SHORT REPORT

ANTI-β2 GLYCOPROTEIN I ANTIBODIES: A USEFUL MARKER FOR THE ANTIPHOSPHOLIPID SYNDROME

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SUMMARY

We studied anti-β2-glycoprotein I antibodies (aβ2-GPI) in autoimmune disease patients to evaluate their relationship to clinical findings. Seventy-nine systemic lupus erythematosus (SLE) patients [44 with antiphospholipid antibodies (aPL)], 21 with primary antiphospholipid syndrome (APS), eight asymptomatic individuals with aPL and 60 controls were studied. Sixteen SLE patients (14 with aPL and two without aPL) and six with primary APS had aβ2-GPI. A significant relationship was found between aβ2-GPI and aPL (P < 0.01). In SLE, a significant correlation was found between previous thrombosis or thrombocytopenia and aβ2-GPI or aβ2-GPI + aPL, but not between fetal losses and aβ2-GPI. These data suggest that aβ2-GPI may be useful in the study of APS.

KEY WORDS: β2-glycoprotein I, Anti-β2-glycoprotein I antibodies, Antiphospholipid antibodies, Anticardiolipin antibodies, Lupus anticoagulant, Systemic lupus erythematosus, Primary antiphospholipid syndrome, Thrombosis, Thrombocytopenia.

ANTIPHOSPHOLIPID antibodies (aPL) are frequently detected in patients with autoimmune diseases or other conditions [1]. In autoimmune disorders, aPL [anticardiolipin antibodies (aCL) and lupus anticoagulant (LA)] are associated with thrombosis, fetal losses and thrombocytopenia constituting the antiphospholipid syndrome (APS) [2]. However, in infectious diseases, aPL are usually not associated with clinical manifestations [1, 2]. The reasons why only some patients with aPL develop APS have not been elucidated.

aCL bind cardiolipin in the presence of their cofactor β2-glycoprotein I (β2-GPI) [3–6]. β2-GPI inhibits the coagulation cascade and platelet function [7, 8], and binds to negatively charged phospholipids [5–6]. Whether aCL recognize a cryptic epitope on β2-GPI or the epitope consists of both β2-GPI and cardiolipin is still open to discussion [4, 9–11]. The discovery of antibodies against β2-GPI (aβ2-GPI) in systemic lupus erythematosus (SLE) and primary APS patients [12–18] suggests that β2-GPI may play a role in the pathogenesis of APS. We determined aβ2-GPI in a series of patients with autoimmune diseases to evaluate their relationship to clinical manifestations.

METHODS

Patients

We studied 108 patients: (i) 79 with SLE [19]: including 44 with aPL (40 female/4 male, mean age 41.3 ± 15.7 yr) and 35 without aPL (32 female/3 male, mean age 37.8 ± 13.2 yr); (ii) 21 with primary APS [2] (18 female/3 male, mean age 29.9 ± 5.4 yr); (iii) eight asymptomatic individuals with aPL (eight female, mean age 32.0 ± 2.7 yr). Twenty-one patients (14 with SLE and seven with primary APS) had a history of thrombosis confirmed by venogram or arteriography. Twenty-five women had fetal losses (nine with SLE and 16 with primary APS) and 32 patients had a history of thrombocytopenia (29 with SLE and three with primary APS). Controls were 60 healthy volunteers (31 female/29 male, mean age 35.7 ± 19.4 yr) without autoimmune diseases, bleeding disorders, thrombosis or pregnancy losses.

Samples were drawn in trisodium citrate and in non-anticoagulated tubes (Becton Dickinson, Rutherford, NJ, USA). Platelet-free plasma was obtained by double centrifugation [first at 2000 g (10 min, 22°C) and then at 5000 g (10 min, 4°C)], frozen, and stored at −70°C.

Detection of aβ2-GPI by ELISA

Microtitre plates Maxisorp (Nunc, Roskilde, Denmark) were coated (16 h, 4°C) with 100 µl of 25 µg/ml human β2-GPI (Behringwerke, Marburg, Germany) in 16 mmol/l Na2CO3 /34 mmol/l NaHCO3 (pH 9.6). This coating concentration showed the best differentiation between specific binding and background. After washing with phosphate-buffered saline + 0.1% Tween 20 (Merk, Munchen, Germany) (PBS–TWEEN), coated plates were blocked (1 h, 22°C) with 300 µl of 5% bovine serum albumin (BSA) (Sigma, St Louis, MO, USA) in PBS–TWEEN. The wells were then incubated (1 h, 22°C) with 200 µl of samples diluted in PBS–TWEEN + 1% BSA (PBS–TWEEN–BSA). Plates were finally incubated (1 h, 22°C) with 200 µl of horseradish peroxidase-conjugated anti-human IgG and IgM (Dako, Glostrup, Denmark) in PBS–TWEEN–BSA. For colour developing, 200 µl of 0.04% o-phenylenediamine dihydrochloride (Sigma) diluted in phosphate citrate buffer (pH 5.0) containing 0.0075% H2O2 (Sigma) were added. After incubation (10 min, 22°C), the reaction was stopped with 50 µl of 2 mol/l H2O.

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Lupus anticoagulant. Anti-β2-glycoprotein I antibodies; APS, antiphospholipid syndrome; LA, antibodies; A-aPL, asymptomatic individuals with aPL; aCL, antiphospholipid antibodies; SLE, systemic lupus erythematosus.

