

Anti- β_2 -Glycoprotein I: Prevalence, Clinical Correlations, and Importance of Persistent Positivity in Patients with Antiphospholipid Syndrome and Systemic Lupus Erythematosus

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ABSTRACT. *Objective.* Antibodies to β_2 -glycoprotein I (anti- β_2 -GPI) are found in a large percentage of patients with primary or secondary antiphospholipid syndrome (APS). Our aim was to identify the prevalence and clinical correlation of these antibodies in patients with APS and systemic lupus erythematosus (SLE), in comparison to anticardiolipin (aCL) and the lupus anticoagulant (LAC). We investigated whether serial samples improve clinical utility.

Methods. Serum samples for anti- β_2 -GPI (IgG, IgM, IgA), aCL (IgG, IgM, IgA), and LAC (by dilute Russell viper venom time; RVVT) were collected from 418 consecutive patients with SLE or APS between October 2002 and March 2003. Clinical and serologic data of these patients were analyzed.

Results. A total of 185 (44.5%) patients were positive for anti- β_2 -GPI, 55.3% were positive for aCL, and 31.1% for LAC. Anti- β_2 -GPI was more common in Caucasians than in African Americans ($p = 0.098$). IgM and IgA were the most frequent isotypes of anti- β_2 -GPI. aCL and anti- β_2 -GPI were highly associated ($p < 0.0001$ to $p = 0.0177$, depending on isotype). A positive association was found between the presence of the LAC by dilute RVVT and anti- β_2 -GPI IgG ($p < 0.0001$), IgM ($p < 0.0001$), and IgA ($p = 0.0002$) antibodies. Persistent positivity increased the association of venous and arterial thrombosis with anti- β_2 -GPI (IgG and IgM isotypes). Pregnancy loss, seizures, and migraines were not associated with anti- β_2 -GPI. IgA anti- β_2 -GPI was not significantly associated with any manifestation of APS.

Conclusion. The prevalence of anti- β_2 -GPI IgM and IgA was very high in our population. Measurement of anti- β_2 -GPI IgG is clinically useful in identifying patients with SLE at higher risk for venous and arterial thrombosis. Persistent positivity increased the association of IgG anti- β_2 -GPI with venous thrombosis and anti- β_2 -GPI IgM with arterial thrombosis. IgA anti- β_2 -GPI was not significantly associated with APS manifestations. (J Rheumatol 2006;33:1775–9)

Key Indexing Terms:

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Antiphospholipid antibodies (aPL) bind to plasma proteins with an affinity for phospholipid surfaces. Many of the identified antigens (β_2 -glycoprotein and prothrombin) are involved in blood coagulation. Anticardiolipin antibodies (aCL) and the lupus anticoagulant (LAC) are included in classification criteria, but the importance of other aPL, including anti- β_2 -glycoprotein I (anti- β_2 -GPI), is not yet accepted. LAC and aCL IgG and IgM antibodies are associated with the clinical features of the antiphospholipid syndrome (APS): venous and arterial thrombosis and pregnancy morbidity. In addition, aCL and LAC have been associated with a spectrum of other

clinical manifestations, such as thrombocytopenia, livedo reticularis, and seizures. The syndrome can occur as a primary disorder or may be secondary to a connective tissue disease, especially systemic lupus erythematosus (SLE).

The major target antigen of aCL and many lupus anticoagulants is β_2 -GPI. β_2 -GPI is a 50 kDa lipophilic plasma protein (formerly called apolipoprotein H) that is composed of 5 fingerlike domains. In 1990 several groups^{1–3} described β_2 -GPI as the cofactor required for antiphospholipid antibody-phospholipid interaction. The lipid-binding site resides in domain 5 but aPL bind to domain 1. *In vivo*, β_2 -GPI binds to negatively charged phospholipids (phosphatidylserine) on activated or apoptotic cell membranes. There is currently no consensus on the clinical utility of anti- β_2 -GPI in comparison to LAC or aCL testing^{4–11}.

