

**COMPARATIVE SENSITIVITY AND SPECIFICITY OF VARIOUS  
ANTIPHOSPHOLIPID ANTIBODIES FOR THROMBOSIS IN SAMPLES WITH LUPUS  
ANTICOAGULANT**

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**ABSTRACT**

Antiphospholipid antibodies form part of a heterogeneous group of autoantibodies associated with the antiphospholipid syndrome (APS) clinically characterized by arterial or venous thrombosis, thrombocytopenia and fetal loss. Elevated serum levels of anticardiolipin (aCL) antibodies measured by ELISA are accepted serologic criteria for the diagnosis of APS. However, assays for these antibodies may show low sensitivity or specificity for thrombosis (APS). Newer assays for antiphospholipid antibodies (i.e. to phosphatidylserine or to protein cofactors) have been developed to enhance their sensitivity and specificity. Several recent reports suggest that anti-B2GPI antibodies may be more specific for thrombosis (and APS) than aCL antibodies. We studied the sensitivity and specificity for thrombosis of various antiphospholipid antibodies in a group of 24 plasma samples, obtained from patients referred to a coagulation laboratory for evaluation of lupus anticoagulant (LA) activity. IgG, IgM and IgA antibodies to cardiolipin (aCL), phosphatidylserine (aPS), beta 2 glycoprotein I (anti-B2GPI) and prothrombin (aPT) were measured by commercial ELISA kits following the manufacturer's instructions. Patients' records were reviewed for clinical manifestations of thrombosis (APS) and/or autoimmune disease (i.e. SLE). While all 24 plasma samples were classified as LA positive, 5 (21%) showed not clinical manifestations of APS. Four of the 5 patients without APS were negative for antiphospholipid antibodies, one tested positive for 3 antibodies. Fifteen of the 24 patients (62%) were positive for anti-B2GPI antibodies, and 11 (46%) were positive for each, aCL, aPS and aPT antibodies. The pattern of reactivity of each patient showed that 3 patients reacted to one antibody, 2 to two antibodies, while the majority reacted to 3 or more antibodies. A 2x2 analysis of positive or negative results for each antibody versus the presence or absence of clinical manifestations of thrombosis (APS) showed the following results: anti-B2GPI antibodies showed the best sensitivity (68%), followed by aPS (58%), and both aCL and aPT with 52%. The best specificity for thrombosis (APS) was observed with aPS (100%) followed by aCL and aPT (80%) and anti-B2GPI (60%). If we combine the results of all antiphospholipid antibodies, the sensitivity for APS was 80% and specificity of 60% giving an overall accuracy of 75%. These results not only confirm the association of antiphospholipid antibodies with thrombosis (APS) but show that the majority of patients developed more than one antiphospholipid antibody in different combinations. This suggests that determining one antibody may result in missing the diagnosis in some patients i.e. 5 APS patients (21%) in this study tested negative for aCL and positive for other antibodies. In this study, the antiphospholipid antibody with the best sensitivity was anti-B2GPI (68%) and best specificity was aPS (100%) suggesting that this may be the best assay combination for the laboratory screening of antiphospholipid antibodies.

**INTRODUCTION**

Antiphospholipid antibodies are a heterogeneous group of autoantibodies associated with the antiphospholipid syndrome (APS), which is clinically characterized by recurrent arterial or venous thrombosis. In addition, APS is the most common acquired risk factor for the development of deep vein thrombosis. High serum levels of anticardiolipin (aCL) antibodies measured by ELISA are accepted serologic criteria for the diagnosis of APS.

In the last decade, it has become apparent that many assays for aCL antibodies demonstrate low sensitivity or specificity for thrombosis (and APS). More recently, new assays for various antiphospholipid antibodies i.e. phosphatidylserine [aPS] or protein cofactors such as B2GPI [anti-B2GPI] and prothrombin [aPT] have been developed with enhanced sensitivity and specificity for thrombosis. Several recent reports suggest that anti-B2GPI antibodies may be more specific for thrombosis (and APS) than aCL antibodies.

**OBJECTIVE**

- To assist the clinical laboratory in becoming familiar with the performance of aCL, aPS, anti-B2GPI and aPT ELISA tests, we studied the sensitivity and specificity for thrombosis of these 4 antiphospholipid antibodies in a group of 24 Lupus Anticoagulant (LA) positive plasma samples.

**MATERIAL AND METHODS**

24 plasma samples were obtained from patients with abnormal coagulation times referred to a coagulation laboratory for evaluation of lupus anticoagulant (LA) activity. Patients' clinical records were reviewed for clinical manifestations of thrombosis (APS) and/or autoimmune disease (i.e. SLE).

