The Value of IgA Antiphospholipid Testing for Diagnosis of Antiphospholipid (Hughes) Syndrome in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective***. It is recognized that the presence of IgG and IgM anticardiolipin antibodies (aCL) and lupus anticoagulant (LAC) is associated with thrombosis in patients with antiphospholipid syndrome (APS). Some reports have shown that testing for IgA anticardiolipin and anti-ß$_2$-glycoprotein antibodies (anti-ß$_2$-GPI) provides extra diagnostic help in patients with APS, while other authors could not support this data. We designed this cross sectional study to determine the prevalence of IgA aCL, anti-ß$_2$-GPI, and antiprothrombin antibodies and to study their clinical significance in a large cohort of patients with systemic lupus erythematosus (SLE).

Methods. This study comprised 134 SLE patients (126 women; median age 37.5 yrs, range 16–72). The median duration of the disease was 9 years, range 0.1–38. Of these, 55 (41%) had a history of thrombotic events: 22 (40%) presented an arterial event, 22 (40%) a venous event, and 11 (20%) both arterial and venous events. Of 49 women who had been pregnant, 18 (37%) gave a history of recurrent pregnancy loss. Thrombocytopenia was present in 14/127 patients (11%). Forty patients (30%) were diagnosed as APS secondary to SLE, 23 (17%) had IgG/M aCL and/or LAC without clinical features of APS, and 71 (53%) were SLE patients who were repeatedly negative for IgG/M aCL or LAC. IgG, IgM, IgA aCL and anti-ß$_2$-GPI were detected by ELISA. Antibodies directed to prothrombin were detected by 2 ELISA using prothrombin coated on irradiated plates (aPT) and phosphatidylserine/prothrombin complex (aPS/PT) as antigen.

Results. IgA aCL were found in 18/134 (13%) patients. Of these, 3 (17%) had IgA aCL as well as IgG/M aCL, and 2 (11%) had IgG/M aCL and anti-ß$_2$-GPI. Of the 18 patients positive for IgA aCL, 8 were also positive for LAC. Of these, one (5%) patient had IgA aCL as well as other isotype of aCL, and 7 (39%) patients had both aCL and anti-ß$_2$-GPI. None of these patients had binding of IgA aPT or aPS/PT. Of the entire group of 18 patients, 5 (28%) had IgA aCL as the sole aPL. Four of 5 of these patients were diagnosed as SLE but had no antiphospholipid (aPL) related clinical manifestations. We found no association between the presence of IgA aCL and clinical manifestations of APS. IgA anti-ß$_2$-GPI were found in 8/134 (6%) patients. Of these, one (12.5%) had IgA anti-ß$_2$-GPI as well as IgG/M anti-ß$_2$-GPI and aCL. Of the 8 patients positive for IgA anti-ß$_2$-GPI, 6 (75%) were also positive for LAC. Of these, one (12.5%) patient presented with IgA anti-ß$_2$-GPI along with other isotypes of aCL, and 4 (50%) patients with aCL and other isotype of anti-ß$_2$-GPI. One patient (12.5%) had IgA anti-ß$_2$-GPI along with LAC only, and one patient (12.5%) who was diagnosed as SLE had no aPL related clinical manifestation but had IgA anti-ß$_2$-GPI as the sole aPL.

Conclusion. IgA aCL and anti-ß$_2$-GPI are found in SLE, usually along with IgG and/or IgM isotypes. Testing for IgA aCL and anti-ß$_2$-GPI is not a helpful screening test and does not contribute to the recognition of APS in SLE. IgA aPT and aPS/PT are not present in patients with SLE, therefore there is no need to test for these antibodies. (J Rheumatol 2001;28:2637–43)

Key Indexing Terms:

