For Diagnostic Use

A patient is considered to have APS if at least one clinical and one laboratory criterion is met. There has been disagreement over whether the criteria are intended for antiphospholipid and anti-prothrombin antibodies, the importance of IgA isotypes and the significance of low levels of anti-B2GPI antibodies. This will be a topic for discussion during the SSEC committee meeting at the International Society on Thrombosis and Hemostasis Congress in Sydney, Australia (August 2005).

Part II of “Anti-Phospholipid Testing 101” will continue in a subsequent issue of THE READER with a review of standardization and performance issues associated with different available methods.

References:

UPCOMING CONFERENCES (cont.)

● The Mayo Clinic and the North American Specialized Coagulation Laboratory Association (NASCOLA) are jointly sponsoring a new conference, “Coagulation Testing Quality: Lessons and Issues from Quality Assessment, Accreditation, and Improvement Programs & Studies.” June 15-17, 2005 in Rochester, MN. Corgenix invites you to stop by our display at the meeting to learn about our Coagulation products. For more information, call 800-533-1710 or visit the Mayo Clinic Website: (www.mayoreferenceservices.org/mmt).

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ACL assays also detect antibodies that bind directly to cardiolipin (non-B2GPI dependent). These antibodies are typically not associated with thrombosis, and may be due to infections or other conditions. With the identification of B2GPI and its association with phospholipid binding, researchers have developed ELISA methods to detect anti-B2GPI antibodies directly, in the absence of cardiolipin. Anti-B2GPI assays have been shown to be more specific for autoantibodies related to thrombosis. However, since more patients test positive for aCL antibodies than for anti-B2GPI antibodies (including some with a history of thrombosis), some investigators consider the aCL assay to be more sensitive than anti-B2GPI testing.

B2GPI is a serum protein found in circulation and associated with several lipids such as chylomicrons, LDL and HDL-cholesterol. For this reason, it is also known as apolipoprotein H. B2GPI is a single chain polypeptide of 326 amino acids with a molecular weight of approximately 50KD, which binds negatively-charged phospholipids and various macromolecular structures including DNA, heparin and platelet membranes. The molecule is made up of five distinctive domains (Figure 2). Domain V binds to the phospholipid surface. When bound to a phospholipid, a tertiary structural change may occur, creating a neoepitope(s) that is recognized by autoantibodies. Many researchers believe there is a very well defined amino acid sequence in domain I where most, if not all, anti-B2GPI antibodies bind. Other studies (see December 2004 READER) demonstrated that though most patients react similarly with different methods, some patients do not. This supports the notion of a heterogeneous group of antibodies that recognize different binding epitopes, possibly in different domains of B2GPI. Whether antibodies to different domain locations have different clinical importance remains to be demonstrated. In vivo, B2GPI acts both as a procoagulant when activated by phospholipid binding (domain V cleavage), down-regulating intrinsic fibrinolysis, and as an anticoagulant, binding factor XI and inhibiting its activation (XI → Xla). Anti-B2GPI antibodies enhance the binding of B2GPI to negatively charged phospholipids on cell membranes, promoting the cleavage of B2GPI, amplifying the procoagulant effect.

There are many other phospholipids and phospholipid binding proteins besides cardiolipin and B2GPI (Table 1, page 3). Though cardiolipin was the first phospholipid identified in association with autoantibodies, it is known that cardiolipin is not contained in the platelet membrane, nor is it involved in the coagulation cascade. The platelet membrane is actually a combination of phosphatidycholine (50-60%), phosphatidylethanolamine (20-30%), phosphatidylserine (10-15%) and phosphatidylinositol (<5%). It has been demonstrated that phosphatidylserine is the phospholipid that binds B2GPI in vivo. Phosphatidylserine is unique because of its direct involvement in the coagulation cascade, but also because it is the only phospholipid located exclusively in the interior of the bilayer membrane in a resting platelet. Phosphatidylserine is externalized by way of a flip-flop mechanism during cell activation (injury). Cardiolipin is found in the membrane of the mitochondria. The molecular structure of cardiolipin is very similar to phosphatidylserine and both have demonstrated the ability to bind B2GPI in solid-phase assays. Because of these similarities, comparisons of antibody detection of ELISA methods that utilize cardiolipin versus phosphatidylserine result in approximately 90% agreement. However, the anti-phosphatidylserine method actually correlates better than aCL testing for the presence of anti-B2GPI antibodies, resulting in a higher positive predictive value.6

