

Comparative Sensitivity and Agreement of 4 Antiphospholipid Antibody Tests on Healthy and Diseased Populations. Dier K, Taylor D, Whittier A, Olsen L, Fink C, Lopez L Corgenix, Inc. Westminster, Colorado

ABSTRACT

Due to the heterogeneity of antiphospholipid antibodies, several assays have been developed to determine different antibodies and introduced to the clinical laboratory. One assay may not be sufficient since patients may present with different antibodies in diverse combinations. The clinical performance (relative sensitivity and agreement) of 4 antiphospholipid assays was evaluated on serum samples from healthy controls (n=40) and diseased states (40 autoimmune, 36 SLE and 11 antiphospholipid syndrome [APS] patients). In addition, plasma samples from healthy controls (n=100) and 2 groups of 81 Lupus Anticoagulant (LA) positive samples and 56 LA controls with abnormal coagulation times but negative for accepted criteria for LA, were evaluated. All serum and plasma samples were tested on commercial ELISA kits (REAAADS) following the manufacturer's instructions (IgG anti-cardiolipin [aCL], anti-phosphatidylserine [aPS], anti-B2GPI and anti-prothrombin [aPT]). To determine the relative sensitivity and agreement (2x2 analysis) of each assay, the results were compared against the antiphospholipid (APL) status of each sample. A sample was classified as APL positive if at least 2 antiphospholipid antibodies (aCL or aPS and strong anti-B2GPI) were present. The prevalence of each antibody and APL status varied between populations. (Table). The results of IgG anti-B2GPI assay in all diseased groups studied (100% relative sensitivity) when APL status was positive, were somewhat biased due to the prominent role of this assay in the APL status criteria. However, the second best overall relative sensitivity (mean 84.5%) was the IgG aPS assay over the most commonly used assay, IgG aCL (mean 77.3%). Similarly, the best % agreement of positive and negative results when compared to APL status was observed with the IgG anti-B2GPI assay (mean 95.8%), followed by the IgG aPS assay (mean 9.4%) and IgG aCL (mean 73.4%). This information should be considered by clinical laboratories when selecting assays to evaluate antiphospholipid antibodies and when interpreting results.

INTRODUCTION

Due to the heterogeneous nature of antiphospholipid antibodies, patients with antiphospholipid syndrome (APS) may present one or several antibodies, and several assays may be required to determine their presence. Antibodies to the phospholipids cardiolipin and phosphatidylserine may be present in patients with diverse medical conditions, including infectious diseases, and are not necessarily associated with thrombosis. Antibodies to the cofactors B2GPI or prothrombin are almost exclusively seen in APS and associated with thrombosis. In addition, patients with APS frequently present with various antiphospholipid and anti-cofactor antibodies in different combinations. Healthy individuals or patients with infectious diseases usually present with one (or two) antiphospholipid antibodies. One assay may not be sufficient to properly assess the presence of antiphospholipid antibodies in the laboratory.

The aCL and aPS assays may be considered better screening methods because of the lower specificity. A positive reaction with high titer on a more specific test like anti-B2GPI or antiprothrombin would be considered indicative of APS.

In a similar study (ISTH abstract, Paris, FRANCE, July 2001), aPS and anti-B2GPI antibodies demonstrated the best relative sensitivity and agreement when compared to clinical criteria (Thrombosis). This study evaluates the performance (relative sensitivity and agreement) of four antiphospholipid assays compared to serologic criteria on serum and plasma samples from healthy and disease state individuals.

OBJECTIVE

To assist the clinical laboratory in becoming familiar with the performance of aCL, aPS, anti-B2GPI and aPT antibody ELISA tests, we studied the relative sensitivity and agreement (2 x 2 analysis) of each assay against a serologic criteria (APL status) of a large population of healthy and disease state samples.

MATERIALS AND METHODS

Serum Specimens:

- 40 healthy blood bank controls
- 40 various autoimmune patients
- 36 unselected SLE patients
- 11 primary antiphospholipid syndrome (APS) patients

Plasma Specimens (3.2% sodium citrate):

- 100 healthy blood bank controls
- 56 abnormal coagulation control patients (negative for LA)
- 81 lupus anticoagulant positive patients (group #1 n=20)(group #2 n=61)

The abnormal coagulation controls and LA positive samples were obtained from two separate institutions. The classification of LA activity was determined by each institution using established criteria (Brandt et al 1995).

