EXTENDED REPORT

Antiphospholipid antibody tests: spreading the net

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Objective: To examine the hypothesis that testing for new antiphospholipid antibody specificities may help to identify the antiphospholipid syndrome (APS) in patients with systemic lupus erythematosus (SLE) with thrombosis who are repeatedly negative for anticardiolipin antibodies (aCL) and/or lupus anticoagulant (LA).

Methods: Three groups of patients with SLE were studied: (*a*) SLE/APS (n = 56): 51 female, mean (SD) age 46 (11) years, fulfilling 1999 Sapporo criteria for the APS; (*b*) SLE/thrombosis (n = 56): 53 female, age 42.6 (12) years, all with a history of thrombosis and persistently negative for aCL and/or LA; (*c*) SLE only (n = 56): 53 female, age 40 (11) years, without a history of thrombotic events. aCL and LA were retested in all samples. All patients were tested for anti-β_2-glycoprotein I (anti-β_2GPI) and antiprothrombin antibodies (aPT) by coating prothrombin on irradiated plates or using phosphatidylserine-prothrombin complex as the antigen (aPS-PT).

Results: Anti-β_2GPI were only present in patients from the SLE/APS group, all of whom were also positive for aCL. aPT and aPS-PT were also more commonly found in SLE/APS than in SLE/thrombosis or SLE only groups (54% *v* 5%, $p<0.0001$ or *v* 16%, $p<0.0001$ for aPT and 63% *v* 2%, $p<0.0001$ or *v* 11%, $p<0.0001$ for aPS-PT, respectively). No differences were found between SLE/thrombosis and SLE only groups ($p = 1.5$ for β_2GPI, $p = 0.1$ for aPT, and $p = 0.1$ for aPS-PT).

Conclusion: Testing for aPT in patients with SLE with thrombosis, but persistently negative for aCL and LA, may be helpful in some selected cases. Anti-β_2GPI are not present in patients who are negative for aCL.

The antiphospholipid syndrome (APS) is a thrombophilic disorder characterised by arterial and/or venous thrombosis and/or pregnancy morbidity, associated with the presence of a specific group of autoantibodies called antiphospholipid antibodies (aPL).[1] In clinical practice, anticardiolipin antibodies (aCL) detected by enzyme linked immunosorbent assay (ELISA) and the lupus anticoagulant (LA) detected by clotting assays are the most widely used and standardised tests for the detection of aPL. The aCL test is positive in about 80% of these patients, the LA is the only positive test in about 20%, and both are positive in about 60% of cases. These assays detect a heterogeneous group of antibodies that bind serum proteins such as β_2-glycoprotein I (β_2GPI) or prothrombin, or protein/phospholipid complexes. The observation that many aCL are directed at an epitope on β_2GPI led to the development of the anti-β_2GPI immunoassay.[2] Anti-β_2GPI are strongly associated with thrombosis and other features of the APS.[3] Indeed, in rare patients with clinical features of APS, anti-β_2GPI antibodies have been reported as the sole antibodies detected.[4]

Several authors have suggested that testing for new aPL specificities may help to identify the APS in patients with systemic lupus erythematosus (SLE) with thrombosis but repeatedly negative for conventional aCL or LA, or both. However, their clinical usefulness remains unclear.[5] We designed this study to analyse the potential clinical usefulness of testing for new aPL specificities in patients with SLE with thrombosis who are persistently negative for the routinely used aCL and LA tests.

PATIENTS AND METHODS

Patients

This study comprised 168 consecutive patients, all attending the Lupus Clinic at St Thomas' Hospital, who fulfilled at least four of the American College of Rheumatology criteria for the classification of SLE.[6] Clinical records were carefully reviewed and patients were interviewed at the time of sample collection. Ethical approval was obtained from the Guy's and St Thomas's ethics committee (EC00/101) and all patients taking part in this study gave their written consent.

Patients were included in three groups according to their clinical and laboratory characteristics: (*a*) SLE/APS: if the patients fulfilled the 1999 Sapporo criteria for the APS,[7] having had a thrombotic event; (*b*) SLE/thrombosis: if they had a history of thrombosis but were persistently negative for aCL or LA, or both (at least three times, 6 weeks apart); and (*c*) SLE only: if the patients did not have a history of thrombotic events or pregnancy morbidity, regardless of their aPL status.

