



THE SIMULTANEOUS DETERMINATION OF ANTI-CARDIOLIPIN, ANTI-PHOSPHATIDYLSERINE AND ANTI-BETA 2 GLYCOPROTEIN I ANTIBODIES: CORRELATION WITH THE ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid antibodies are a heterogeneous group of autoantibodies associated with the clinical manifestations of the antiphospholipid syndrome (APS). One factor contributing to the heterogeneity of these antibodies is their immunologic reactivity to various anionic phospholipids (cardiolipin, phosphatidylserine, etc.) or to phospholipid-protein (cofactor) complexes. The most commonly studied serum protein cofactors are Beta 2 Glycoprotein I (B2GPI) and prothrombin. The presence of antibodies to B2GPI has been regarded as more specific for thrombosis than anti-cardiolipin (aCL) or anti-phosphatidylserine (aPS) antibodies, and ELISA kits for anti-B2GPI antibodies are now being incorporated into test menus in clinical laboratories. A practical question arising from many laboratories is: how many tests are required to properly diagnose APS or assess the risk for thrombosis? Taking into consideration the heterogeneous nature of these antibodies, one can assume that a single test may not be sufficient, but how many are necessary for a cost efficient evaluation?

In an attempt to answer these questions, Corgenix tested 114 serum samples from various patient populations simultaneously for aCL, aPS and anti-B2GPI antibodies. Serum from the following populations were included in the study: 1) Forty-nine serum samples from patients with various autoimmune diseases including SLE and antiphospholipid syndrome, and suspected of having positive reactivity to antiphospholipid antibodies; 2) Twenty-eight from consecutive (unselected) SLE patients; 3) Sixteen from SLE patients selected for the absence of any clinical manifestation of the APS and used as disease controls; 4) Twelve from selected SLE patients with history of thrombosis and miscarriages (secondary APS); and 5) Nine from primary APS patients. The results are summarized in the table in the next column.

Patient Population	n	% with 3 positive*	% with 2 positive*	% with 1 positive*
1) Autoimmune	49	22	45	14
2) Unselected SLE	28	14	14	11
3) Control SLE	16	0	33	50
4) Secondary APS (SLE)	12	58	0	17
5) Primary APS	9	56	44	0

* when tested for aCL, aPS and anti-B2GPI

In the autoimmune and unselected SLE groups, 22% and 14% of the samples were positive on 3 assays while the remaining samples were either negative or positive on 1 or 2 assays. Of the samples that were positive for anti-B2GPI, most (98%) were also positive for at least one of the other 2 assays. These results clearly show that some patients present 3 antibodies while other patients may present only 1 or any combination of 2 antibodies. To further evaluate the significance of having 3 versus 2 or 1 antibodies, serum samples from secondary APS (SLE) and primary APS patients were tested. In the secondary APS (SLE) group, 58% of the samples had 3 antibodies while only 2 patients (17%) had 1 antibody. In the primary APS group, 56% of the samples showed 3 and the remainder (44%) 2 antibodies. In the SLE control population, none of the patients had all 3 antibodies present. In healthy controls (data not shown), occasional weak positive reactions to 1 or 2 but not to all 3 antibodies were seen. These results suggest that most patients with primary or secondary APS frequently present with 3 antiphospholipid antibodies. However, some APS patients (17%-44%) presented 1 or 2 antibodies in different combinations. Hence, the diagnosis of a significant number of patients could be missed by performing only one assay; in addition, positive results in at least 2 assays may enhance the diagnosis of APS. (cont. on p. 2)

THE READER RESPONSE

Q. We frequently see very high anti-B2GPI levels in patients with thrombosis. Since we report anti-B2GPI results in G, M, or A units, can you suggest interpretive ranges for the REAADS assays which correlate with clinical significance?

A. We recently completed a major clinical study to identify appropriate interpretive ranges for REAADS IgG, IgM, and IgA anti-B2GPI assays. The results of our testing and our recommendations are summarized in the special **Technical Update Supplement** included with this edition of **THE READER**.

(cont. from p. 1)

Since anti-B2GPI antibodies have been reported to show a higher correlation with thrombosis than classic aCL or aPS antibodies, testing directly for anti-B2GPI antibodies may provide information which is more clinically relevant. However, due to the fact that some patients may present with only one, or with any combination of aCL, aPS and anti-B2GPI antibodies, it is recommended that classic assays be included along with the more specific anti-B2GPI assay to support the diagnosis of APS.

A clinical case which supports this proposal was recently identified. A young woman with a clinical history suggestive of primary APS had a previous diagnosis of questionable SLE, two documented episodes of cerebro-vascular accidents, skin ulcers and livedo reticularis. Her initial antiphospholipid antibody studies showed positive Lupus Anticoagulant, negative aCL, strong positive aPS and anti-B2GPI tests. On follow-up testing over several months, the aCL test became positive while aPS and anti-B2GPI remained positive. This case illustrates that additional testing beyond the classic aCL was needed to confirm the initial serologic diagnosis of APS in this patient. In addition, the aCL sero-conversion seen in this patient also suggests that different antibodies may be present at various stages of the disease. Additional follow-up studies on larger patient populations will be required to confirm the frequency of these findings. This information will be helpful in the development of better and more cost effective diagnostic testing for APS.

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REAADER PRODUCT FEATURE

REAADS Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kit

For *In Vitro* Diagnostic Use

Assay format -	96-well microtiter plate (8 x 12 strips) with breakaway wells
Antigen substrate -	Purified Beta 2 Glycoprotein I (human)
Conjugate -	Horseradish peroxidase (HRP) goat anti-human IgG, IgM, or IgA
Chromogenic substrate -	TMB (single component)
Stopping solution -	0.36 N Sulfuric acid
Sample -	Human serum, 1:50 dilution
Incubations	
Sample -	15 min @ room temperature
Conjugate -	15 min @ room temperature
Substrate -	10 min @ room temperature
Wavelength -	450 nm
Clinical specificity -	IgG 100%; IgM 93%; IgA 95%
Clinical sensitivity -	autoimmune population: IgG 28%; IgM 23%; IgA 27%
Product number -	037-001 IgG anti-B2GPI 038-001 IgM anti-B2GPI 039-001 IgA anti-B2GPI

REAADER ANNOUNCEMENTS

• **Corgenix** received FDA Clearance on four new products during the final quarter of 1998. All three of the REAADS anti-Beta 2 Glycoprotein I Test Kits, IgG anti-B2GPI, IgM anti-B2GPI, and IgA anti-B2GPI, are now available for *In Vitro* Diagnostic use. All three B2GPI isotypes are packaged separately in individual kits to allow laboratories the option of choosing which isotypes they wish to include in their anti-B2GPI testing. In addition, the REAADS Monoclonal Free Protein S Kit, our second generation assay for the measurement of free Protein S in human plasma, was also cleared for marketing. With the new kit, Free Protein S is measured directly, avoiding the time consuming and non-specific PEG precipitation step. Please call **Corgenix** Customer Service for additional information or to evaluate any of these new assays.

• Of particular interest to our customers with automated ELISA analyzers or who wish to automate REAADS aCL assays, **Corgenix** is now including additional wash buffer concentrate and larger volumes of kit sera in the REAADS 288 well aCL IgG/IgM Test Kit. We now offer both REAADS aCL IgA Kit and REAADS aCL IgG/IgM Kit in a 3-plate format with reagent volumes to accommodate automated testing.

