CONCISE REPORT

Antiphospholipid antibodies and non-thrombotic manifestations of systemic lupus erythematosus

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Objectives: The aim of this study was to investigate the association between antiphospholipid antibodies and non-thrombotic and non-gestational manifestations of systemic lupus erythematosus.

Methods: Systemic lupus erythematosus patients with persistently positive antiphospholipid antibodies or lupus anticoagulant were identified and grouped as systemic lupus erythematosus with antiphospholipid syndrome (SLE-APS), systemic lupus erythematosus with positive antiphospholipid antibodies/lupus anticoagulant without antiphospholipid syndrome (SLE-aPL), and systemic lupus erythematosus with negative aPLs (SLE-No aPL). Groups were compared in terms of non-thrombotic systemic lupus erythematosus manifestations and laboratory features retrospectively.

Results: A total of 150 systemic lupus erythematosus patients, 26 with SLE-APS, 25 with SLE-aPL, and 99 with SLE-No aPL, were identified. Livedo reticularis, neurologic involvement, and thrombocytopenia were more common in antiphospholipid antibody positive systemic lupus erythematosus cases. Malar rash, arthritis, and pleuritis were more common in the SLE-No aPL, SLE-APS, and SLE-aPL groups, respectively. Positivity rates and titers of specific antiphospholipid antibodies did not differ between the SLE-APS and SLE-aPL groups.


Key words: Antiphospholipid antibody; antiphospholipid syndrome; lupus anticoagulant; systemic lupus erythematosus

Introduction

Antiphospholipid antibodies (aPLs) were first described as predictors of thrombosis in patients with systemic lupus erythematosus (SLE).1 Antiphospholipid syndrome (APS) was defined as a distinct disease entity not much later, with frequent overlap with SLE,2 although some considered it as a SLE-spectrum disease.3 Since then, clinical and laboratory features of both primary APS and APS secondary to SLE have been studied extensively in APS and SLE cohorts, and in metaanalyses.4–6 Apart from thrombosis and pregnancy complications, which are the principal clinical features of APS, aPLs in SLE have been shown to be associated with valvular heart disease,7 livedo reticularis,8 thrombocytopenia,9 hemolytic anemia,10 renal impairment,11 and neuropsychiatric disease.12 aPL associations of non-thrombotic manifestations of SLE are still less well studied.

The aim of this study was to investigate the association between aPLs and non-thrombotic and non-gestational manifestations of SLE.

Patients and methods

Past laboratory results of all SLE patients meeting 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria,13 aged 18 years or older, referred between...
January 2014 and January 2016, followed up at the Department of Rheumatology, Ankara University Medical School Ibn-i Sina Hospital, were checked for persistently positive (at least twice and 12 weeks apart) aPLs (anticardiolipin [aCL] IgG and IgM, anti-β2-glycoprotein I [aβ2GPI] IgG, IgM, and IgA, and antiphosphatidylyserine [aPS] IgG, IgM, and IgA) and lupus anticoagulant (LA). Patients were grouped as SLE with APS (SLE-APS), SLE with persistently positive aPLs without APS (SLE-aPL), and SLE with negative aPLs (SLE-No aPL). APS classification was based on the 2006 Sydney criteria.Age, sex, durations of SLE, APS, and aPL positivity, presence of photosensitivity, malar rash, discoid rash, alopecia, livedo reticularis, oral ulcers, Raynaud’s phenomenon, arthritis, lupus nephritis, proteinuria (>500 mg per gram of urinary creatinine), hematuria, peritonitis, pancreatitis, interstitial lung disease, pleuritis, pulmonary hypertension, alveolar hemorrhagia, splenomegaly, and use of steroids, hydroxychloroquine, and potent immunosuppressive medications were recorded for each patient. Diagnoses of SLE manifestations relied upon standard clinical, laboratory, radiological, and histopathological methods when symptoms or signs were not best explained by other possible causes like infections or drugs. Positivity, titer, and staining pattern of antinuclear antibodies (ANA), anti-dsDNA titer, presence of autoantibodies against nuclear antigens (Sm, nucleosome, histone, PCNA, PO, SS-A 60 kDa, SS-A 52 kDa, SS-B, CENP-B, Scl-70, U1-RNP, Jo-1, PM/Scl, Mi-2, Ku), serum titers of C3 and C4, and direct Coombs test were also evaluated. All laboratory tests were performed at the Biochemistry, Hematology, and Immunology Laboratories of Ankara University Medical School, Ibn-i Sina Hospital using standard methods. Patients with overlap with other connective tissue diseases were not included in the study.

aPL and LA assays

Serum titers of all aPLs were measured using standardized commercial ELISA kits (Euroimmun AG, Lübeck, Germany). Respective cut-off values of 40 GPL and 40 MPL for aCL IgG and IgM, and >99th percentile cut-off for aβ2GPI IgG and IgM were used both as laboratory criteria for APS and titer criteria for “criteria test positivity” in the SLE-APS group. Persistent non-criteria aPL test positivity in the SLE-APS group was considered in cases of any degree of positivity above the reference range of aβ2GPI IgA, aPS IgG, IgM, and IgA at least 12 weeks apart. Persistent aPL positivity in the SLE-aPL group was considered if any degree of positivity above the reference range of any aPL was present at least 12 weeks apart. Integrated activated partial thromboplastin time (aPTT) test was used for LA detection according to updated International Society on Hemostasis and Thrombosis (ISHT) guidelines. Every sample outside the normal range was considered positive, as advised by the international APS consensus, and LA ratio was used as a quantitative measure.