LUPUS ANTICOAGULANT	THROMBOSIS	ANTIPROTHROMBIN
PREGNANCY LOSS	ANTICARDIOLIPIN	ANTI-ß$_2$-GLYCOPROTEIN I

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The antiphospholipid antibody (aPL) family includes a heterogeneous population of autoantibodies whose specificity is directed against phospholipids and their complex with plasma proteins. Anticardiolipin antibodies (aCL) and lupus anticoagulant (LAC) tests are widely performed to screen aPL, which is associated with thrombotic complications in patients with systemic lupus erythematosus (SLE) or antiphospholipid syn-

drome (APS). It is recognized that the presence of IgG and IgM aCL and LAC is associated with thrombosis and pregnancy loss, making these antibodies essential to classify a patient as APS[1]. It has been shown that these antibodies are directed to plasma proteins bound to anionic phospholipids. The phospholipids may induce some conformational changes in protein structure, thus many of the antibodies against phospholipid binding proteins can be detected in the presence of phospholipids[2-4]. So far, ß$_2$-glycoprotein I (ß$_2$-GPI) and prothrombin are the best known and characterized phospholipid binding proteins[5].

Along with the development of new assays, like those detecting antibodies directed to ß$_2$-GPI (anti-ß$_2$-GPI) or prothrombin (aPT), attention has been focused on IgA aCL and their relationship with thrombotic events. There is still controversy whether patients with features of the APS, negative to other aCL isotypes or LAC, have IgA aCL antibodies and, if so, their clinical significance[6,7]. Some experimental work suggests that IgA aCL are as prothrombotic as the IgG or IgM isotypes[8]. Although some reports showed that testing for IgA aCL was of additional benefit in patients with APS[7], other authors could not support these data[6,9,10].

A number of studies have highlighted the significance of anti-ß$_2$-GPI by ELISA as an alternative assay of conventional aCL ELISA with higher specificity[11-13], but very little is known about the prevalence and clinical significance of the IgA isotype in patients with SLE.

We designed this cross sectional study to determine the prevalence of IgA aCL and anti-ß$_2$-GPI and study their clinical significance in a large cohort of patients with SLE.

MATERIALS AND METHODS

Patients. Our study comprised 134 patients (76% Caucasian, 13% Afro-Caribbean, and 10% Middle-Eastern and Asian), all fulfilling at least 4 of the American College of Rheumatology criteria for the classification of SLE[14]. The median disease duration was 9 years (range 0.1–38). In all, 126 patients were female, with a median age of 37.5 years (range 16–72). Fifty-five patients (41%) gave a history of thrombosis. Of these, only 35 fulfilled the new preliminary criteria for the classification of APS[1]. Twenty-two patients (40%) suffered arterial thrombosis, 22 (40%) venous thrombosis, and 11 (20%) both arterial and venous events. All thrombotic events were confirmed by imaging studies. Forty-nine out of 63 women had been pregnant. Out of 49 women with obstetric history available, 18 (37%) gave a history of pregnancy loss [2 or more miscarriages (≤ 10th week of gestation) and/or fetal loss (≥ 10th week of gestation)]. Of them, 11 patients fulfilled the criteria for APS: 6/11 had a history of pregnancy loss and thrombotic events; 5/11 had only a history of pregnancy loss. Data regarding thrombocytopenia were available in 127/134 patients. Of them, 14 (11%) had a history of thrombocytopenia with a platelet count < 80,000/mm^3 on at least 2 occasions, 3 weeks apart.

Of the entire group of 134 patients, 40 patients (30%) fulfilled the new preliminary criteria for definite APS[1], 23 (17%) had aCL and/or LAC without clinical features of APS, and 71 (53%) were repeatedly negative for aCL/LA.

aCL ELISA. IgG and IgM aCL were determined according to the standardized aCL ELISA[15].

Due to the lack of standardized sera in our laboratory, IgA aCL were tested by an in-house ELISA technique. Briefly, microtiter ELISA plates (Immulon 1, Dynatech Inc., Virginia, USA) were coated with 50 μg/ml bovine cardiolipin (Sigma, Dorset, UK) in ethanol and dried. After blocking with 10% fetal calf serum (Sigma) in phosphate buffered saline (10% FCS-PBS), serum diluted 1:50 in 10% FCS-PBS was added in duplicate. After incubation and washes with PBS, alkaline phosphatase conjugated goat anti-human IgA was added in the appropriate dilution. Color was developed by adding 100 μl of 1 mg/ml of p-nitrophenylphosphate disodium in 1 M diethanolamine buffer (pH 9.8). IgA aCL titer of each sample was derived from the standard curve according to the dilutions of positive IgA control, which showed high IgA binding to cardiolipin but low binding to control well without antigen, suggesting that IgA antibody was appropriately detected, and converted to units. The cutoff point for IgA assays was established by the mean + 5 SD of 100 controls.

anti-ß$_2$-GPI ELISA. IgG and IgM anti-ß$_2$-GPI were detected by ELISA using irradiated ELISA plates (Nunc Maxisorp, Denmark) as described[13].

