

# Anti- $\beta_2$ -Glycoprotein I and Antiphosphatidylserine Antibodies Are Predictors of Arterial Thrombosis in Patients With Antiphospholipid Syndrome

Luis R. Lopez, MD,<sup>1</sup> Ken J. Dier,<sup>1</sup> Daniel Lopez,<sup>1</sup> Joan T. Merrill, MD,<sup>2</sup> and Catherine A. Fink, PhD<sup>1</sup>

**Key Words:** Antiphospholipid syndrome; Thrombosis; Enzyme-linked immunosorbent assay; ELISA; Autoantibodies

DOI: 10.1309/YVQ6PX76XMYM3J29

## Abstract

*The predictive value (PV) and association of 4 antiphospholipid antibodies with clinical manifestations of the antiphospholipid syndrome (APS) were evaluated in 90 patients with systemic lupus erythematosus (SLE) and 100 with APS. Patients with APS were classified into arterial thrombosis, venous thrombosis, and pregnancy morbidity subgroups. IgG, IgM, and IgA anticardiolipin (aCL), antiphosphatidylserine (aPS), anti- $\beta_2$ -glycoprotein I (anti-B2GPI), and antiprothrombin (aPT) antibodies were determined by enzyme-linked immunosorbent assay. Individually, anti-B2GPI and aPS antibodies had the strongest PV for APS (86.4%-94.1%;  $P < .001$ ) in patients with SLE. The PV for APS reached 100% when 2 or more antibodies were present. Similarly, anti-B2GPI and aPS antibodies had a stronger PV and association for arterial thrombosis (87%-95%;  $P < .001$ ) compared with venous thrombosis (80%-92%;  $P = .01$ ). Weak PV and association with pregnancy morbidity were seen with all antibodies. These results suggest an important pathogenic role of anti-B2GPI antibodies in arterial thrombosis. In addition, anti-B2GPI and aPS antibodies seem to provide the best diagnostic value for the laboratory assessment of APS.*

Antiphospholipid antibodies, anticardiolipin (aCL) antibodies or lupus anticoagulants, are a heterogeneous group of autoantibodies characterized by their reactivity to anionic phospholipids, phospholipid-protein complexes, and certain proteins presented on suitable surfaces (ie, activated cell membranes and oxygenated polystyrene).<sup>1-4</sup> Several plasma proteins with coagulation functions that interact with anionic phospholipids have been reported as antiphospholipid cofactors.  $\beta_2$ -Glycoprotein I (B2GPI) and prothrombin are the most extensively studied cofactors.<sup>5,6</sup> These protein cofactors have been shown to be relevant antigenic targets for antiphospholipid antibodies.<sup>6-8</sup> In addition, a significant relationship with and increased specificity for thrombosis of anti-B2GPI over aCL antibodies in patients with antiphospholipid syndrome (APS) has been reported.<sup>9-11</sup>

IgG and IgM aCL enzyme-linked immunosorbent assays (ELISAs), introduced during the early 1980s, are the most commonly used methods to detect antiphospholipid antibodies. The presence of these antibodies is one of the major serologic criteria for the classification of APS.<sup>12,13</sup> APS may be subclassified as primary if there is no coexistent autoimmune disease or secondary when present in the context of an autoimmune disorder (eg, systemic lupus erythematosus [SLE]). Significant variability of aCL results among clinical laboratories has been reported. Numerous efforts by many groups to standardize the aCL assay have been unsuccessful,<sup>14</sup> likely reflecting the heterogeneity of these antibodies, the complex nature of the interaction between cofactors and phospholipids, and the lack of participation of cardiolipin in coagulation, including its absence from the phospholipid bilayer of cell membranes. An additional diagnostic problem with APS is the low specificity of the aCL

ELISA, which might lead to possible misdiagnosis and unnecessary anticoagulation.<sup>15</sup>

In a recent prospective study of patients with autoimmune disorders and healthy adults, an elevated aCL antibody level was not a risk factor for venous thromboembolism.<sup>16</sup> ELISAs for other antiphospholipid antibodies, such as those directed to phosphatidylserine (a more physiologically relevant phospholipid), have been developed and introduced to clinical laboratories.<sup>17</sup> In addition, several groups have explored the usefulness of IgA antiphospholipid antibodies, with many suggesting their possible pathogenic role and diagnostic value.<sup>18</sup> It also has been suggested that different antiphospholipid antibodies might have different clinical associations, and the combination of these antibodies provides a stronger risk for thrombosis in patients with autoimmune disorders.<sup>19-21</sup>

High serum levels of antiphospholipid antibodies in patients with APS have been associated with arterial and venous thromboembolic events, pregnancy morbidity (miscarriages and fetal loss), and thrombocytopenia.<sup>22,23</sup> Venous thrombosis (eg, deep venous thrombosis, pulmonary embolism) is the most common initial clinical manifestation in APS. However, more than 25% of the patients enrolled in a European cohort of 1,000 patients with APS had an arterial thrombotic event (eg, myocardial infarction, cerebrovascular accident, angina) as the initial clinical manifestation.<sup>24</sup>

In the present study, serum levels of 4 antiphospholipid antibodies were measured by ELISA in patients with SLE and APS. These included aCL, antiphosphatidylserine (aPS), anti-B2GPI, and antiprothrombin (aPT) antibodies. The goal was to assess the diagnostic value and association of each antibody specificity with the clinical manifestations of APS. Anti-B2GPI and aPS antibodies had a stronger predictive value and association with APS and with thrombosis compared with aCL and aPT antibodies. Anti-B2GPI and aPS antibodies also demonstrated the strongest association with arterial thrombosis. These results suggest that the determination of anti-B2GPI and aPS antibodies might provide the best diagnostic value for the serologic assessment of APS. In addition, these results support the concept that the B2GPI molecule has an important pathogenic role in the development of thrombosis, particularly in arterial thrombosis in patients with SLE and APS.