In this study, anti- β_2 -GPI (IgG, IgM, and IgA) was measured in patients with SLE and APS in order to clarify the prevalence of the antibodies, their association with the clinical features of the APS syndrome, and the importance of persistent positivity.

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MATERIALS AND METHODS

Serum samples were obtained from 418 consecutive and unselected patients (396 women, 24 men) from the Lupus Center at the Johns Hopkins Hospital, Baltimore, between October 2002 and March 2003. Both aCL and anti- β_2 -GPI were measured using Inova assays (Inova Diagnostics, San Diego, CA, USA). The dilute Russell viper venom time (dRVVT) test was performed as described¹², with mixing and confirmatory studies.

Interassay precision for the anti- β_2 -GPI assay was assessed at 4 levels of antibody activity using 4 standards supplied by the manufacturer. These were run with each run and the mean, standard deviation, and coefficient of variation percentage calculated for cumulative runs. These data are summarized for 10 runs in Table 1.

Patients were classified as having primary APS (1.2%), APS secondary to SLE (17%), SLE with any aPL (54.3%), and SLE without aPL (27.5%). All the patients classified as having SLE fulfilled at least 4 of the 1982 revised criteria of the American College of Rheumatology¹³. To be classified as APS, patients had to meet the Sapporo criteria proposed by the international consensus committee¹⁴. There were 237 Caucasians (56.7%), 157 African Americans (37.5%), and 24 other ethnicity, including Hispanic and Asian (5.7%).

Medical records of each patient were reviewed to ascertain the clinical manifestations of APS, including: venous thrombosis, arterial thrombosis, pregnancy loss, thrombocytopenia, and livedo reticularis. Migraines and seizures were also recorded. Venous thrombosis was confirmed by venogram or ultrasound. Arterial thrombosis was confirmed by computerized tomography, magnetic resonance imaging, or arteriogram.

Statistical analysis. Statistical analyses were done using chi-square and Fisher's exact tests (JMP v 5.0.1a, SAS Institute Inc., Cary, NC, USA). A p value of 0.05 was taken as statistically significant. All analyses were pre-specified, and Bonferroni corrections were not applied.

RESULTS

Prevalence of aCL, LAC, and anti- β_2 -GPI. Of the 418 patients, 186 tested positive for anti- β_2 -GPI of at least one isotype, representing 44.5% of the entire population (Table 2). Among the patients who tested positive for any anti- β_2 -GPI (186 patients), 29.6% were IgG, 65% IgM, and 50% IgA; 137 (73.7%) patients were positive for both anti- β_2 -GPI and aCL antibodies; 77 (41.4%) were positive for both anti- β_2 -GPI and the LAC (dRVVT). The prevalence of LAC (by dRVVT) in our population was 31.1% (130 patients). Only 5 patients with primary APS were included: 4 were positive for anti- β_2 -GPI, 3 were positive for aCL, and all 5 were positive for LAC.

Table 3 shows the distribution of anti- β_2 -GPI isotypes by ethnicity. There was a striking difference in IgM anti- β_2 -GPI, which was more common in Caucasians.

Thirty-seven of 418 (9%) were positive for anti- β_2 -GPI, but negative for both aCL and LAC. Of 187 patients negative

Table 2. Prevalence of anti- β_2 -GPI. Comparison of anti- β_2 -GPI titers, by isotype, in 418 patients.

	IgG+	IgM+	IgA+	Any +
Anti- β_2 -GPI-positive	55 (13.2%)	121 (28.9%)	93 (22.2%)	186 (44.5%)

for aCL, 49 (26%) had anti- β_2 -GPI (7 IgG, 24 IgM, and 27 IgA) and 44 (23.5%) had LAC.

Prevalence of clinical features in anti- β_2 -GPI-positive patients. Table 4 shows the prevalence of clinical features by anti- β_2 -GPI status.