IgA anti-ß$_2$-GPI were detected by an in-house ELISA. Briefly, microtiter ELISA plates (Maxisorp) were coated with 4 μg/ml human ß$_2$-GPI (Yamasa Co., Choshi, Japan) in PBS or PBS alone and incubated overnight at 4°C. After blocking with 1% bovine serum albumin (BSA; Sigma), 0.1% Tween 20® (Sigma) in PBS (1% BSA-0.1% Tween-PBS), serum diluted 1:100 in 1% BSA-0.1% Tween-PBS was added in duplicate. After incubation and washes with PBS-0.1% Tween, alkaline phosphatase conjugated goat anti-human IgA was added in the appropriate dilution. Color was developed by adding 100 μl of 1 mg/ml of p-nitrophenylphosphate disodium in 1 M diethanolamine buffer (pH 9.8). IgA anti-ß$_2$-GPI titer of each sample was derived from the standard curve according to the dilutions of a positive IgA control that showed high IgA binding to ß$_2$-GPI but low binding to control well without antigen, suggesting that IgA antibody was appropriately detected, and converted to units. The cutoff point for IgA anti-ß$_2$-GPI assays was established by the mean + 5 SD of 112 controls.

Antiprothrombin antibodies. Antibodies directed against prothrombin are commonly detected by ELISA methods, using irradiated plates (aPT)[16] or in complex with phosphatidylserine (aPS/PT)[17]. aPT and aPS/PT were detected by ELISA as reported[16,17].

Lupus anticoagulant. As many of the patients were taking warfarin at the time of the study, data regarding LAC were those historically present in the patients' clinical records before starting anticoagulation therapy. LAC was screened using activated partial thromboplastine time (aPTT) and dilute Russell's viper venom time (dRVVT), and confirmed according to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibodies[18].

Statistical analysis. Statistical analysis was performed using SPSS 7.5 program. Categorical analysis was determined by chi-square test. Comparisons between patients groups were expressed as relative risk with its 95% confidence interval. Comparisons between patients and controls were expressed as odds ratio with its 95% CI. All p values were determined by Fisher's exact test. A p value < 0.05 was considered statistically significant.

RESULTS

Prevalence of aCL and anti-ß$_2$-GPI. aCL were present in 55/134 (41%) SLE patients. Of these, 28/55 (51%) had IgG aCL only, 3 (5%) IgM aCL only, and 5 patients (9%) IgA aCL only. Eight patients (15%) were positive for IgG, IgM, and IgA aCL isotypes, 6/55 (11%) for IgG and IgM aCL, and 5/55 (9%) for IgG and IgA isotypes. IgA aCL were also more frequent in patients with SLE than in controls [18/134 vs 2/100; OR 7.6 (95% CI 1.7-33.6); p = 0.0017]. Distribution of IgA aCL in patients with SLE and controls is shown in Figure 1A.

Anti-ß$_2$-GPI were present in 28/134 (21%) patients with SLE. Of these, 16 (57%) were positive for IgG isotype only, 3 (12%) for IgM only, and 3 (12%) for IgA anti-ß$_2$-GPI only. Two (7%) patients were positive for IgG, IgM, and IgA anti-ß$_2$-GPI isotypes, 1/28 (3%) for IgG and IgM anti-ß$_2$-GPI, and