Statistics

Clinical and laboratory findings were compared among groups. All the statistical analyses were performed using IBM SPSS Statistics, version 21. Data were described as numbers and percentages for categorical variables, and as means ± standard deviations or medians with interquartile ranges (IQRs) for continuous variables. Analyses between two or more categorical variables were performed using chi-square or Fisher’s exact tests. Odds ratios (ORs) with 95% confidence intervals were given where appropriate. Mann Whitney U or Kruskal Wallis tests were used for comparison of variables with non-normal distributions and t-tests or one-way ANOVA for normally distributed ones. p values less than 0.05 were considered statistically significant.

Results

A total of 295 SLE patients were identified, referred within the study period. Of these, 150 had serial (at least twice and 12 weeks apart) aPL measurements and were included in the study. Fifty-one had persistently positive aPLs or LA. A total of 26, 25, and 99 patients were classified into the SLE-APS, SLE-aPL, and SLE-No aPL groups, respectively. In the SLE-APS group, nine (34.6%) and 16 (61.5%) patients had arterial and venous thromboses, respectively, and nine of 23 females (39.1%) in this group had pregnancy-related complications. Demographic data and clinical and laboratory
features that differed between groups are given in Table 1. Pooled analysis of the SLE-APS and SLE-aPL groups and comparison with the SLE-No aPL group revealed, in addition to higher rates of livedo reticularis, pleuritis, neurologic involvement, thrombocytopenia, lower rate of malar rash and lower serum C4, that SLE patients with positive aPLs or LA have higher rates of endocarditis (OR = 5.2 [1–28.2], p = 0.045) and cytoplasmic staining patterns in an ANA test (OR = 2.2 [1–4.6], p = 0.039). Other clinical manifestations, laboratory features, and medication uses have similar distributions among groups in both separate analyses between APS and livedo reticularis, although positivity rates, titers, and durations of positivity of specific aPLs are similar between groups (Table 2). This is why we felt the need to further classify aPL positive SLE patients as SLE-APS and SLE-aPL. The associations between APS and livedo reticularis, thrombocytopenia, and neurologic disease are well known. They are also known to be more positive LA (p = 0.03), and respective mean LA ratios were 1.91 ± 0.44 and 1.77 ± 0.39 for LA positive cases (p = 0.43). Worthy of note, all patients with livedo reticularis (n = 5) had positive LA and no patient had livedo reticularis without LA positivity (p < 0.001).

**Discussion**

aPL or LA positive SLE cases differ from aPL or LA negative cases in terms of clinical and laboratory manifestations other than thrombosis or pregnancy complications. Not all of these differences are explainable by the higher prevalence of features that may be APS-related (i.e. the non-criteria APS features like cardiac valvular disease, livedo reticularis, thrombocytopenia, and neuropsychiatric disease) in aPL positive SLE patients. Moreover, some features differ significantly between SLE-APS patients and aPL positive SLE patients with no accompanying APS (SLE-aPL group) in our cohort (Table 1), although positivity rates, titers, and durations of positivity of specific aPLs are similar between groups (Table 2). This is why we felt the need to further classify aPL positive SLE patients as SLE-APS and SLE-aPL. The associations between APS and livedo reticularis, thrombocytopenia, and neurologic disease are well known. They are also known to be more