Importance of persistent positivity for anti- β_2 -GPI. Patients with SLE were seen routinely at 3-month intervals; at the second visit, repeat samples were collected for anti- β_2 -GPI. Thirty-four out of 138 tested twice were persistently positive for anti- β_2 -GPI. Table 5 shows that persistent positivity for anti- β_2 -GPI was more strongly associated with arterial and venous thrombosis for the IgG isotype and with arterial thrombosis for the IgM isotype. Pregnancy loss was not associated with anti- β_2 -GPI of any isotype. Livedo reticularis was only associated with IgG and IgA isotypes.

In data not shown, no assay was associated with seizures or migraines.

Thrombosis. In those with a history of thrombosis (93 patients), anti- β_2 -GPI was detected in 44 patients (47.3%), aCL in 52 (55.9%), and LAC in 53 (57%). In 21 patients with a history of thrombosis negative for both LAC and aCL, 4 had anti- β_2 -GPI (0 IgG, 2 IgM, 3 IgA). In 41 patients with a history of thrombosis who were negative for aCL antibodies, 20 had the LAC and 9 had anti- β_2 -GPI (1 IgG, 2 IgM, and 8 IgA). In 40 patients with a history of thrombosis who were negative for LAC, 10 had anti- β_2 -GPI and 19 had aCL (6 IgG, 17 IgM, and 1 IgA).

DISCUSSION

Anti- β_2 -glycoprotein I was highly prevalent in our series. Strikingly, it was more prevalent in Caucasians than in African Americans. IgM and IgA anti- β_2 -GPI were the most prevalent isotypes. The high prevalence of IgA over other isotypes has been found in SLE¹⁵, APS¹⁶, and cancer¹⁷. Anti- β_2 -GPI was found rarely in our patients who were negative for both aCL and LAC, but was found more often in those negative for just aCL, in agreement with previous studies^{16,18,19}.

Anti- β_2 -GPI is associated with arterial and venous thrombosis. These associations are greater when anti- β_2 -GPI is persistently positive. Thus, the requirement of the Sapporo classification criteria that aCL and LAC assays be positive twice (6 weeks apart) should now be extended to anti- β_2 -GPI. Several groups²⁰⁻²³ have found no association of anti- β_2 -GPI with thrombosis, but many have^{5-9,11,24-30}, as reviewed in Galli, *et al*³¹.

Livedo reticularis was associated with IgG and IgA anti-

Table 1. Precision of the anti- β_2 -GPI assay.

	18.75	Anti- β_2 -GPI Concentration 37.5	75	150
IgG	18.7, 37, 2.1	37, 3.2, 6.5	78, 3.9, 7.6	144, 1.1, 3.2
IgM	18.5, 98, 5.5	39.1, 1.0, 2.7	79, 3.2, 5.8	141, 1.8, 3.3
IgA	17.8, 0.94, 5.3	39, 0.82, 2.1	79.9, 0.77, 4.2	147, 1.0, 3.4

The mean, standard deviation, and coefficient of variation percentage for 10 cumulative runs for each antibody concentration.

Table 3. Distribution of anti- β_2 -glycoprotein I, by ethnicity. Number positive for each anti- β_2 -GPI isotype (percentage of total, N = 418).

Race	Total no.	Anti- β_2 -GPI, IgG+	Anti- β_2 -GPI, IgM+	Anti- β_2 -GPI, IgA+	Anti- β_2 -GPI, Any +
Caucasian	238 (60.3)	35 (8.9)	86 (21.8)	52 (13.2)	113 (27.1)
African American	157 (39.7)	17 (4.3)	32 (8.1)	35 (8.9)	61 (14.6)
p		0.29	0.0008	1.0	0.0982

Table 4. Comparison of clinical features in anti- β_2 -GPI positives and negatives.