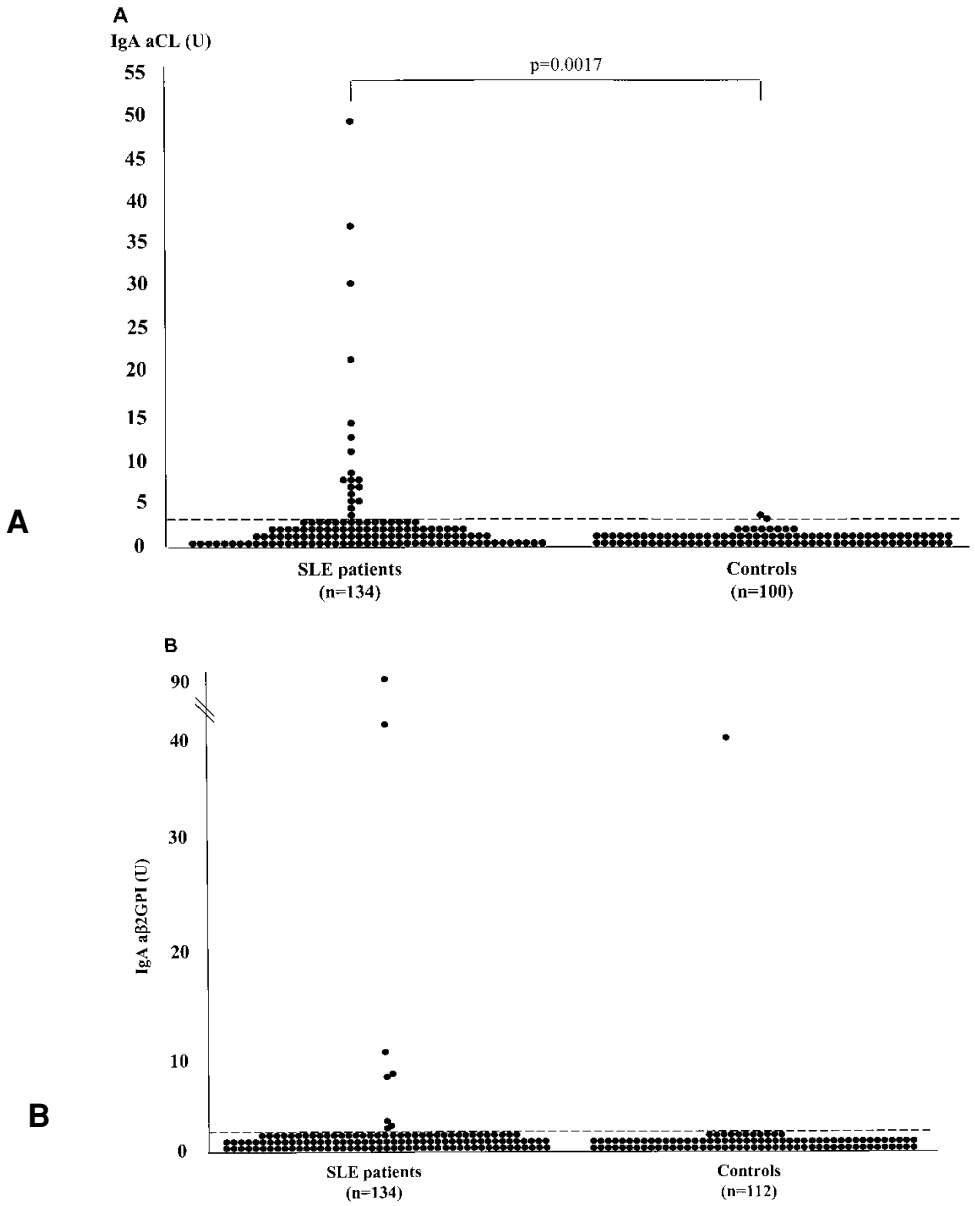

Figure 1. Distribution of IgA anticardiolipin (A) and anti-ß$_2$-glycoprotein I (B) in patients with SLE and in controls.

3/28 (9%) for IgG and IgA isotypes. IgA anti-ß$_2$-GPI was also more frequent in patients with SLE than in controls but the difference was not statistically significant [8/134 (6%) vs 1/112 (0.9%); OR 7.0 (95% CI 0.8-57.3); p = NS]. Distribution of IgA anti-ß$_2$-GPI in patients with SLE and controls is shown in Figure 1B.