Blood collection

Blood was collected by venepuncture from the antecubital vein into precooled tubes containing 0.105 M sodium citrate and in non-anticoagulated tubes (Hemogard 9NC and Hemogard Z, respectively; Becton Dickinson, Rutherford, USA). Platelet-free plasma was obtained by centrifugation at 2500 *g* for 20 minutes and filtration using a 0.2 μm surfactant free cellulose acetate membrane (Nalgene, Rochester, NY, USA). Plasma was stored at −80°C until used. All samples from the groups with thrombosis were taken at least 3 months after the thrombotic event.

aPL testing

All patients were retested for IgG and IgM aCL according to the standardised technique.[8] The cut off point for aCL was established at 2GPL and 3.2MPL, respectively. LA was screened using activated partial thromboplastin time (from synthetic phospholipids; IL test APTT-SP; Instrumentation

Abbreviations: aCL, anticardiolipin antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; aPS-PT, phosphatidylserine-prothrombin complex; aPT, antiprothrombin antibodies; β_2GPI, β_2-glycoprotein I; CI, confidence interval; LA, lupus anticoagulant; OR, odds ratio; SLE, systemic lupus erythematosus

Table 1 Demographic and clinical characteristics of patients with SLE

Characteristics	SLE/APS (n = 56)	SLE/thrombosis (n = 56)	SLE only (n = 56)
Female	51	53	53
Age, mean (SD)	46 (11)	42.6 (12)	40 (11)
Thrombosis	50	56	0
Arterial only	22	10	0
Venous only	14	41	0
Arterial+venous	14	5	0

Laboratory, Italy) and dilute Russell's viper venom time (dRVVT test; American Diagnostica Inc), and confirmed according to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid dependent Antibodies.[9] Testing of samples was carried out using the Automated Coagulation Laboratory 700 (Instrumentation Laboratory, Milan, Italy).

All patients were tested for IgG and IgM anti-β_2GPI using human purified β_2GPI coated on irradiated plates[3] and antiprothrombin antibodies (aPT) by coating human purified prothrombin on irradiated plates or using phosphatidylserine-prothrombin complex as the antigen (aPS-PT), as previously reported.[10–12]

Statistical analysis

All statistical analysis was performed using the SPSS 11.0 program (Microsoft software). Comparisons between patients groups were expressed as an odds ratio with its 95% confidence interval (OR (95% CI)), where a lower limit >1.0 was considered significant. Differences between means were analysed by the Mann-Whitney test. All p values were determined by Fisher's exact test. A p value of <0.05 was considered significant.

RESULTS

Demographic and clinical characteristics of patients with SLE

Patients were included in three groups comprising (*a*) SLE/APS (n = 56): 51 female, mean (SD) age 46 (11) years, all fulfilling 1999 Sapporo criteria for the APS[7]; (*b*) SLE/thrombosis (n = 56): 53 female, mean (SD) age 42.6 (12) years, all with history of thrombosis (41 venous, 10 arterial, and 5 both venous and arterial thrombosis) and persistently negative for aCL or LA, or both; and (*c*) SLE only (n = 56): 53 female, mean age 40 (11) years, without a history of thrombotic events. Table 1 summarises the demographic and clinical characteristics of the patients.

Patients with SLE/APS were significantly older than patients with SLE only (p = 0.003). However, no differences in age were found between patients with SLE/thrombosis and SLE/APS (p = 0.1) or SLE only (p = 0.2).

There was no difference in the number of patients with thrombotic events between the SLE/APS and SLE/thrombosis groups (89% *v* 100%; p = 0.06). However, more patients in the SLE/APS group had arterial thrombosis than in the SLE/thrombosis group (64% *v* 27%; p<0.0001) and more patients had venous thrombosis in the SLE/thrombosis group than in the SLE/APS group (82% *v* 50%; p = 0.0006).

Apart from aPL, 34/56 (61%) patients with SLE/APS had other risk factors for thrombosis, including: hyperlipidaemia (n = 9), diabetes (n = 1), hypertension (n = 21), oral contraceptive pill/hormone replacement therapy (n = 7), smoking (n = 8), low protein S or protein C (n = 2), and factor V Leiden (n = 2).

Other conditions known to increase the risk of thrombosis were present in 36/56 (64%) patients with SLE/thrombosis. Acquired, often transient, conditions were present in 33 patients: immobilisation/surgery (n = 3), hyperlipidaemia (n = 5), diabetes (n = 2), hypertension (n = 17), oral contraceptive pill/hormone replacement therapy (n = 9), breast cancer (n = 2), pregnancy (n = 2), and obesity (n = 1). Coagulation abnormalities were present in five patients: low protein S or protein C (n = 3), prothrombin mutation (n = 1), and factor V Leiden (n = 1). Twenty patients from the SLE/thrombosis group had no recognisable risk factor for thrombosis.

There was no difference in the prevalence of other risk factors for thrombosis between patients with SLE/APS and SLE/thrombosis.