## Materials and Methods

### Samples

Serum samples from 2 autoimmune populations were included in the study. One population consisted of 90 patients with SLE and was used to evaluate the association of

4 IgG, IgM, and IgA antiphospholipid antibodies with the clinical manifestations of APS. This population was classified into 2 subgroups: 40 patients with SLE and secondary APS and 50 patients with SLE without a clinical history of antiphospholipid antibodies. The diagnosis of SLE was established according to the American College of Rheumatology Classification Criteria,<sup>25</sup> and the diagnosis of APS was established according to the Sapporo Criteria for the Classification of APS.<sup>26</sup> Of the patients with SLE, 82 were females and 8 were males. The mean age was 38.7 years (range, 17-74 years).

A second population consisted of 100 patients with APS enrolled in the Oklahoma Registry for the Antiphospholipid Syndrome (Oklahoma Medical Research Foundation, Oklahoma City [<http://www.slraps.org>]); their serum samples were used to evaluate the association of the antiphospholipid antibodies with thrombosis (arterial or venous) and pregnancy morbidity. The clinical diagnosis of APS also was based on the Sapporo Criteria for the Classification of APS.<sup>26</sup> All patients had a previous positive lupus anticoagulant and/or IgG B2GPI-dependent aCL ELISA result on 2 or more occasions. Of the patients with APS, 88 were women and 12 were men. The mean age was 44.6 years (range, 18-82 years). Twenty-four patients were classified as having primary APS and 76 as having APS secondary to SLE.

Samples from the group of 50 patients with SLE without APS served as control samples. In addition, 43 serum samples from healthy blood bank donors were included as control samples. All samples were stored at  $-70^{\circ}\text{C}$  until assayed.

Three major clinical manifestations for the patients with APS were recorded: venous thrombosis, arterial thrombosis, and pregnancy morbidity. Of the patients with APS, 85 had a history of a thrombotic event (40 venous, 31 arterial, 14 both venous and arterial) and 15 pregnancy morbidity only. Venous thrombotic events included deep venous thrombosis, pulmonary embolism, and superficial phlebitis confirmed by Doppler ultrasound, venography, or ventilation-perfusion scanning. Arterial thrombotic events included myocardial infarction and angina, cerebrovascular accident, and transient ischemic attack confirmed by angiography, computed tomography, or magnetic resonance imaging. Pregnancy morbidity included fetal death, miscarriage, and preeclampsia as previously defined.<sup>26</sup> Fourteen patients with APS had thrombocytopenia (platelet count,  $<100 \times 10^3/\mu\text{L}$  [ $<100 \times 10^9/\text{L}$ ]). In all cases, thrombocytopenia was present in combination with any of the aforementioned clinical manifestations. Informed consent was obtained from all patients with APS, and institutional review board approval for this project was obtained from the Oklahoma Medical Research Foundation.

### Antiphospholipid Antibody ELISAs

IgG, IgM, and IgA antibody isotypes were determined for aCL, aPS, and anti-B2GPI antibodies and only IgG and IgM isotypes for aPT antibodies. Commercially available ELISA test kits (Corgenix, Westminster, CO) were used according to the manufacturer's instructions. Both aCL and aPS ELISA tests require exogenous (bovine) B2GPI; thus, they measure B2GPI-dependent antiphospholipid antibodies. Anti-B2GPI and aPT ELISAs used purified human B2GPI and prothrombin as antigens, detecting antibodies in the absence of exogenous phospholipids. Results were reported in antiphospholipid unit values, and the cutoff level for positive or negative classification of results was used as recommended by the manufacturer for each assay.

### Statistical Analysis

Statistical analysis was performed with a SigmaStat 3.0 program (SPSS Science, Chicago, IL). The Mann-Whitney rank sum test was used to compare the results between different groups and the  $\chi^2$  test (with Yates correction) to assess the relationship between antibodies and clinical manifestations. Relative sensitivity, specificity, positive predictive value (PPV), and odds ratio (OR) for each APL antibody were evaluated by  $2 \times 2$  contingency table analysis. The 95% confidence intervals for the ORs also were calculated. A *P* value of .05 or less was considered as significant.

## Results

### Antiphospholipid Antibodies in Patients With SLE

All 90 SLE serum samples were assayed for IgG, IgM, and IgA aCL, aPS, and anti-B2GPI and IgG and IgM aPT antibodies. The distribution, mean value, and prevalence of positive reactors for both groups (40 with secondary APS and 50 SLE control samples) are summarized in **Figure 1**. Except for IgM aPT antibodies (*P* = .938), the mean IgG, IgM, and IgA antiphospholipid antibody values for patients with SLE with secondary APS were significantly higher (*P* < .05) compared with values for the SLE control group without a history of APS.