LAC was present in 34/117 (29%) patients. Twenty (59%) presented LAC along with other aPL, whereas 14 patients (41%) presented only LAC.

Prevalence of aPT and aPS/PT. aPT were present in 36/134 (27%) patients with SLE. Of these, 20/36 (55.5%) had IgG aPT only, 9 (25%) IgM aPT only, and 7 patients (19.5%) IgG and IgM aPT. No patient showed IgA binding to prothrombin coated on irradiated plates.

aPS/PT were tested in 87 SLE patients. Of these, 26 (30%) had aPS/PT. Ten out of 26 patients (38%) had IgG aPS/PT only, 8 (31%) IgM aPS/PT only, and 8 patients (31%) IgG and IgM aPS/PT. No patient showed IgA binding to prothrombin-phosphatidylserine complex.

Prevalence of IgA aCL and anti-β$_2$-GPI and association with other aPL. IgA aCL were found in 18/134 (13%) patients. Of these, 3 (17%) had IgA aCL along with IgG/M aCL and 2 (11%) with IgG/M aCL and anti-ß$_2$-GPI. Of the 18 patients positive for IgA aCL, 8 were also positive for LAC. Of these, one (5%) patient had IgA aCL along with another isotype of aCL and 7 (39%) patients along with aCL and anti-ß$_2$-GPI. Of the entire group of 18 patients, 5 (28%) presented IgA aCL as the sole aPL.

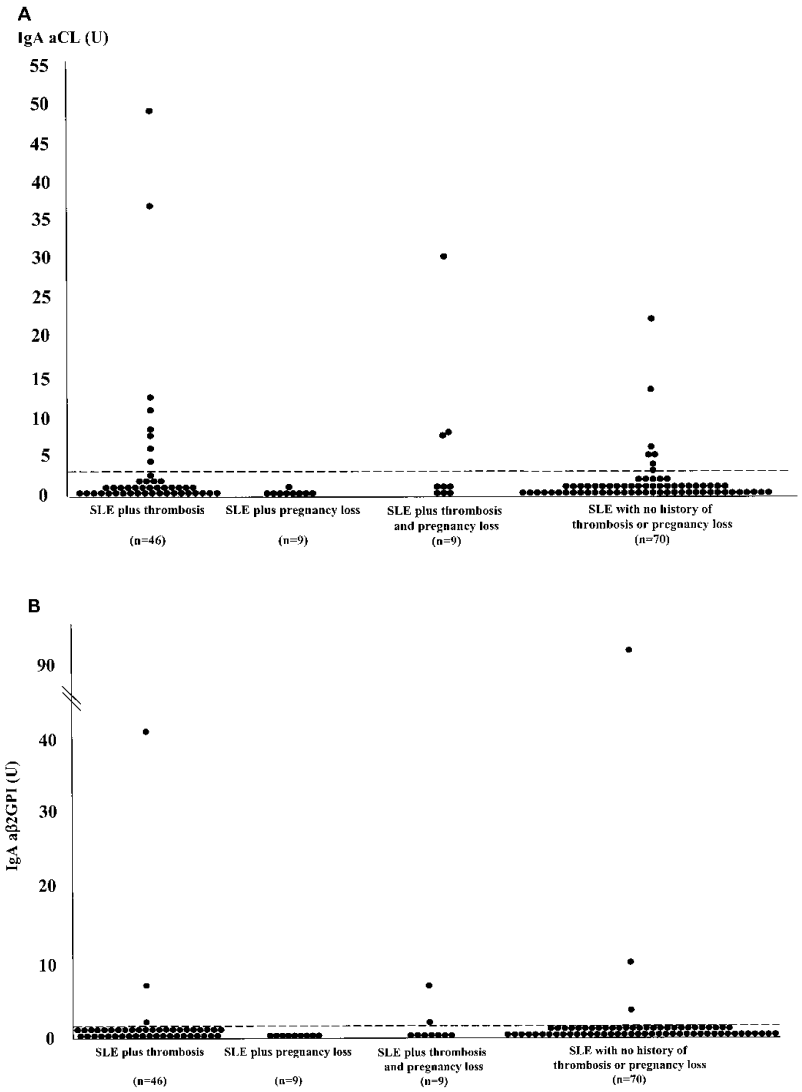

Figure 2. Distribution of IgA anticardiolipin (A) and anti-ß$_2$-glycoprotein I (B) in SLE patients with/without thrombosis and/or pregnancy loss.