Prevalence of aPL in SLE subgroups

Table 2 shows the prevalence of aPL in the three SLE subgroups. By inclusion criteria no patients from the SLE/thrombosis group had aCL or LA. As expected, aCL were more frequently found in patients with SLE/APS than in those with SLE only (84% *v* 27%, OR = 14.3 (95% CI 5.6 to 36), p<0.0001). IgG and IgM aCL were more frequently found in patients with SLE/APS than in those with SLE only (77% *v* 25%, OR = 9.9 (95% CI 4.2 to 23), p<0.0001 and 41% *v* 2%, OR = 38.3 (95% CI 4.9 to 297), p<0.0001; respectively). Levels of IgG and IgM aCL were higher in patients with SLE/APS than in SLE only (mean (SD) 30.1 (33.5) *v* 1.5 (1.2), p<0.0001 and 15.4 (30) *v* 0.3 (0.4), p = 0.0004; respectively). Figure 1 shows the aCL distribution.

LA was present in 25/56 (45%) patients with SLE/APS and 8/56 (14%) patients with SLE only.

IgG and IgM anti-β_2GPI were only positive in patients from the SLE/APS group, all of whom were also positive for aCL. Levels of IgG anti-β_2GPI were higher in patients with SLE/APS than in those with SLE/thrombosis and SLE only (mean (SD) 20.4 (31) *v* 1.3 (0.01), p<0.0001 and *v* 1.4 (0.3), p<0.0001; respectively). Levels of IgM anti-β_2GPI were also higher in patients with SLE/APS than in those with SLE/thrombosis and SLE only (6.6 (21) *v* 1.0 (0.2), p = 0.05 and *v* 0.7 (0.4), p = 0.04; respectively) (fig 2).

aPT were more frequently found in SLE/APS than in SLE/thrombosis or SLE only groups (54% *v* 5%, OR = 20 (95% CI 6 to 73), p<0.0001 or *v* 16%, OR = 6 (95% CI 2.5 to 15), p<0.0001; respectively).

Table 2 Prevalence of aPL in SLE

aPL	SLE/APS (n = 56) (%)	SLE/thrombosis (n = 56) (%)	SLE only (n = 56) (%)
aCL	47 (84)	0 (0)	15 (27)
IgG	43	0	14
IgM	23	0	1
LA	25 (45)	0 (0)	8 (14)
Anti-β_2GPI	33 (59)	0 (0)	0 (0)
IgG	27	0	0
IgM	10	0	0
aPT	30 (54)	3 (5)	9 (16)
IgG	28	2	8
IgM	3	1	1
aPS-PT	35 (63)	1 (2)	6 (11)
IgG	26	0	5
IgM	22	1	1

aCL, anticardiolipin antibodies; LA, lupus anticoagulant; anti-β_2GPI, antibodies to β_2-glycoprotein I; aPT, antibodies to solid phase prothrombin; aPS-PT, antibodies to phosphatidylserine-prothrombin complex. By inclusion criteria no patients from the SLE/thrombosis group had aCL or LA.

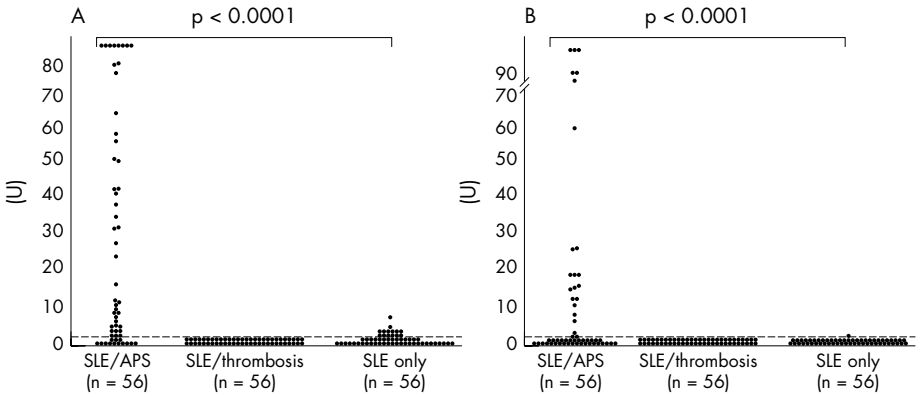

Figure 1 Distribution of (A) IgG and (B) IgM aCL in SLE.