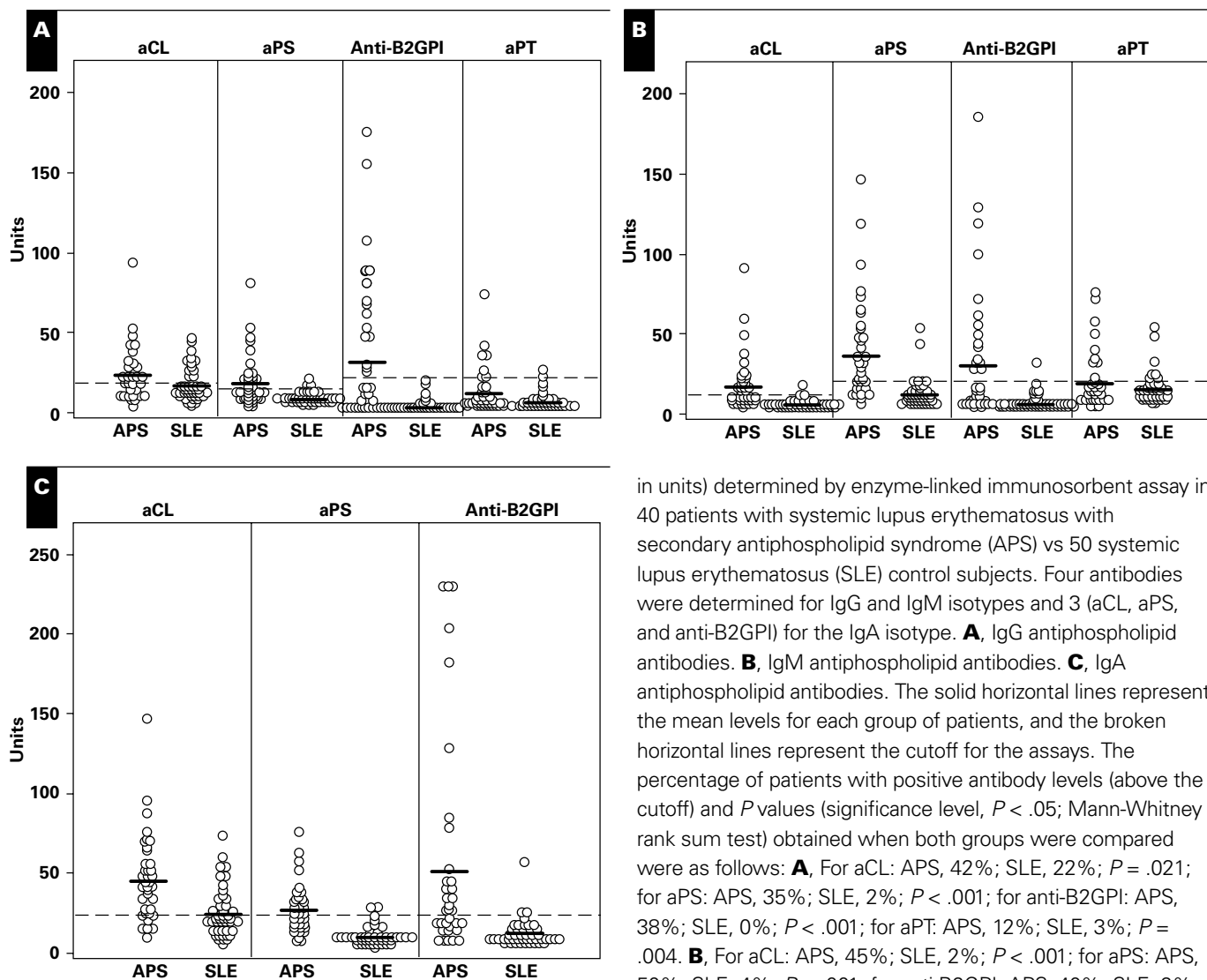
In addition to higher antibody levels, SLE patients with secondary APS frequently had 2 or more IgG, IgM, or IgA antiphospholipid antibodies present in their serum. In contrast, SLE patients without a history of APS mostly presented only 1 antibody **Figure 2**. When 2 antiphospholipid antibodies were present in a patient, the most frequent combination was aCL and aPS, followed by aPS and anti-B2GPI. These results are in agreement with the polyclonal nature of the antiphospholipid autoimmune response and emphasize the possible diagnostic value of detecting 2 or more antibodies when evaluating APS.

The clinical performance (relative specificity, PPV, and OR) of 4 antiphospholipid antibodies for APS is summarized in **Table 1**. The IgG aPS and IgG anti-B2GPI PPVs were significant, while the IgG aCL and IgG aPT PPVs were not. The IgM aCL, IgM aPS, and IgM anti-B2GPI PPVs were significant, while the IgM aPT PPV was not. All IgA PPVs reached statistical significance. In summary, in this group of patients with SLE, the antiphospholipid antibodies with the best PPV (>90%) for APS were as follows: IgM aCL; IgG, IgM, and IgA aPS; and IgG and IgM anti-B2GPI.

### Antiphospholipid Antibodies in Patients With APS

All 100 APS serum samples (24 with primary APS and 76 with secondary APS) were assayed for IgG, IgM, and IgA aCL, aPS, and anti-B2GPI antibodies and for IgG and IgM aPT antibodies. No difference was found between the mean aCL, aPS, anti-B2GPI, or aPT antibody level of the patients with primary vs secondary APS (data not shown). The patients with APS were classified into 3 groups: 45 with arterial thrombosis, 40 with venous thrombosis, and 15 with pregnancy morbidity. The antiphospholipid antibody distribution, prevalence, and mean values for each group are summarized in **Figure 3**. Although the mean antibody levels for the pregnancy morbidity group were consistently lower, only the mean levels of IgG aCL, aPS, and anti-B2GPI antibodies for the arterial and venous thrombosis groups were significantly higher (*P* < .05). In most cases, the mean antiphospholipid antibody levels for patients with arterial thrombosis were higher than for patients with venous thrombosis. However, none of the differences between the arterial and venous thrombosis groups reached statistical significance.