	No Anti- β_2 -GPI, n = 233 (%)	Any Anti- β_2 -GPI, n = 185 (%)	Anti- β_2 -GPI, IgG, n = 45 (%)	Anti- β_2 -GPI, IgM, n = 123 (%)	Anti- β_2 -GPI, IgA, n = 98 (%)
Arterial thrombosis	26 (11.1)	23 (12.4)	4 (8.8)	12 (9.7)	16 (16.3)
Venous thrombosis	22 (9.4)	29 (15.7)	8 (17.7)	20 (16.2)	17 (17.3)
Pregnancy loss	61 (26)	42 (22.7)	8 (17.7)	28 (22.8)	22 (22.4)
Thrombocytopenia	45 (19.3)	35 (18.9)	12 (26.6)	20 (16.2)	22 (22.4)
Seizures	16 (6.8)	11 (5.9)	3 (6.6)	5 (4)	7 (7.1)
Migraine	31 (13.3)	31 (16.7)	8 (17.7)	21 (17)	13 (13.2)
Livedo reticularis	38 (16.3)	56 (30)	13 (28.8)	38 (30.8)	30 (30.6)

Table 5. Association of anti- β_2 -GPI with clinical features of APS, based on persistent positivity versus intermittent positivity.

	Persistently Positive vs Intermittently Positive or Negative			Ever Positive vs Negative		
	OR	95% CI	p	OR	95% CI	p
Anti- β_2 -Glycoprotein I IgG						
Arterial thrombosis	5.32	1.33, 21.2	0.0497	3.44	1.14, 10.38	0.0392
Venous thrombosis	6.37	1.82, 22.31	0.0125	2.02	0.78, 5.23	NS
Pregnancy loss	0.38	0.05, 2.92	NS	0.47	0.15, 1.43	NS
Livedo reticularis	1.80	0.43, 7.54	NS	2.91	1.21, 7.00	0.0231
Anti- β_2 -Glycoprotein I IgM						
Arterial thrombosis	5.63	1.40, 22.6	0.0455	1.93	0.52, 7.19	NS
Venous thrombosis	1.73	0.43, 6.98	NS	2.04	0.79, 5.23	NS
Pregnancy loss	1.19	0.30, 4.82	NS	1.25	0.50, 3.10	NS
Livedo reticularis	0.82	0.17, 4.06	NS	1.30	0.50, 3.35	NS
Anti- β_2 -Glycoprotein I IgA						
Arterial thrombosis	3.30	0.90, 12.1	NS	3.04	0.86, 10.7	NS
Venous thrombosis	2.30	0.83, 6.36	NS	1.60	0.61, 4.20	NS
Pregnancy loss	0.61	0.19, 1.93	NS	0.62	0.23, 1.68	NS
Livedo reticularis	1.89	0.69, 5.17	NS	1.30	0.50, 3.35	NS

NS: nonsignificant.

β_2 -GPI. Previous studies have been inconsistent, with one finding an association with IgA²⁶, one with anti- β_2 -GPI, unspecified isotype³², and one finding no correlation³³.

Pregnancy loss was not associated with any isotype of anti- β_2 -GPI, confirming previous negative studies^{25,34,35}. Others have found an association with pregnancy loss^{7,36,37} or preeclampsia³⁸.

This series found a high prevalence of the IgA isotype in anti- β_2 -GPI. Persistent positivity of the IgA isotype was not associated with thrombosis, pregnancy loss, or livedo reticularis. Other studies of IgA anti- β_2 -GPI did not address the issue of persistent positivity^{4,26,39}. These results suggest that

the IgA isotype of anti- β_2 -GPI should not be added to classification criteria.

In the Sydney revision of the Sapporo APS classification criteria, both IgG and IgM anti- β_2 -GPI have been added to the laboratory requirement. Our data do not support the addition of IgM anti- β_2 -GPI in the SLE population. The Sydney revision uses the "3-month" rule of persistent positivity, in contrast to the Sapporo 6-week rule. Ours is the first series to use the 3-month persistent positivity rule.

Comparison of our results with past studies is limited by differences in assays, ethnicity, and selection of patients. We could not address the issue of low versus high affinity anti- β_2 -

GPI. However, the results strongly support the association of persistently positive anti- β_2 -GPI (IgG) with venous and arterial thromboses, and suggest that the addition of anti- β_2 -GPI (IgG) to the classification criteria for APS is valid⁴⁰.

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