Lupus anticoagulant and anticardiolipin antibodies in systemic lupus erythematosus

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ABSTRACT

Background. Lupus anticoagulant and anticardiolipin antibodies are antiphospholipid antibodies which have been independently associated with a high incidence of thrombotic diseases. However, the importance of their combined occurrence has not yet been examined.

Methods. We investigated 70 patients with systemic lupus erythematosus for the presence of anticardiolipin antibodies paying particular attention to a history of thrombosis and abortion. Lupus anticoagulant was detected using the kaolin clotting time, its mixing tests with normal plasma and the inosithin neutralization test. Anti-cardiolipin antibodies were detected using the ELISA technique.

Results. Lupus anticoagulant was detected in 11 patients (16%) and anticardiolipin antibodies in 13 (19%). Six patients were positive for both lupus anticoagulant and anticardiolipin antibodies. These patients had a higher incidence of thrombosis or recurrent abortion (5 of 6) compared to those with lupus anticoagulant (2 of 5) or anticardiolipin antibodies alone (1 of 7). The amount of inosithin required to neutralize lupus anticoagulant was greater [mean (SD) 27.5 (20.5) µg] in patients with both lupus anticoagulant and anticardiolipin antibodies than in patients with lupus anticoagulant alone [mean (SD) 4.0 (5.4) µg].

Conclusion. The presence of lupus anticoagulant is associated with thrombosis and recurrent abortion which are more frequent when both lupus anticoagulant and anticardiolipin antibodies are present and these patients probably have more severe disease as the amount of inosithin required to neutralize the lupus anticoagulant was greater.


INTRODUCTION

Lupus anticoagulant (LA) and anticardiolipin antibodies (ACA) are both acquired antiphospholipid antibodies present in systemic lupus erythematosus (SLE) or related autoimmune diseases. They have been found to be associated with thrombosis and recurrent foetal wastage. However, most large series are accumulations of smaller reports from different parts of the world and have not correlated the occurrence of LA and ACA with thrombotic manifestations or severity of disease.

PATIENTS AND METHODS

We investigated 70 patients (67 females and 3 males) aged between 13 and 40 years, for the presence of LA and ACA. They had been diagnosed to have SLE by the American Rheumatism Association criteria. We excluded those who had taken drugs such as chlorpromazine, procainamide, quinidine or immunosuppressants.

Detection of LA

Blood samples were collected in trisodium citrate (9:1 ratio of blood: citrate) and platelet poor plasma (PPP) was prepared by centrifugation at 2000 g for 15 minutes. The activated partial thromboplastin time (APTT) was carried out in all patients. Inosithin (Asoletin), a soya bean-derived phospholipid (Associate concentrates, New York, USA) was used as the phospholipid source for activated partial thromboplastin time (APTT). Patients who had a prolonged DRVVT (>27 seconds) or KCt (normal 70–120 seconds) which was not corrected by the addition of an equal volume of normal plasma were considered to be positive for LA. In these patients the LA concentration was measured by the inosithin neutralization test as described earlier. Briefly, after increasing concentrations of inosithin were added to KCt, the LA concentration was calculated from the minimum amount of inosithin required to correct the prolonged KCt.

Detection of ACA

The presence of ACA was detected using the sandwich ELISA technique wherein the wells on the microtitre plate were coated with cardiolipin. Binding of ACA to the plate was detected using alkaline phosphatase conjugated IgG. The degree of binding was assessed by the extent of substrate turnover which resulted in a yellow colour. Estimation of ACA in the test sera was done in 15 patients, using an ACA kit (Cheshire Diagnostics, England). The optical density (OD) of the test sample was measured using standard dilutions plotted against the IgG concentration in IgG phospholipid (GPL) units. A value of more than 10 GPL was considered to be positive. In the other 55 patients (because the date of our Cheshire Diagnostics kit had expired), ELISA was performed using ACA (Sigma Chemicals, New Jersey, USA).
TABLE I. Patient profile and results of tests in the 11 patients positive for lupus anticoagulant