Antiphospholipid Antibody Testing

All serum and plasma samples were tested on four IgG antiphospholipid antibody assays described below. The positive and negative results of each sample was used to determine their prevalence and antiphospholipid (APL) status.

anti-Cardiolipin ELISA: Purified bovine cardiolipin was coated onto 96-microwell plates, blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and absorbance read at 450/650 nm.

anti-Phosphatidylserine ELISA: Purified bovine phosphatidylserine was coated onto 96-microwell plates blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine B2GP was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG was added for another 15 minute incubation followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and absorbance read at 450/650 nm.

anti-Beta 2 Glycoprotein I ELISA: Purified human B2GPI (purity > 95% SDS-PAGE) was coated onto 96 well micro-plates, blocked, and stabilized in the absence of exogenous B2GPI. 100uL of diluted patient serum (1:50) in sample diluent containing no B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and optical density read at 450/650 nm.

Anti-Prothrombin ELISA: Purified human prothrombin was coated onto 96 well micro-plates, blocked and stabilized. 100uL of diluted patient serum (1:51) in sample diluent was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and optical density read at 450/650 nm.

Serologic Criteria for APL Status

Based on positive or negative results from IgG antiphospholipid antibody testing, each sample was classified for "antiphospholipid (APL) status" (positive or negative). The criteria used to classify the APL status was:

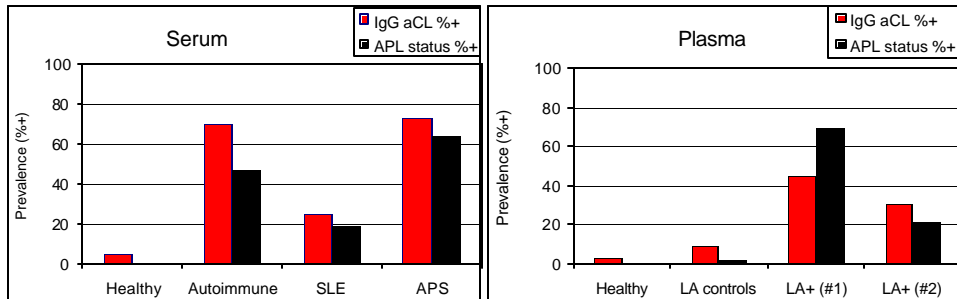
1. Negative APL status if aCL, aPS and anti-B2GPI tests are negative.
2. Negative APL status if aCL and/or aPS tests are positive but anti-B2GPI is negative.
3. Positive APL status if aCL and/or aPS and anti-B2GPI tests are positive.
4. Positive APL status if aCL and aPS tests are negative but anti-B2GPI is moderate to strong positive (>50 G units).

RESULTS

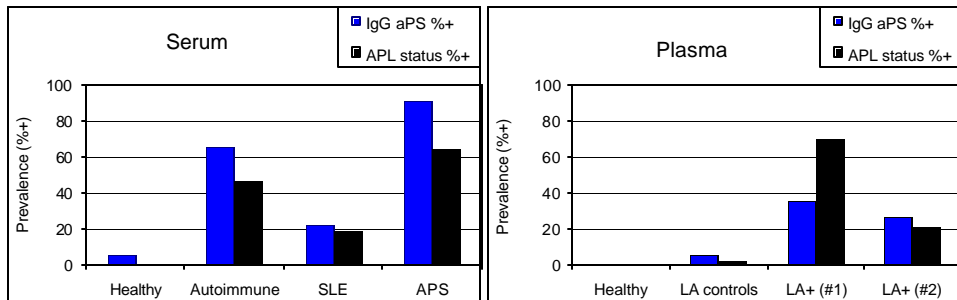
A summary of the relative sensitivity (RS) and % agreement (2 x 2 analysis) of each assay against the APL status of diseased samples is shown below:

Test Sample	IgG aCL		IgG aPS		IgG anti-B2GPI		IgG aPT		APL Status % +
	RS %	% ag	RS %	% ag	RS %	% ag	RS %	% ag	
Autoimmune	95	72	95	77	100	92	53	72	47
SLE	71	83	86	91	100	94	14	69	19
APS	71	54	100	72	100	100	28	54	64
LA+ (grp 1)	64	75	50	65	100	100	57	65	70
LA+ (grp 2)	84	83	92	92	100	93	33	77	20
Mean	77	73	85	79	100	96	37	67	