**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, IgM, or IgA 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 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, IgM, or IgA 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, IgM, or IgA 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, IgM, or IgA 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.

## RESULTS

While all 24 plasma samples were classified as LA positive by accepted classification criteria (Brandt et al. 1995 and Triplett 1998), five (21%) showed no clinical manifestations of APS (thrombosis, or autoimmune disease). The majority (79%) had a diagnosis of APS (SLE, or a history of thrombosis in their clinical records). Three (#'s 2,3,11) of the five patients without APS were negative for antiphospholipid antibodies; one (# 23) was positive for anti-B2GPI only and the other patient (# 9) tested positive for three different assays.

Fifteen of the 24 patients (62%) were positive for anti-B2GPI antibodies, and 11 (46%) were positive for aCL, aPS and aPT antibodies. However, four patients with history of thrombosis (#'s 8,16,21,22) showed no reactivity in any of the assays.

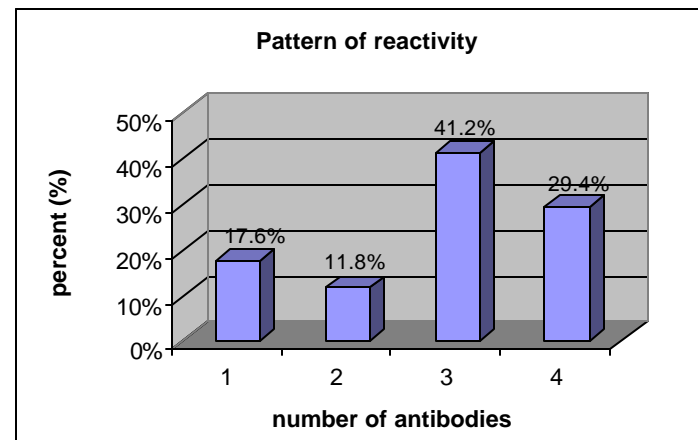
### Correlation between APS (thrombosis) and antiphospholipid antibody reactivity of selected Lupus Anticoagulant positive plasma samples

LA sample	APS (thrombosis)	aCL*	aPS*	anti-B2GPI*	aPT*
1	+			+	+
2					
3					
4	+			+	
5	+	+	+	+	
6	+	+	+	+	
7	+	+		+	
8	+				
9		+		+	+
10	+	+	+	+	+
11					
12	+		+	+	+
13	+	+	+	+	+
14	+	+	+	+	+
15	+	+	+		+
16	+				
17	+			+	
18	+		+	+	+
19	+	+	+	+	+
20	+	+	+	+	+
21	+				
22	+				
23				+	
24	+	+	+		+
% positive	79%	46%	46%	62%	46%

APS = antiphospholipid syndrome

\* indicates positive IgG, IgM and/or IgA isotypes.

The pattern of reactivity showed that 3 patients (17.6%) reacted to one antibody, 2 patients (11.8%) to two antibodies, while the majority (70.6%) reacted to 3 or more antibodies in various combinations.



A 2x2 analysis of positive or negative results for each antibody versus the presence or absence of clinical manifestations of thrombosis (APS) showed that anti-B2GPI antibodies had the best sensitivity (68%), followed by aPS (58%), and both aCL and aPT antibodies with 52%.

The best specificity for thrombosis (APS) was observed with aPS antibodies (100%), followed by aCL and aPT (80%), and anti-B2GPI antibodies (60%). When the results of all four antiphospholipid antibody assays were combined, the sensitivity for APS was 80% with a specificity of 60%, giving an overall accuracy of antiphospholipid antibodies of 75%.

### Comparative clinical performance of 4 antiphospholipid antibody assays in selected Lupus Anticoagulant positive plasma samples

	aCL	aPS	anti-B2GPI	aPT	all assays combined
Sensitivity %	52	58	68	52	80
Specificity %	80	100	60	80	60
Accuracy %	58	67	67	58	75

## SUMMARY AND CONCLUSIONS

- These results not only confirm the association of antiphospholipid antibodies with thrombosis (APS), but also demonstrated that the majority of patients develop more than one antiphospholipid antibody in different combinations.
- These results suggest that measuring only one antibody may result in a missed diagnosis in some patients. For example, 5 patients with APS (21%) in this study tested negative for aCL and positive for other antibodies.
- In this study, the antiphospholipid antibody with the best specificity was aPS (100%) while anti-B2GPI demonstrated the best sensitivity (68%), suggesting that this may be the best assay combination for the laboratory screening of antiphospholipid antibodies.