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Anticardiolipin antibodies</th>
<th>Amount of inosithin required (μg per ml of plasma)</th>
<th>Thrombosis or recurrent abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>F</td>
<td>+</td>
<td>50</td>
<td>Recurrent abortions (5/5)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>M</td>
<td>+</td>
<td>12.5</td>
<td>Deep vein thrombosis, pulmonary embolism</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>F</td>
<td>+</td>
<td>12.5</td>
<td>Recurrent abortions (8/8)</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>F</td>
<td>+</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>F</td>
<td>+</td>
<td>50</td>
<td>Recurrent abortions (3/4)</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>F</td>
<td>+</td>
<td>12.5</td>
<td>Recurrent abortions (9/9)</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>F</td>
<td>-</td>
<td>0.39</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>F</td>
<td>-</td>
<td>0.19</td>
<td>Livido reticularis</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>F</td>
<td>-</td>
<td>0.78</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>F</td>
<td>-</td>
<td>12.5</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>13</td>
<td>F</td>
<td>-</td>
<td>6.25</td>
<td>Thrombotic seizures</td>
</tr>
</tbody>
</table>

Figures in parentheses are the number of recurrent abortions/number of total pregnancies nd not done

The OD of the test samples was compared with those of 20 normal samples [mean (SD) OD: 3498 (0.05)] and a value greater than mean ±3SD (OD>0.5) was considered to be positive. In the absence of standards, this however, could not be further quantified.

Statistical methods
The differences between the groups were assessed using Wilcoxon's Rank Sum test.

RESULTS
LA was detected in 11 out of 70 (16%, 10 females, 1 male) patients. In all of them, the inosithin neutralization test also confirmed the presence of LA. Its assay revealed that the minimum amount of inosithin required to convert the prolonged KCT varied between 0.19 μg and 50 μg per ml of plasma. Seven of these 11 patients had a history of thrombosis and recurrent abortions (more than 3 consecutive foetal losses; Table I). ACA were present in 13 (19%; 12 females, 1 male) patients of the 70 and a history of recurrent abortions was obtained in 6. Based on the presence of LA or ACA, the following groups were identified:

Group I, positive LA and ACA
Six patients were positive for both LA and ACA. In these patients, the amount of inosithin required to correct the prolonged KCT varied from 12.5 μg to 50 μg per ml of plasma [mean (SD) 27.5 (20.5)]. A history of thrombosis or recurrent abortions was obtained in 5 patients. One of them had a history of deep vein thrombosis with pulmonary embolism and 4 had had more than three abortions (Table I).

Group II, positive LA only
Five patients had a positive LA and negative ACA test. The amount of inosithin required to correct the KCT in them varied from 0.19 μg to 12.5 μg per ml of plasma [mean (SD) 4.02 (5.37)]. The difference in the inosithin required to neutralize KCT between groups I and II was statistically significant (p<0.05). In contrast to group I a history suggestive of thrombosis was present in only 2 patients.

Group III, positive ACA only
Seven patients had a positive ACA but negative LA test. A history of recurrent abortions was present in 1.

Group IV, negative ACA and LA
Fifty-two patients were negative for both ACA and LA. Only 1 of them had had recurrent abortions (Table II).

DISCUSSION
Our findings suggest that although LA and ACA are distinct antiphospholipid antibodies, their presence together reflects severe disease and their serial quantitation might be of help in the management of patients with SLE.

The prevalence of LA and ACA in these patients was very similar, being 16% and 18% respectively. The average prevalence of LA in SLE has been reported to vary from 10% to 34% in other studies done in western populations, whereas that for ACA is 44%. Although the prevalence of LA in our study was comparable to what others have reported, we found a lower prevalence of ACA. This is known to occur in patients who are treated with steroids but none of ours had received steroids prior to being tested. We need to study
more patients to determine whether or not this is an ethnic variation.

We found that 6 patients had both ACA and LA, 7 had only ACA and 5 only LA. Similar observations have been made earlier.\textsuperscript{10} LA and ACA have been physically separated, suggesting that the two antibodies are biochemically distinct.\textsuperscript{11,12}

We found an increased prevalence of thrombosis and/or recurrent abortions associated with the presence of LA or ACA. and this has been reported by other workers.\textsuperscript{7} These complications occurred most often in patients who had both antibodies and were less frequent in those with LA or ACA alone. This may be because group I had a higher concentration of LA indicating they had more severe disease. Thus, quantitative assay of LA by the inosithin neutralization test may provide additional information to assess the severity of the disease and its response to therapy. A similar test to quantitate ACA is needed to compare its concentration with that of LA.

REFERENCES