### Table 1 Comparison of demographic, clinical, and laboratory features of groups

<table>
<thead>
<tr>
<th>Feature</th>
<th>SLE-APS n = 26</th>
<th>SLE-aPL n = 25</th>
<th>SLE-No aPL n = 99</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.7 ± 12</td>
<td>44.5 ± 14</td>
<td>46.6 ± 12</td>
</tr>
<tr>
<td>Female sex (n, %)</td>
<td>23 (92)</td>
<td>22 (84.6)</td>
<td>23 (92.9)</td>
</tr>
<tr>
<td>SLE duration (years)</td>
<td>7.2 ± 6.5</td>
<td>9.1 ± 6.9</td>
<td>10.9 ± 8.8</td>
</tr>
<tr>
<td>Livedo reticularis n (%, IQR)</td>
<td>4 (15)%</td>
<td>1 (4)%</td>
<td>0%</td>
</tr>
<tr>
<td>Malar rash n (%, IQR)</td>
<td>7 (26.9)%</td>
<td>9 (36)%</td>
<td>60 (60.6)%</td>
</tr>
<tr>
<td>Arthritis n (%, IQR)</td>
<td>14 (53.8)%</td>
<td>5 (20)%</td>
<td>35 (35.4)</td>
</tr>
<tr>
<td>Pleuritis n (%, IQR)</td>
<td>2 (7.7)%</td>
<td>5 (20)%</td>
<td>3 (3)%</td>
</tr>
<tr>
<td>Neurologic involvement n (%, IQR)</td>
<td>5 (19.2)%</td>
<td>5 (20)%</td>
<td>6 (6.1)%</td>
</tr>
<tr>
<td>Nucleolar ANA n (%, IQR)</td>
<td>9 (34.6)</td>
<td>11 (44)%</td>
<td>18 (18.2)%</td>
</tr>
<tr>
<td>Serum C4 &lt; 8 mg/L</td>
<td>0.13</td>
<td>0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>&lt;U-IgG (GPL U/C6)</td>
<td>(IQR 0.12)</td>
<td>(IQR 0.12)</td>
<td>(IQR 0.19)</td>
</tr>
</tbody>
</table>

*Age and SLE duration are given as means ± standard deviations, and serum C4 as median with interquartile range (IQR).
+a and b indicate pairs with significant differences in the same row (adjusted p value < 0.05 for post hoc comparisons).

### Table 2 Comparison of aPL profiles of SLE-APS and SLE-aPL groups

<table>
<thead>
<tr>
<th>Feature</th>
<th>SLE-APS n = 26</th>
<th>SLE-aPL n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positivity n (%, Median titer)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCL IgG</td>
<td>11 (42.5)</td>
<td>11 (44)</td>
</tr>
<tr>
<td>aCL IgM</td>
<td>10 (38.5)</td>
<td>14 (56)</td>
</tr>
<tr>
<td>aβ2GPI IgG</td>
<td>11 (42.5)</td>
<td>12 (48)</td>
</tr>
<tr>
<td>aβ2GPI IgM</td>
<td>8 (30.8)</td>
<td>11 (44)</td>
</tr>
<tr>
<td>aβ2GPI IgA</td>
<td>7 (26.9)</td>
<td>6 (24)</td>
</tr>
<tr>
<td>aPS IgG</td>
<td>3 (11.5)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>aPS IgM</td>
<td>6 (23.1)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>aPS IgA</td>
<td>2 (7.7)</td>
<td>2 (8)</td>
</tr>
</tbody>
</table>

*Median titers are calculated for only positive cases for specific aPL tests, given with interquartile ranges (IQRs), and expressed as GPL U/ml for IgG class aPLs, MPL U/ml for IgM class aPLs, U/ml for aβ2GPI IgA, and RU/ml for aPS IgA.

There are no statistically significant differences between positivity rates and titers of aPLs between groups.
common in aPL positive SLE patients than aPL negative ones and have been suggested for inclusion in APS criteria. The findings of lower rates of malar rash and higher rates of arthritis in the SLE-APS group, and higher rates of pleuritis, and lower C4 serum levels in the SLE-aPL group in our cohort (Table 1) reflect the fact that presence of APS and aPLs may, additionally, have some relationship with SLE manifestations other than non-criteria APS features. The presence of APS or aPLs without APS seems not to have the same impact on the above mentioned SLE manifestations. The differences in the ANA staining profiles may be a clue to the different underlying immunopathogenetic mechanisms in the SLE-APS and SLE-aPL groups, considered together with the observation that most patients with SLE plus aPLs do not develop APS. aPLs are known to have an effect on the cutaneous expression of SLE but a negative association between malar rash and aPLs has not been reported before. It is an interesting observation that the prevalence of aPLs among patients with subacute or chronic cutaneous lupus was similar to that of the general population. Hypocomplementemia was previously shown to be more frequent in SLE patients with positive aPLs than those with negative aPLs and to be associated with disease features like hemolytic anemia that may be APS-related. Data on inflammatory features of SLE like arthritis and pleuritis and aPL positivity are not consistent between studies. Interestingly, despite the use of stricter criteria, aPL titer did not predict APS retrospectively in SLE cases with long standing aPL positivity in this cohort. This may be due to the mostly transient nature of low titer aPLs in our SLE cohort, preventing us from classifying these cases as SLE-aPL.

Due to the relatively low number of patients and retrospective cross-sectional design, it was not possible to show a causal relationship between aPLs and non-thrombotic manifestations in this study.

In conclusion, presence of APS or persistent aPLs may be related to non-thrombotic and non-gestational SLE manifestations. Patients with SLE plus APS and persistent aPLs without APS also differ in terms of SLE manifestations.

### Declaration of conflicting interests

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### References

Technical Task Force Report on Antiphospholipid Syndrome