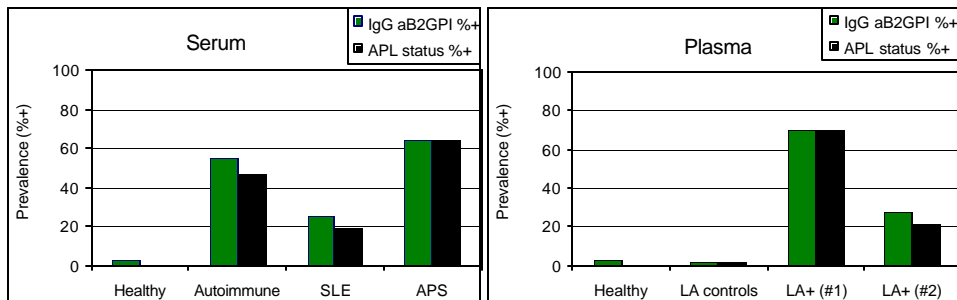
Graph #1: IgG AntiCardiolipin (aCL) Antibodies



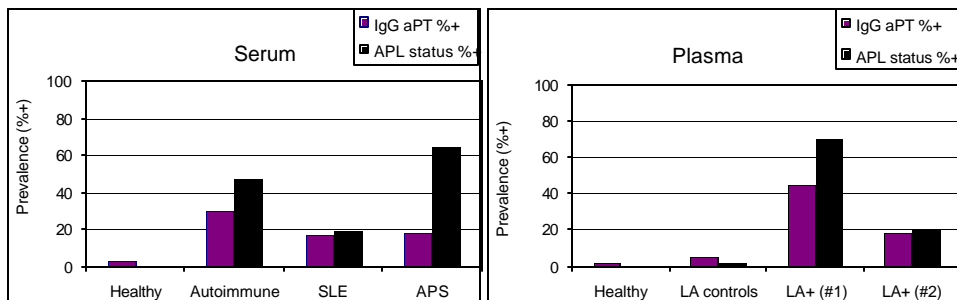
Graph #2: IgG AntiPhosphatidylserine (aPS) Antibodies



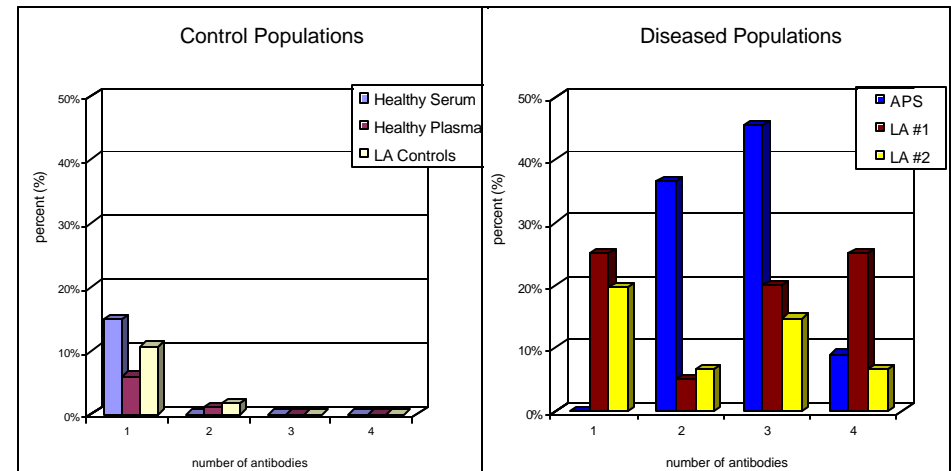
Graph #3: IgG anti-Beta 2 Glycoprotein I (aB2GPI) Antibodies



Graph #4: IgG antiProthrombin (aPT) Antibodies



Graph #5: Distribution of the Number of Antibodies Present in Control and Diseased Populations



SUMMARY AND CONCLUSIONS

- The prevalence of both aCL and aPS antibodies in most of the groups tested was greater than the APL status. This indicates that these tests determine more positive reactors (false positives) than the APL status. This makes these assays good screening methods for antiphospholipid antibodies.
- The aPS assay had a higher relative sensitivity and agreement with the APS status compared to the aCL assay, indicating that testing for aPS antibodies may be more relevant.
- The anti-B2GPI assay resulted with the best relative sensitivity and agreement with the APL status compared to all of the other assays. These results may be biased due to the prominent role of anti-B2GPI antibodies in determining the APL status. However, these results are comparable to a previous study using a clinical (thrombosis) criteria (ISTH abstract, Paris, FRANCE, July 2001).
- The lowest relative sensitivity and agreement with the APL status was found with the antiprothrombin (aPT) assay, indicating that fewer patients have antibodies to prothrombin compared to antibodies to B2GPI.
- The distribution of the number of antibodies present in each sample of control population was skewed toward the presence of one antibody compared to the diseased populations where most of the samples had more than one antibody present.