The clinical performance (PPV and OR) for thrombosis (arterial, venous, or both), the arterial and venous thrombosis subgroups, and pregnancy morbidity is summarized in **Table 2**. The following antibodies were associated significantly with thrombosis (arterial and venous combined), with predictive values of more than 90%: IgM aCL; IgG and IgM aPS; IgG, IgM, and IgA anti-B2GPI; and IgG aPT. When the arterial and venous thrombosis groups were compared, the best PPVs were found for arterial thrombosis. The IgG PPV for aPS was 95% and for anti-B2GPI was 94.7%. The arterial thrombosis PPV was low for IgG aCL at 64.5% and for aPT at 87.5%. Similar results were obtained with the arterial thrombosis PPVs for IgM antibodies: the PPV for IgM aCL was 95.2%, for IgM aPS was 90.4%, and for IgM anti-B2GPI antibodies was 94.7%. The only significant PPV for IgA was seen with anti-B2GPI antibodies. The arterial thrombosis PPVs, ORs, and statistical associations were consistently stronger than the values for venous thrombosis in this group of patients with APS. In summary, these results indicate that the following antibodies might be better predictors for arterial



**Figure 1** Distribution of anticardiolipin (aCL), antiphosphatidylserine (aPS), anti-β<sub>2</sub>-glycoprotein I (anti-B2GPI), and antiprothrombin (aPT) antibody levels (expressed

in units) determined by enzyme-linked immunosorbent assay in 40 patients with systemic lupus erythematosus with secondary antiphospholipid syndrome (APS) vs 50 systemic lupus erythematosus (SLE) control subjects. Four antibodies were determined for IgG and IgM isotypes and 3 (aCL, aPS, and anti-B2GPI) for the IgA isotype. **A**, IgG antiphospholipid antibodies. **B**, IgM antiphospholipid antibodies. **C**, IgA antiphospholipid antibodies. The solid horizontal lines represent the mean levels for each group of patients, and the broken horizontal lines represent the cutoff for the assays. The percentage of patients with positive antibody levels (above the cutoff) and *P* values (significance level, *P* < .05; Mann-Whitney rank sum test) obtained when both groups were compared were as follows: **A**, For aCL: APS, 42%; SLE, 22%; *P* = .021; for aPS: APS, 35%; SLE, 2%; *P* < .001; for anti-B2GPI: APS, 38%; SLE, 0%; *P* < .001; for aPT: APS, 12%; SLE, 3%; *P* = .004. **B**, For aCL: APS, 45%; SLE, 2%; *P* < .001; for aPS: APS, 50%; SLE, 4%; *P* < .001; for anti-B2GPI: APS, 40%; SLE, 2%; *P* < .001; for aPT: APS, 20%; SLE, 10%; *P* = .938. **C**, For aCL: APS, 80%; SLE, 40%; *P* < .001; for aPS: APS, 57%; SLE, 4%; *P* < .001; for anti-B2GPI: APS, 47%; SLE, 6%; *P* < .001.

**Table 1** Clinical Performance\* of Four Antiphospholipid Antibodies in 90 Patients With SLE†

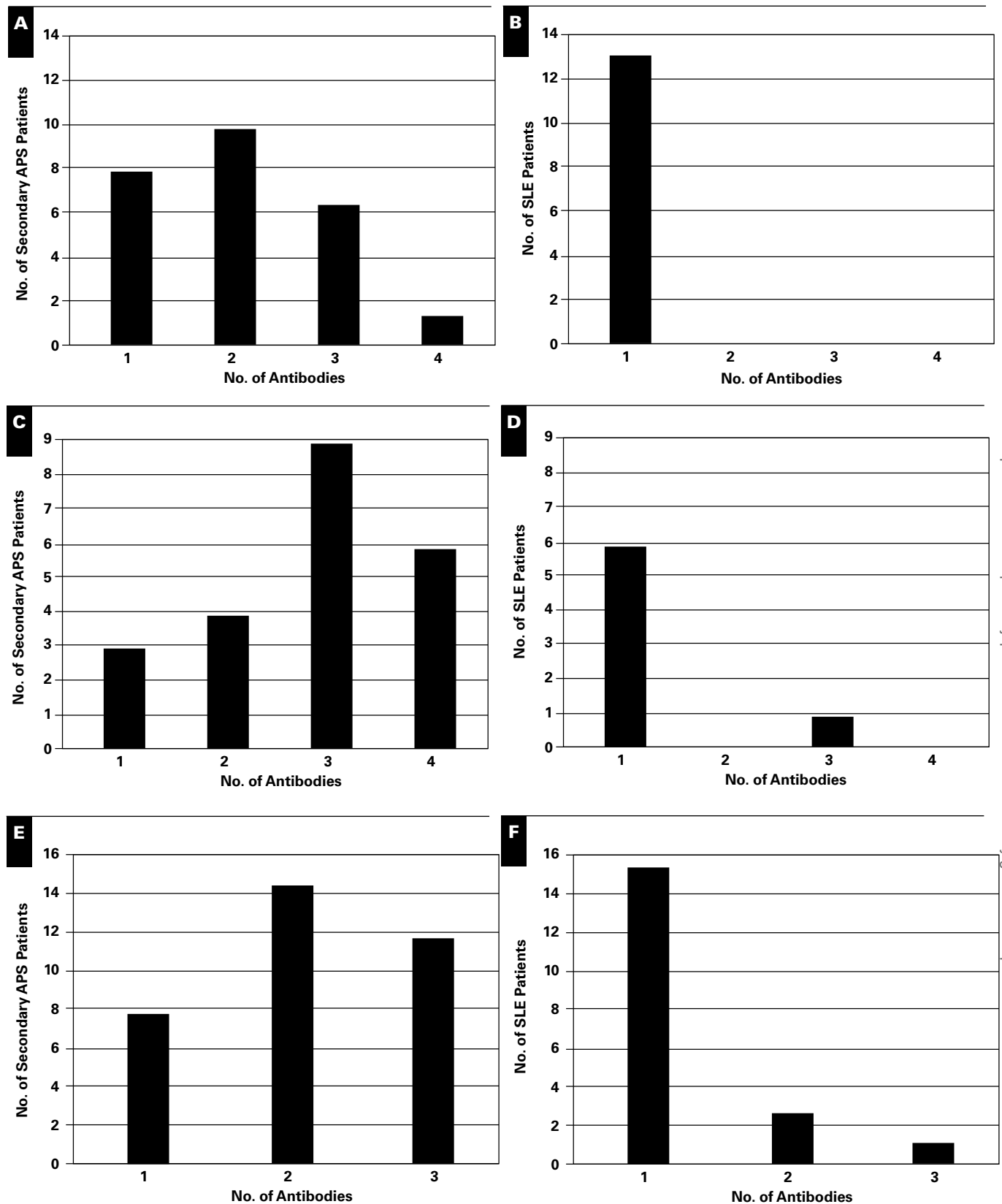
	Specificity (%)	PPV (%)	<i>P</i> ‡	Odds Ratio	95% CI
IgG					
aCL	78.0	60.7	.063	2.6	1.0-6.5
aPS	98.0	93.3	<.001	26.4	3.3-212.1
aB2GPI	98.0	93.3	<.001	29.4	3.7-236.3
aPT	97.5	83.3	.203	5.6	0.6-50.1
IgM					
aCL	98.0	94.7	<.001	40.1	5.0-319.5
aPS	95.7	90.9	<.001	22.5	4.8-105.6
aB2GPI	97.9	94.1	<.001	32.0	4.0-255.8
aPT	89.9	61.5	.317	2.2	0.6-7.3
IgA					
aCL	60.0	61.5	<.001	6.0	2.3-15.6
aPS	95.5	92.0	<.001	31.8	6.7-149.5
aB2GPI	94.0	86.4	<.001	14.2	3.8-53.2