IgG aPT were more frequently found in patients with SLE/APS than in the SLE/thrombosis or SLE only groups (50% *v* 4%, OR = 27 (95% CI 6 to 122), p<0.0001 or *v* 14%, OR = 6 (95% CI 2.5 to 15), p<0.0001, respectively). However, the prevalence of IgM aPT did not differ between the SLE/APS group and the SLE/thrombosis or the SLE only group (5% *v* 2%, OR = 3 (95% CI 0.3 to 31), p = 0.6 for both comparisons). Levels of IgG aPT were higher in patients with SLE/APS than in those with SLE/thrombosis and SLE only (mean (SD) 16.5 (27) *v* 2.1 (2.2), p<0.0001 and *v* 2.9 (2.8), p = 0.0003; respectively). Although levels of IgM aPT were also higher in patients with SLE/APS than in those with SLE/thrombosis and SLE only (mean (SD) 3.7 (5.9) *v* 1.9 (3.4) and *v* 2.2 (1.6)), the differences were not statistically significant (fig 3).

aPS-PT were also more frequently found in SLE/APS than in SLE/thrombosis or SLE only groups (63% *v* 2%, OR = 92 (95% CI 12 to 712), p<0.0001 or *v* 11%, OR = 14 (95% CI 5 to 38) p<0.0001, respectively).

IgG aPS-PT were more frequently found in patients with SLE/APS than in the SLE/thrombosis or SLE only groups (46% *v* 0%, OR = 50 (95% CI 6 to 383), p<0.0001 or *v* 9%, OR = 9 (95% CI 3 to 25), p<0.0001, respectively). IgM aPS-PT were also more frequently found in patients with SLE/APS than in the SLE/thrombosis or SLE only groups (39% *v* 2%, OR = 36 (95% CI 5 to 276), p<0.0001 or *v* 2%, OR 36 (95% CI 5–276), p<0.0001, respectively). Levels of IgG aPS-PT were higher in patients with SLE/APS than in those with SLE/thrombosis and SLE only (mean (SD) 25.6 (40) *v* 1.5 (0.1), p<0.0001 and *v* 1.7 (0.7), p<0.0001; respectively). Levels of IgM aPS-PT were also higher in patients with SLE/APS than in those with SLE/thrombosis and SLE only (mean (SD) 22.9 (33) *v* 3.8 (10), p = 0.0001 and *v* 2.9 (2.2), p<0.0001; respectively)(fig 4).

No differences in the prevalence of anti-β_2GPI, aPT, or aPS-PT were found between SLE/thrombosis and SLE only groups (0% *v* 0%, p = 1.5 for β_2GPI; 5% *v* 16%, p = 0.1 for aPT, and 2% *v* 11%, p = 0.1 for aPS-PT).

DISCUSSION

In this study we assessed the value of testing for new aPL specificities as an aid to identify the APS in patients with SLE with thrombosis but repeatedly negative for conventional aCL or LA, or both.

Laboratory diagnosis of APS is based on a positive aCL antibody or LA test. The aCL test is positive in about 80% of these patients, the LA is the only positive test in about 20%, and both are positive in about 60% of cases. Correct identification of these patients is important, because prophylactic anticoagulant treatment can prevent thrombosis from recurring,[13] and treatment of affected women during pregnancy can improve fetal and maternal outcome.[14]

A history of thrombosis has been reported in 7.2–12% of patients with SLE.[15] Further, the mortality from thrombosis in SLE has been found to be 27%.[16] However, in daily practice it is not unusual to find patients with these manifestations but persistently negative for aCL or LA, or both. In these cases, testing for other aPL specificities such as β_2GPI or prothrombin could help in recognising further patients with APS. Although their presence is not currently included in the criteria for the APS, anti-β_2GPI and aPT have been associated with thrombosis and other features of the APS.[3 10 11] Indeed,

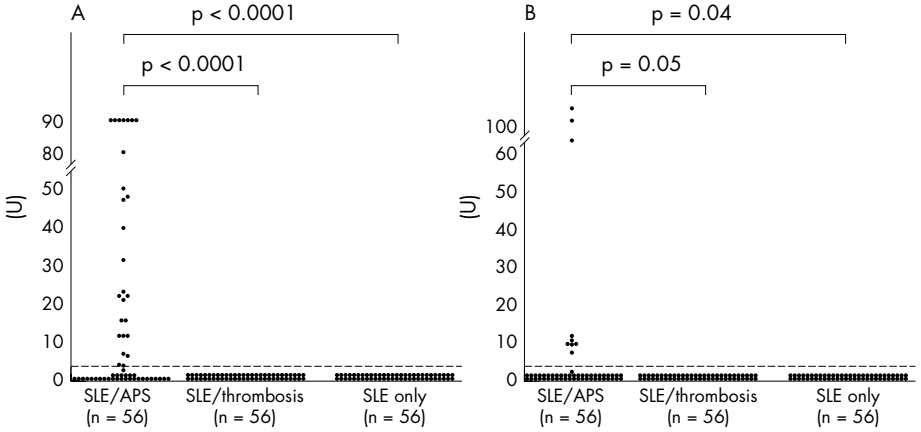

Figure 2 Distribution of (A) IgG and (B) IgM anti-β_2GPI in SLE.