The presence of aCL antibodies was initially associated with SLE and lupus like disorders, but a number of patients with SLE lacking other disease specific markers were also diagnosed with APS. Many researchers believe there is a very well defined amino acid sequence in domain I where most, if not all, anti-B2GPI antibodies bind. Other studies (see December 2004 READER) demonstrated that though most patients react similarly with different methods, some patients do not. This supports the notion of a heterogeneous group of antibodies that recognize different binding epitopes, possibly in different domains of B2GPI. Whether antibodies to different domain locations have different clinical importance remains to be demonstrated. In vivo, B2GPI acts both as a procoagulant when activated by phospholipid binding (domain V cleavage), down-regulating intrinsic fibrinolysis, and as an anticoagulant, binding factor XI and inhibiting its activation (XI → Xla). Anti-B2GPI antibodies enhance the binding of B2GPI to negatively charged phospholipids on cell membranes, promoting the cleavage of B2GPI, amplifying the procoagulant effect.

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The presence of human source B2GPI in aCL test systems rather than bovine has been the topic of discussion. Bovine B2GPI has about 85% identical amino acid sequence with human B2GPI and binds similarly to aCL antibodies in ELISA methods. Human B2GPI is always present in aCL test methods from the patient sample itself. Endogenous B2GPI can bind to the cardiolipin surface during the reaction step and therefore bind autoantibodies. The advantage of using purified human B2GPI on the coated cardiolipin surface may result in better consistency and control of its quantity (density), without introducing other bovine antigens to the test system. Accordingly, many aCL methods now use human B2GPI in the plate coating process.

The preliminary classification criteria for definite APS (Table 2) are based on the presence of aCL antibodies associated with vascular thrombosis and/or pregnancy morbidity. The overall prevalence of APS in the general population is estimated to be 1 in 1,000 women. APS has been documented in women of all ages, but most cases occur in women of childbearing age. The prevalence of APS is higher in women with SLE and antiphospholipid antibodies. The incidence of APS is higher in women with SLE and antiphospholipid antibodies. The incidence of APS is highest in women with SLE and antiphospholipid antibodies. Antiphospholipid antibodies are associated with increased risk of venous and arterial thrombosis, pregnancy morbidity, and recurrent miscarriage. The recommended treatment for APS is anticoagulation with warfarin or alternative anticoagulants, such as direct thrombin inhibitors or factor Xa inhibitors. Other treatments may include immunosuppressive agents, such as low-dose steroids, or intravenous immunoglobulin. Prevention strategies for APS include avoiding prothrombotic risk factors, such as smoking and obesity. The presence of aCL antibodies was initially associated with SLE and lupus like disorders, but a number of patients with SLE lacking other disease specific markers were also diagnosed with APS. Many researchers believe there is a very well defined amino acid sequence in domain I where most, if not all, anti-B2GPI antibodies bind. Other studies (see December 2004 READER) demonstrated that though most patients react similarly with different methods, some patients do not. This supports the notion of a heterogeneous group of antibodies that recognize different binding epitopes, possibly in different domains of B2GPI. Whether antibodies to different domain locations have different clinical importance remains to be demonstrated. In vivo, B2GPI acts both as a procoagulant when activated by phospholipid binding (domain V cleavage), down-regulating intrinsic fibrinolysis, and as an anticoagulant, binding factor XI and inhibiting its activation (XI → Xla). Anti-B2GPI antibodies enhance the binding of B2GPI to negatively charged phospholipids on cell membranes, promoting the cleavage of B2GPI, amplifying the procoagulant effect.