aB2GPI, anti-β<sub>2</sub>-glycoprotein I; aCL, anticardiolipin; aPS, antiphosphatidylserine; aPT, antiprothrombin; 95% CI, confidence interval of the odds ratio; PPV, positive predictive value; SLE, systemic lupus erythematosus.

\* Relative specificity, PPV, and odds ratio for antiphospholipid syndrome.

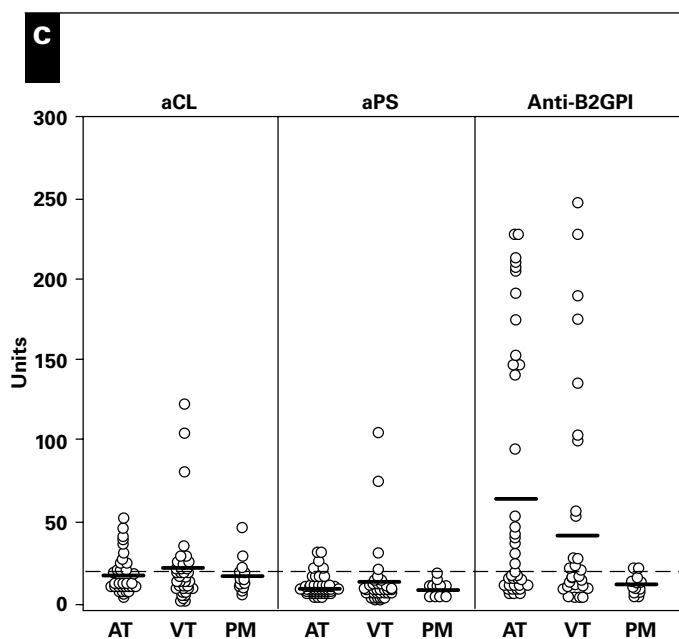
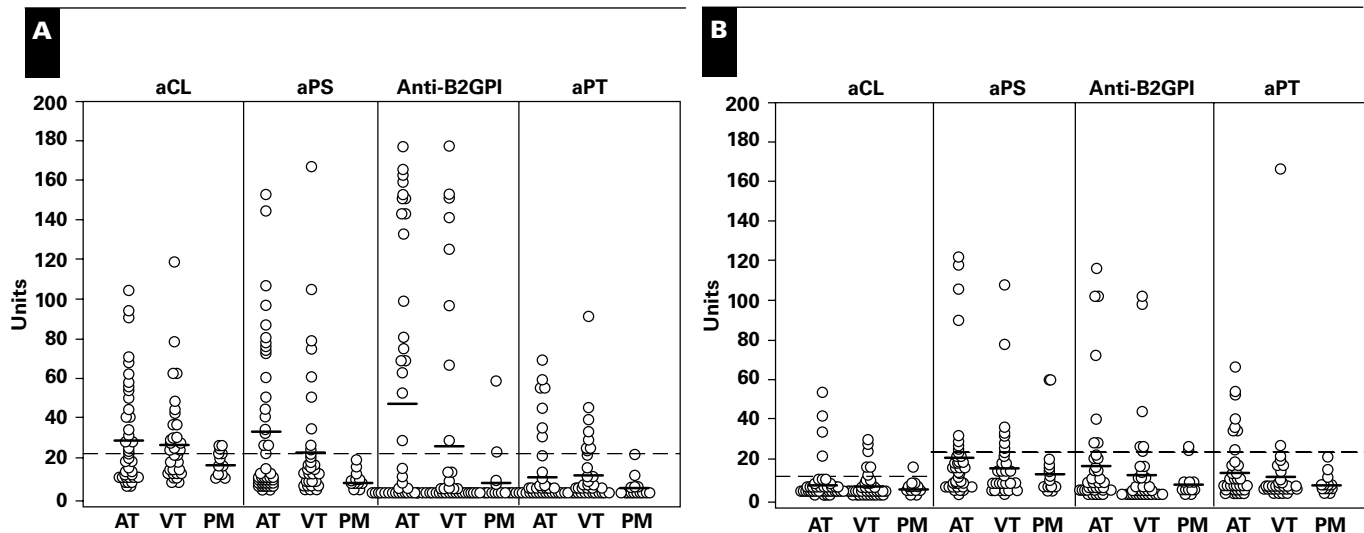
† Secondary antiphospholipid syndrome, n = 40; SLE control subjects, n = 50.

‡ χ<sup>2</sup> test with Yates correction; significance level, *P* < .05.