IgA anti-ß$_2$-GPI were found in 8/134 (6%) patients. Of these, one (12.5%) had IgA anti-ß$_2$-GPI along with IgG/M anti-ß$_2$-GPI and aCL. Of the 8 patients positive for IgA anti-ß$_2$-GPI, 6 (75%) were also positive for LAC. Of these, one (12.5%) had IgA anti-ß$_2$-GPI along with other isotype of aCL and 4 (50%) patients along with aCL and other isotype of anti-ß$_2$-GPI. One patient (12.5%) had IgA anti-ß$_2$-GPI along with LAC only. Of the entire group of 8 patients, only one (12.5%) had IgA anti-ß$_2$-GPI as the sole aPL.

Relationship of IgA aCL and anti-ß$_2$-GPI to thrombosis and to pregnancy loss. The presence of IgA aCL was more frequent in patients with thrombosis (11/55, 20%) than in those without (7/79, 9%), but the difference was not statistically significant ($p > 0.5$). IgA aCL was more frequent in patients with arterial thrombosis than in those without [9/33, 27% vs 9/101, 9%; relative risk (RR) 2.4 (95% CI 1.3-4.3); $p = 0.015$], but the association was not retained when only the thrombosis group (n = 55) was analyzed [9/33, 28% with venous thrombosis vs 2/22, 9% with arterial thrombosis; RR 1.5 (95% CI 1.0-2.2); p = NS]. No differences were found when the presence of IgA aCL was analyzed in patients with venous thrombosis. The frequency of IgA aCL was similar in patients with venous thrombosis compared to those without (5/33, 15% vs 13/88, 14%; p = NS). When only the thrombosis group was analyzed, we found no significant association between the presence of IgA aCL in patients with venous thrombosis compared to those without (arterial thrombosis) (5/33, 15% vs 2/22, 27%; p = NS). No associations were found between the presence of IgA aCL and pregnancy loss. The frequency of IgA aCL was not different in patients with pregnancy loss compared to those without (3/18, 17% vs 5/31, 16%, respectively; $p > 0.5$).

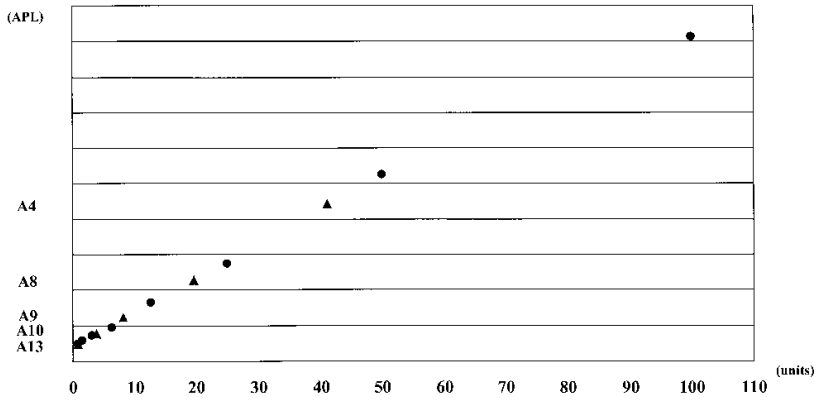

Figure 3. Correlation between APL standard calibrators and in-house standard. ●: in-house standard; ▲: APL calibrators (Louisville APL Diagnostics, Inc.). A4 = 120 APL, A8 = 40 APL, A9 = 20 APL, A10 = 10 APL, and A13 = 2.7 APL.

Four out of 5 patients who had IgA aCL as the sole aPL were diagnosed as SLE without APS and they did not present any aPL related clinical features. Only Patient 5 was diagnosed as SLE and had had arterial thrombosis in the past. IgA aCL distribution is shown in Figure 2A.