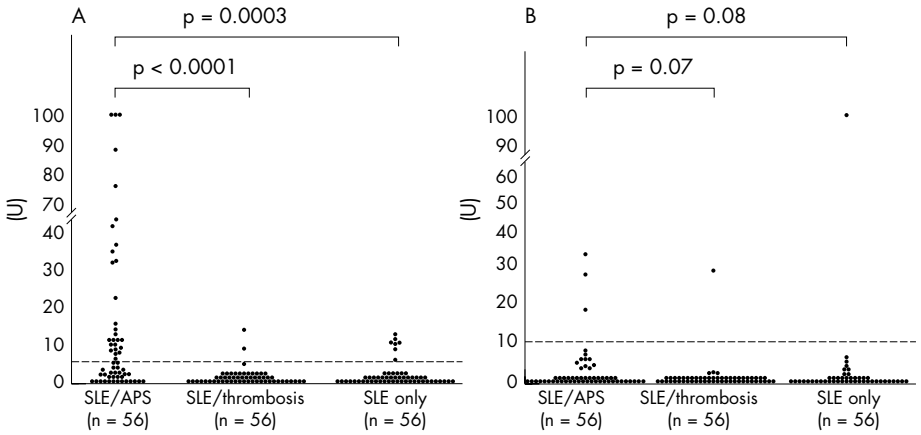

Figure 3 Distribution of (A) IgG and (B) IgM aPT in SLE.

in rare patients with clinical features of APS, anti-β_2GPI antibodies are the sole antibodies detected.[4 17 18]

Antibodies against β_2GPI in the absence of cardiolipin were shown in patients with SLE or primary APS, and they correlated with aCL.[19 20] Cabiedes *et al* found that anti-β_2GPI are present in 89.7% of patients with SLE with clinical manifestations of APS and especially in aPL negative patients (88.9%),[17] suggesting that in some patients with APS anti-β_2GPI differ from aCL.[21] Other authors have reported the presence of anti-β_2GPI in aCL negative patients,[22] suggesting that clinically important autoantibodies to β_2GPI may not be detected by the standard aCL assay. In this study, anti-β_2GPI were not found in the absence of aCL, supporting the hypothesis that aCL associated with APS recognise cryptic epitope expressed on β_2GPI.[3]

Although some of the patients from the SLE/thrombosis group had other congenital or acquired risk factors for thrombosis, a high percentage did not have any concomitant factor that would explain the thrombotic event by itself. A close follow up of those patients may help to clarify this point.

Overall, our data suggest that tests for aCL and LA, the only antibodies strongly associated with thrombosis, should be carried out for the laboratory diagnosis of APS. The inclusion of an isolated positivity for anti-β_2GPI as laboratory criterion for the diagnosis of the APS and in the absence of aCL is not supported by our data. However, testing for aPT may be helpful in some selected cases.

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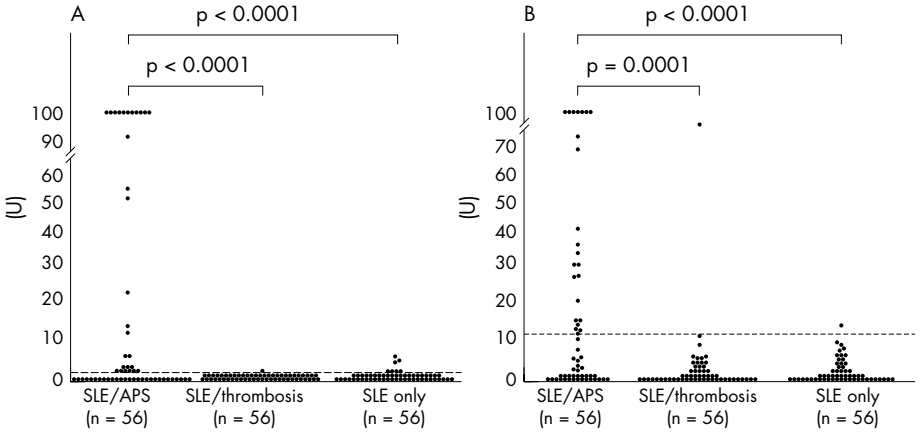

Figure 4 Distribution of (A) IgG and (B) IgM aPS-PT in SLE.

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