Downloaded from https://academic.oup.com/ajcp/article/121/1/142/1759225 by guest on 23 April 2024

**Figure 2** Distribution of patients with systemic lupus erythematosus with secondary antiphospholipid syndrome (APS; n = 40; **A, C, E**) vs systemic lupus erythematosus (SLE) control subjects (n = 50; **B, D, F**) according to the number of antiphospholipid antibodies present. Four antibodies (anticardiolipin [aCL], antiphosphatidylserine [aPS], anti-β<sub>2</sub>-glycoprotein I [anti-B2GPI], and antiprotease [aPT]) were determined for IgG (**A** and **B**) and IgM (**C** and **D**) isotypes, and 3 antibodies (aCL, aPS, and anti-B2GPI) for the IgA isotype (**E** and **F**) by enzyme-linked immunosorbent assay.



**Figure 3** Distribution of anticardiolipin (aCL), antiphosphatidylserine (aPS), anti- $\beta_2$ -glycoprotein I (anti-B2GPI), and antiprothrombin (aPT) antibody levels (expressed in units) determined by enzyme-linked immunosorbent assay in 100 patients with antiphospholipid syndrome (APS) subclassified according to a history of arterial thrombosis (AT, n = 45), venous thrombosis (VT, n = 45), or pregnancy morbidity (PM, n = 15). Four antibodies were determined for IgG (**A**) and IgM (**B**) isotypes and 3 (aCL, aPS, and anti-B2GPI) for the IgA isotype (**C**). The solid horizontal lines represent the mean levels for each group of patients, and the broken horizontal lines represent the cutoff for the assays. The percentage of patients with positive antibody levels (above the cutoff) for each group are as follows: **A**, For aCL: AT, 44%; VT, 42%; PM, 20%; for aPS: AT, 42%; VT, 30%; PM, 6%; for anti-B2GPI: AT, 40%; VT, 20%; PM, 13%; for aPT: AT, 15%; VT, 20%; PM, 0%. **B**, For aCL: AT, 9%; VT, 12%; PM, 6%; for aPS: AT, 20%; VT, 20%; PM, 6%; for anti-B2GPI: AT, 18%; VT, 15%; PM, 13%; for aPT: AT, 18%; VT, 5%; PM, 0%. **C**, For aCL: AT, 22%; VT, 25%; PM, 13%; for aPS: AT, 6%; VT, 7%; PM, 0%; for anti-B2GPI: AT, 44%; VT, 30%; PM, 0%.

thrombosis in patients with APS: IgM aCL; IgG and IgM aPS; and IgG, IgM, and IgA anti-B2GPI.

### Discussion

This is a cross-sectional study of serum samples from autoimmune patients with a history of antiphospholipid antibodies, thrombosis, or pregnancy morbidity. Two autoimmune populations were included. One population of patients with SLE was used to assess the relationship of each antiphospholipid antibody with the history of APS. The distribution of the antibodies in 40 patients with SLE with secondary APS vs 50 SLE control subjects in Figure 1 shows that the levels of antibodies were higher in patients

with SLE with APS than in patients with SLE without APS. These results suggest the coexistence of multiple antiphospholipid antibodies and isotypes in patients with secondary APS. The most significant antibodies found were IgG, IgM, and IgA aPS and anti-B2GPI antibodies. The aCL antibody had a lower specificity for APS, as reported by others.<sup>15</sup>

Figure 2 demonstrates that patients with secondary APS frequently had more than 1 antiphospholipid antibody and that patients with SLE without APS most likely had only 1 antibody. These results are consistent with the polyclonal nature of autoimmune responses and the possibility that increased autoantibody spreading might lead to increased pathologic risk. This information might be valuable in the laboratory evaluation of a patient for antiphospholipid antibodies to establish the diagnosis of APS. von

Downloaded from https://academic.oup.com/ajcp/article/121/1/142/1759225 by guest on 23 April 2024

**Table 2**  
**Clinical Performance\* of Four Antiphospholipid Antibodies in 100 Patients With Antiphospholipid Syndrome†**

	IgG				IgM				IgA			
	PPV (%)	P‡	OR	95% CI	PPV (%)	P‡	OR	95% CI	PPV (%)	P‡	OR	95% CI
aCL												
Thrombosis	77.1	.019	2.7	1.2-6.0	97.4	<.001	37.8	4.9-286.3	50.0	.067	0.4	0.2-0.9
Arterial	64.5	.035	2.8	1.1-6.9	95.2	<.001	39.2	4.9-309.2	33.3	.101	0.4	0.1-1.0
Venous	60.7	.063	2.6	1.0-6.5	94.4	<.001	36.2	4.5-288.7	33.3	.202	0.5	0.2-1.2
Pregnancy morbidity	21.4	.999	0.9	0.2-3.7	75.0	.036	12.2	1.2-128.4	9.1	.109	0.2	0.1-1.1
aPS												
Thrombosis	96.8	<.001	28.1	3.7-213.8	93.9	<.001	12.9	2.9-56.9	75.0	.747	1.8	0.3-9.1
Arterial	95.0	<.001	35.8	4.5-282.7	90.4	<.001	13.8	2.9-63.9	60.0	.654	1.7	0.2-10.5
Venous	92.3	<.001	21.0	2.6-170.1	85.7	.003	9.6	2.0-46.3	60.0	.668	1.9	0.3-11.9
Pregnancy morbidity	50.0	.411	3.5	0.2-59.5	33.3	.571	1.6	0.1-19.1	33.3	.558	1.7	0.1-19.9
aB2GPI												
Thrombosis	96.3	<.001	21.6	28.3-164.8	96.3	<.001	21.1	2.8-161.5	91.4	<.001	9.4	2.7-32.9
Arterial	94.7	<.001	32.6	4.1-258.2	94.7	<.001	32.0	4.0-253.1	86.9	<.001	12.5	3.3-46.9
Venous	88.9	.009	12.2	1.4-106.8	88.9	.01	12.0	1.4-100.6	80.0	.006	6.7	1.7-25.8
Pregnancy morbidity	66.7	.131	7.5	0.6-89.8	66.6	.134	7.4	0.6-87.9	25.0	.998	1.1	0.1-11.6
aPT												
Thrombosis	93.7	.038	8.3	1.1-65.7	75.0	.361	1.9	0.6-5.5	—	—	—	—
Arterial	87.5	.061	7.2	0.8-61.1	58.3	.317	1.6	0.5-5.5	—	—	—	—
Venous	88.9	.029	9.7	1.2-82.1	61.5	.640	2.2	0.6-7.3	—	—	—	—
Pregnancy morbidity	50.0	.475	2.8	0.1-47.5	16.7	.390	0.6	0.1-5.8	—	—	—	—