The presence of IgA anti-ß$_2$-GPI was more frequent in patients with thrombosis (5/55, 9%) than in those without (3/79, 3.8%), but the difference was not statistically significant [RR 1.6 (95% CI 0.8-2.8); p = NS]. IgA anti-ß$_2$-GPI was also more frequent in patients with arterial thrombosis but the difference was not statistically significant [3/33, 9% vs 5/101, 5%; RR 1.6 (95% CI 0.6-4.1); p = NS].

No differences in frequency were found when the presence of IgA anti-ß$_2$-GPI was analyzed in patients with venous thrombosis or pregnancy loss and compared with those without (3/33, 9% and 2/18, 11% vs 5/101, 5% and 1/31, 3%, respectively; p = NS).

The patient who had IgA anti-ß$_2$-GPI as the sole aPL was diagnosed as SLE without APS and did not present any aPL related clinical features. IgA anti-ß$_2$-GPI distribution is shown in Figure 2B.

DISCUSSION

In our study we evaluated the prevalence and clinical significance of IgA aCL, anti-ß$_2$-GPI, and antiprothrombin antibodies as alternative additive risk factors for the well established IgG and/or IgM aCL and LAC in a large cohort of patients with SLE.

Our data showed a low prevalence of IgA aCL in patients with SLE. IgA aCL were present in 13% of this SLE population. There have been few studies of the prevalence and clinical associations of IgA aCL. Gharavi, *et al*[6] were the first to determine the distribution of immunoglobulin isotypes and phospholipid specificities of aCL in 40 patients with one or more of the following "aPL associated clinical complications," namely, thrombosis, fetal loss, and thrombocytopenia. They found IgA aCL in 52% of their population. Twelve of 40 patients had IgG, IgM, and IgA aCL, 5 patients had IgG and IgA, and 3 patients had IgM and IgA aCL. Only one patient had IgA aCL as the sole aPL; thus this test was concluded to be useful to identify occasional patients with APS. Kalunian, *et al*[19] studied 85 patients with SLE. They found IgA aCL to be the most predictive test of thrombosis and fetal loss. However, their results could not be confirmed[20-22]. Weidmann, *et al*[20] found no association between the presence of IgA aCL and clinical features of APS in a series of 92 patients with SLE. Merkel, *et al*[21] studied 386 patients with a variety of connective tissue diseases (CTD) (including 70 SLE patients) and 33 patients with APS. Although they found that IgA aCL were more frequent in patients with APS (15%) than in patients with CTD (0.5%), they failed to demonstrate any association between IgA aCL and clinical features of APS. Escalante, *et al*[22] reported that IgA aCL have poor diagnostic accuracy compared to IgG aCL when they studied 113 patients with SLE.

On the other hand, Lopez, *et al*[7] suggested that IgA aCL had value in identifying patients at risk for thrombosis or fetal loss. However, this observation was based on results from 6/27 patients with SLE, where low positive values were found in 3/6 of the "positive" patients. Molina, *et al*[23] studied 152 African-American, 136 Afro-Caribbean (Jamaican), and 163 Hispanic (Colombian) unselected patients with SLE. The major finding of this study was the higher prevalence of IgA aCL in the Afro-Caribbean population (21%), IgA aCL being the sole isotype, detected in 82% of these positive patients. This isotype was usually detected at low titers and did not seem to be associated with clinical features of APS. However, in 1999 Diri, *et al*[24] reported 8 Afro-American female patients with the APS, in which IgA aCL were present in 7, co-occurring with IgG or IgM isotype in 4 of them. In the same study they also found IgA anti-ß$_2$-GPI in 4/8 patients, co-occurring with IgM isotype in 3 of them. In a recent review, Wilson, *et al*[25] indicated that IgA aCL are common in SLE, often at low/moderate titers and often transient, requiring longitudinal

studies to facilitate the assessment of their clinical significance.