Tests were also performed in mixtures with control plasmas or phospholipids [22].

Statistical analysis
Results are shown as mean ± s.d. Associations were assessed by χ² test with Yates’ correction. Sensitivity, specificity and odds ratios (OR) were determined by contingency tables. Intra- and interassay coefficients of variation were calculated as recommended [23].

RESULTS
Results of aβ2-GPI are shown in Table I. Sixteen SLE patients (20.2%) and six primary APS (28.6%), but no asymptomatic individuals with aPL or controls, had aβ2-GPI. Patients with aPL had a significantly higher prevalence of aβ2-GPI than those without aPL (P < 0.01). This relationship was also found in the SLE group (P < 0.01). Only two patients (5.7%) without aPL had aβ2-GPI. A relationship was found between aCL and aβ2-GPI in both IgG and IgM isotypes (P < 0.01). However, no increased presence of aβ2-GPI was observed in patients with LA but not aCL.

In SLE, thrombotic episodes were related to aβ2-GPI (P < 0.01) in both IgG and IgM aβ2-GPI isotypes, and to aβ2-GPI + aPL (P < 0.01) (Table II). Specificity for thrombosis was higher using aβ2-GPI than aPL. A combination of both aPL and aβ2-GPI showed the best OR for thrombosis. No relationship was found between thrombosis and aβ2-GPI in patients with primary APS (2/6 with aβ2-GPI vs 5/15 without aβ2-GPI).

Thrombocytopenia was related to aβ2-GPI (P < 0.01) and to aβ2-GPI + aPL (P < 0.01) in SLE (Table II), but not in primary APS patients (2/6 vs 1/15, NS). IgM aβ2-GPI (P < 0.05), but not IgG, was related to thrombocytopenia. Specificity for thrombocytopenia was higher using aβ2-GPI. Positivity in both aPL and aβ2-GPI showed the best OR for thrombosis. No relationship was observed between aβ2-GPI and fetal losses either in SLE (Table II) or in primary APS (5/5 with aβ2-GPI vs 11/13 without aβ2-GPI).

DISCUSSION
aβ2-GPI have been found in APS with prevalence ranging from 0 to 90% [4, 9–10, 12–18, 24]. In our series, aβ2-GPI prevalence was 32% in SLE aPL-positive patients, 6% in SLE aPL-negative, and 29% in

### TABLE I

<table>
<thead>
<tr>
<th>aβ2-GPI</th>
<th>Total</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG + IgM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Controls</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A-aPL</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SLE</td>
<td>79</td>
<td>2</td>
<td>11</td>
<td>3</td>
<td>16 (20.2%)</td>
</tr>
</tbody>
</table>

Table I: Anti-β2-glycoprotein 1 antibodies

| aβ2-GPI + aPL | 9      | 0   | 1   | 2         | 5     |

### TABLE II

<table>
<thead>
<tr>
<th>aβ2-GPI</th>
<th>Thrombosis</th>
<th>Thrombocytopenia</th>
<th>Fetal losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>S (%)</td>
<td>E (%)</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>14</td>
<td>50.0</td>
</tr>
<tr>
<td>aβ2-GPI</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aβ2-GPI</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aβ2-GPI</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01.
aβ2-GPI, anti-β2-glycoprotein 1 antibodies; aPL, antiphospholipid antibodies; S, sensitivity; E, specificity; OR, odds ratio.
primary APS. Differences in patient populations and in laboratory methodology, mainly β2GPI concentration and the use of irradiated plates, may be the reason for these discrepancies [24]. It has been suggested that high coating β2GPI concentrations might magnify the effect of phospholipid contamination [24]. However, aβ2GPI have been found using low concentrations of β2GPI [15, 16]. aβ2GPI have been determined in APS patients using irradiated [13–14, 18] and non-irradiated [12, 15–17] polystyrene plates. Although a more specific binding has been suggested in irradiated plates [13], it seems that irradiation should work by increasing the density of antigen sites [25]. In our assay, we obtained a good differentiation between specific binding and background using irradiated plates.

Despite the association between aPL and aβ2GPI [12–18], two aPL-negative patients had aβ2GPI and 70% patients with aβ2GPI. These results, and the differences in aCL and aβ2GPI isotype distribution, indirectly suggest that aCL and aβ2GPI conform to two populations of antibodies that are related but not equivalents.

We observed an association between aβ2GPI and thrombosis, as reported by others [12–14, 16–18], and also between aβ2GPI and thrombocytopenia, which has been less commonly described [14, 17, 18]. It is interesting that positivity in both aPL and aβ2GPI showed the best specificity and OR for thrombosis and for thrombocytopenia. Despite the relationship found between aβ2GPI and recurrent fetal losses [14], we and others [13, 17] have not confirmed this association.

The effect of aβ2GPI in the pathophysiology of thrombosis or thrombocytopenia is unknown. It may be attributed to β2GPI properties inhibiting the intrinsic coagulation pathway [7] and ADP-induced platelet aggregation [8], aβ2GPI could participate in the thrombogenesis through the inhibition of these anticoagulant properties.

In conclusion, it seems that both aPL and aβ2GPI are useful in the APS, but the higher specificity for clinical manifestations of aβ2GPI determination supports their use after aPL determination.

ACKNOWLEDGEMENTS

Supported by Spanish grants FIS94/0323, FIS95/0250 and FIS96/0380.

REFERENCES