aB2GPI, anti-β<sub>2</sub>-glycoprotein I; aCL, anticardiolipin; aPS, antiphosphatidylserine; aPT, antiprothrombin; 95% CI, confidence interval of OR; OR, odds ratio; PPV, positive predictive value.

\* PPV and OR for thrombosis and pregnancy morbidity.

† Thrombosis (arterial and venous); arterial, n = 45; venous, n = 40; pregnancy morbidity, n = 15.

‡ χ<sup>2</sup> test with Yates correction; significance level, P < .05.

Landenberg and colleagues<sup>27</sup> retrospectively studied different antiphospholipid antibodies in patients with autoimmune diseases and concluded that the combination of these antibodies was a predictor for thrombosis. Furthermore, the presence of aPS without aCL or anti-B2GPI was not associated with thrombosis.

The specificity, PPV, and OR for APS of aCL, aPS, anti-B2GPI, and aPT (for IgG, IgM, and IgA) were determined in 90 patients with SLE (Table 1). Anti-B2GPI and aPS antibodies had the strongest predictive value for APS (86.4%-94.1%). The presence of 2 or more antibodies had a predictive value of 100%. These results not only support the conclusions of von Landenberg et al<sup>27</sup> but also indicate that the combination of anti-B2GPI and aPS antibodies is a stronger predictor for APS and thrombosis in patients with SLE.

Several clinical studies have suggested that different antiphospholipid antibodies are associated with different features of APS. Recent prospective studies have suggested that aCL, particularly B2GPI-dependent, and anti-B2GPI antibodies are important predictors for myocardial infarction (and stroke) in men,<sup>28-30</sup> and aPT antibodies have been correlated with arterial thrombosis in patients with autoimmune disorders.<sup>31</sup> To further assess the relationship of each antiphospholipid antibody with manifestations of APS, the population of patients with APS was classified into subgroups according to their history of arterial or venous thrombosis and pregnancy morbidity. Figure 3 illustrates that higher antiphospholipid antibody titers were found in

patients with arterial and venous thrombosis than in those with pregnancy morbidity. Also, the mean antiphospholipid antibody levels were higher for patients with arterial thrombosis compared with those for patients with venous thrombosis. The lower prevalence in patients with pregnancy morbidity could be due to the event being further away from the sample tested, and, given the cross-sectional sampling, our results can be considered a preliminary observation.

The PPVs and ORs for thrombosis (arterial and venous) and pregnancy morbidity of aCL, aPS, anti-B2GPI, and aPT antibodies are shown in Table 2. IgG and IgM for aPS and IgG, IgM, and IgA for anti-B2GPI antibodies demonstrated stronger predictive values and associations with arterial than with venous thrombosis. The aPS antibody ELISA uses bovine B2GPI as the cofactor, while the anti-B2GPI assay uses human B2GPI, suggesting a role for B2GPI in the development of arterial thrombosis.<sup>32</sup> In the present study, IgM more than IgA aCL showed a significant association with thrombosis (arterial more than venous), while aPT showed no association with arterial thrombosis.

This article contributes to the ongoing evaluation of the role of antiphospholipid antibodies in diagnosing APS and in assessing the role of these antibodies in the development of vascular thrombosis or fetal losses. In addition, these results confirm that different antiphospholipid antibodies (and isotypes) might have different clinical associations. Given the heterogeneity in existing clinical tests, it would be important to know the clinical significance of the particular test to

Downloaded from https://academic.oup.com/ajcp/article/121/11/1421/759225 by guest on 23 April 2024

be used. This information should help to elucidate the usefulness of assays for the laboratory diagnosis of APS.

From <sup>1</sup>Corgenix, Westminster, CO; and <sup>2</sup>Oklahoma Medical Research Foundation, Oklahoma City.

Address reprint requests to Dr Lopez: Corgenix, 12061 Tejon St, Westminster, CO 80234.

Acknowledgment: We thank Eiji Matsuura, PhD, for thoughtful discussions.

