of birth, collection date, collection time, & collector's initials. Write the patient's full name as it appears on the laboratory requisition, along with the date of birth on the crack and peel labels and place the labels on the blood collection tube/s. Failure to comply with the above requirements will necessitate re-collection.

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