A recent study by Lockshin, *et al*[26] suggested that measurement of IgA aCL may identify APS in some patients. In our study we found no consistent association between the presence of IgA aCL and clinical features of APS. Although IgA aCL were more frequently present in patients with arterial thrombosis than in those without, this isotype was frequently found along with IgG and/or IgM aCL or LAC. In the study, of those 5 patients who presented IgA aCL as the sole aPL, 4 were diagnosed as SLE without APS and they did not present any aPL related clinical features.

Regarding anti-$ß_2$-GPI, recent studies showed that the IgA isotype is very frequent in patients with SLE. Fanopoulos, *et al*[27] reported a prevalence of IgA anti-$ß_2$-GPI of 58% in patients with SLE. Tsutsumi, *et al*[28] found IgA anti-$ß_2$-GPI in 25% of SLE patients. Cucurull, *et al*[29] reported that IgA anti-$ß_2$-GPI was the most prevalent isotype (19%) among the African-American patients with SLE. However, our results in a large cohort of SLE showed a small percentage of positives, since IgA anti-$ß_2$-GPI were present in 6% of our population. These discrepancies may be due to heterogeneity of the population included in the studies (i.e., ethnicity) and/or differences in the methods of detection. Of interest, in our population, 76% of the patients were Caucasian with a very low prevalence of Afro-Caribbean (13%), therefore, our findings are in agreement with those of Weidmann, *et al*[20] and Escalante, *et al*[22], in which no clinical correlation of the IgA isotype was found and the predominant ethnic groups were Caucasian. Regarding methodology, in this study all samples were tested in duplicate in the presence/absence of antigen and subtraction of nonspecific binding was performed to evaluate the positivity of the samples. In our IgA assays, a positive control showed high IgA binding to $ß_2$-GPI [optical density (OD) 1.5 ± 0.07), but low binding to control well without antigen (OD 0.16 ± 0.003) (data not shown), suggesting that IgA antibody was appropriately detected. However, the standardized sera for this assay were lacking and the sensitivity of the detection may differ between laboratories. Figure 3 shows the correlation between APL standard calibrators and our in-house standard.

Clinically, the presence of IgA anti-$ß_2$-GPI has been associated with APS manifestations. Abinader, *et al*[30] described a patient with SLE who developed extensive vascular thrombosis with positivity for IgA anti-$ß_2$-GPI, suggesting that IgA anti-$ß_2$-GPI could be responsible for catastrophic APS. Fanopoulos, *et al*[27] reported that IgA anti-$ß_2$-GPI but not aCL was associated with APS. Tsutsumi, *et al*[28] studied 124 Japanese patients with SLE and found that the presence of IgA anti-$ß_2$-GPI was significantly related to the presence of thromboses in these patients. IgA anti-$ß_2$-GPI have been also reported in patients with pregnancy loss, one of the features of APS. Yamada, *et al*[31] studied 63 women with 3 or more spontaneous abortions and compared their results with 52 nonpregnant healthy, fertile women. They found that the frequency of IgA anti-$ß_2$-GPI was higher in patients with recurrent spontaneous abortion (13.9%) than in controls (0%). Lee, *et al*[32] recently reported that IgA anti-$ß_2$-GPI are significantly elevated and frequent in patients with recurrent spontaneous abortion and fetal death who initially tested negative for aCL and LAC, concluding that testing for IgA anti-$ß_2$-GPI may identify women with APS who are not identified by traditional screening tests (i.e., aCL and LAC). In our study, we found no association between the presence of IgA anti-$ß_2$-GPI and arterial/venous thrombosis or pregnancy loss.

To our knowledge, this is the first report where IgA antiprothrombin antibodies have been studied. We failed to observe binding of aPT or aPS/PT to prothrombin coated on irradiated plates or to phosphatidylserine-prothrombin complex, respectively. Therefore, we suggest that these antibodies are not present in patients with SLE.

From our cross sectional data we can conclude that IgA aCL and anti-$ß_2$-GPI are found in SLE, usually along with IgG and/or IgM isotypes. Testing for IgA aCL and anti-$ß_2$-GPI is not suitable for screening purposes and does not further contribute to the recognition of APS in SLE. IgA aPT and aPS/PT are not present in patients with SLE, therefore there is no need for routine testing for these antibodies.

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