## References

- Hughes GRV, Harris EN, Gharavi AE. The anticardiolipin syndrome. *J Rheumatol*. 1986;13:486-489.
- Gharavi AE, Harris EN, Asherson RA, et al. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. *Ann Rheum Dis*. 1987;46:1-6.
- Roubey RAS. Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulants and other "antiphospholipid" antibodies. *Blood*. 1994;84:2858-2867.
- Matsuura E, Igarashi Y, Yasuda T, et al. Anticardiolipin antibodies recognize  $\beta_2$ -glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J Exp Med*. 1994;179:457-462.
- McNeil HP, Simpson RJ, Chesterman CN, et al. Antiphospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation:  $\beta_2$ -glycoprotein I (apolipoprotein H). *Proc Natl Acad Sci U S A*. 1990;87:4120-4124.
- Bever EM, Galli M, Barbui T, et al. Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb Haemost*. 1991;66:629-632.
- Matsuura E, Igarashi Y, Fujimoto M, et al. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease [letter]. *Lancet*. 1990;336:177-178.
- Sheng Y, Kandiah DA, Krilis SA.  $\beta_2$ -glycoprotein I: target antigen for "antiphospholipid" antibodies: immunological and molecular aspects. *Lupus*. 1998;7(suppl 2):S5-S9.
- Tsutsumi A, Matsuura E, Ichikawa K, et al. Antibodies to  $\beta_2$ -glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus. *Arthritis Rheum*. 1996;39:1466-1474.
- Guerin J, Feighery C, Sim R, et al. Antibodies to  $\beta_2$ -glycoprotein I: a specific marker for the antiphospholipid syndrome. *Clin Exp Immunol*. 1997;109:304-309.
- Day HM, Thiagarajan P, Ahn C, et al. Autoantibodies to  $\beta_2$ -glycoprotein I in systemic lupus erythematosus and primary antiphospholipid syndrome: clinical correlations in comparison with other antiphospholipid antibody tests. *J Rheumatol*. 1998;25:667-674.
- Loizou S, McCrea JD, Rudge AC, et al. Measurement of anticardiolipin antibodies via an enzyme-linked immunosorbent assay (ELISA): standardization and quantitation of results. *Clin Exp Immunol*. 1985;62:738-745.
- Harris EN, Chan JKH, Asherson RA, et al. Thrombosis, recurrent fetal loss and thrombocytopenia: predictive value of the anticardiolipin antibody test. *Arch Intern Med*. 1986;146:2153-2156.
- Tincani A, Allegri F, Sanmarco M, et al. Anticardiolipin antibody assay: a methodological analysis for a better consensus in routine determinations. *Thromb Haemost*. 2001;86:575-583.
- Reddel SW, Krilis SA. Testing for and clinical significance of anticardiolipin antibodies. *Clin Diagn Lab Immunol*. 1999;6:775-782.
- Runchey SS, Folsom AR, Tsai MY, et al. Anticardiolipin antibodies as a risk factor for venous thromboembolism in a population-based prospective study. *Br J Haematol*. 2002;119:1005-1010.
- Keedy K, Santos M, Lopez LR. Prevalence and clinical significance of anti-phosphatidylserine antibodies [abstract]. *Arthritis Rheum*. 1994;37(suppl):S392.
- Wilson WA, Faghiri Z, Taheri F, et al. Significance of IgA antiphospholipid antibodies. *Lupus*. 1998;7:110-113.
- Lopez LR, Santos ME, Espinoza LR, et al. Clinical significance of immunoglobulin A versus immunoglobulins G and M anti-cardiolipin antibodies in patients with systemic lupus erythematosus: correlation with thrombosis, thrombocytopenia, and recurrent abortion. *Am J Clin Pathol*. 1992;98:449-454.
- Fanopoulos D, Teoderescu MR, Varga J, et al. High frequency of abnormal levels of IgA anti- $\beta_2$ -glycoprotein I antibodies in patients with systemic lupus erythematosus: relationship with antiphospholipid syndrome. *J Rheumatol*. 1998;25:675-680.
- Greco TP, Amos MD, Conti-Kelly AM, et al. Testing for the antiphospholipid syndrome: importance of IgA anti- $\beta_2$ -glycoprotein I. *Lupus*. 2000;9:33-41.
- Ginsburg KS, Liang MH, Newcomer L, et al. Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. *Ann Intern Med*. 1992;117:997-1002.
- Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. *N Engl J Med*. 2002;346:752-763.
- Cervera R, Piette JC, Font J, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum*. 2002;46:1019-1027.
- Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1982;25:1271-1277.
- Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum*. 1999;42:1309-1311.
- von Landenberg P, Scholmerich J, von Kempis J, et al. The combination of different antiphospholipid antibody subgroups in the sera of patients with autoimmune diseases is a strong predictor for thrombosis: a retrospective study from a single center. *Immunobiology*. 2003;207:65-71.
- Romero FI, Amengual O, Atsumi T, et al. Arterial disease in lupus and secondary antiphospholipid syndrome: association with anti- $\beta_2$ -glycoprotein I antibodies but not with antibodies against oxidized low-density lipoprotein. *Br J Rheumatol*. 1998;37:883-888.
- Brey RL, Abbott RD, Curb JD, et al.  $\beta_2$ -Glycoprotein I dependent anticardiolipin antibodies and the risk of ischemic stroke and myocardial infarction: the Honolulu Heart Program. *Stroke*. 2001;32:1701-1706.
- Swadzba J, De Clerck LS, Stevens WJ, et al. Anticardiolipin, anti- $\beta_2$ -glycoprotein I, antiprothrombin antibodies, and lupus anticoagulants in patients with systemic lupus erythematosus with a history of thrombosis. *J Rheumatol*. 1997;24:1710-1715.
- Vaara O. Antiphospholipid antibodies in myocardial infarction. *Lupus*. 1998;7(suppl 2):S132-S134.
- Kobayashi K, Kishi M, Atsumi T, et al. Circulating oxidized LDL forms complexes with  $\beta_2$ -glycoprotein I: implication as an atherogenic autoantigen. *J Lipid Res*. 2003;44:716-726.



# First and Only FDA Cleared Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager



## Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer  
with the Advanced Technology of the  
Genius™ Digital Diagnostics System



Click or Scan  
to discover more

ADS-04159-001 Rev 001 © 2024 Hologic, Inc. All rights reserved. Hologic, Genius, and associated logos are trademarks and/or registered trademarks of Hologic, Inc. and/or its subsidiaries in the United States and/or other countries. This information is intended for medical professionals in the U.S. and other markets and is not intended as a product solicitation or promotion where such activities are prohibited. Because Hologic materials are distributed through websites, podcasts and tradeshows, it is not always possible to control where such materials appear. For specific information on what products are available for sale in a particular country, please contact your Hologic representative or write to [diagnostic.solutions@hologic.com](mailto:diagnostic.solutions@hologic.com).

**genius™**  
DIGITAL DIAGNOSTICS