Significance of anticardiolipin and anti-β₂-glycoprotein I antibodies in lupus nephritis

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Abstract

Objective. To investigate whether anticardiolipin (aCL) and anti-β₂-glycoprotein I (anti-β₂GPI) antibodies are associated with lupus nephritis (group II patients), and whether there are differences in the prevalence of these two autoantibodies between group II patients and patients with non-nephritis SLE (group I) and primary antiphospholipid syndrome (PAPS) patients (group III).

Methods. IgG and IgM aCL were measured in 31 patients and anti-β₂GPI in 30 patients with systemic lupus erythematosus (SLE) nephritis and 25 without SLE nephritis and in 36 PAPS patients by validated enzyme immunoassays. Relationships of anti-double-stranded DNA (anti-dsDNA) antibodies and antibodies to the collagenous region of C1q (anti-C1q) with SLE nephritis were also examined.

Results. The prevalence and levels were higher for aCL, but not for anti-β₂GPI, antibodies in group II than in group I patients. Absolute values of aCL and anti-β₂GPI in all three patient groups correlated with each other. The prevalences of aCL, anti-dsDNA and anti-C1q antibodies were significantly higher in group II than in group I and group III patients.

Conclusion. The observations in this paper suggest that raised levels of aCL antibodies are associated with lupus nephritis. We were not able to demonstrate an association between anti-β₂GPI antibodies and kidney disease either in patients with lupus or in patients with primary antiphospholipid syndrome. In SLE, we demonstrated that the presence of anticardiolipin antibodies in conjunction with elevated levels of anti-dsDNA and anti-C1q antibodies is highly specific for glomerulonephritis in patients with lupus.

Key words: Lupus nephritis, Anticardiolipin, Anti-β₂-glycoprotein I.

Raised blood levels of antiphospholipid (aPL) antibodies in autoimmune diseases and especially in systemic lupus erythematosus (SLE), in which levels of these autoantibodies are raised in 20–60% of patients, have been associated with recurrent venous and arterial thromboses, fetal loss, thrombocytopenia and neurological disorders [1–5]. A separate and distinct syndrome, the primary antiphospholipid syndrome (PAPS), has been recognized in which patients have raised aPL levels and at least one of the clinical complications associated with these antibodies, but no other overt feature of SLE or another autoimmune disease [6–8].

Recently, three independent groups have reported that, in PAPS patients, anticardiolipin antibodies (aCL) need a circulating plasma protein as a cofactor, which binds to the cardiolipin antigen in solid-phase ELISA assays. This cofactor has been identified as β₂-glycoprotein I (β₂GPI) [9–11]. It has been shown subsequently that β₂GPI is able to bind to activated polystyrene plates as an antigen and can be used to facilitate the detection of specific anti-β₂GPI antibodies in patient sera [12]. This has led to a number of studies in which these autoantibodies have been reported to occur in 5–82% of SLE and PAPS patients [13–16].

Although a number of studies have examined levels of aCL [3–5, 17] and anti-β₂GPI [14,18] in lupus nephritis patients, no association has been reported between autoantibody levels and this clinical complication; a negative association has, however, been reported for aCL in lupus nephritis by two independent groups [19, 20].

The present study was undertaken to establish validated ELISA assays for the ascertainment of the prevalence of IgG and IgM aCL and anti-β₂GPI antibodies in SLE patients with and without nephritis; we have also examined the value of these antibodies as markers for SLE nephritis in conjunction with the simultaneous measurement of antibodies to double-stranded DNA.
(anti-dsDNA) and the collagenuous region of C1q (anti-C1q antibodies). For comparative purposes, all these autoantibodies were also measured in a group of PAPS patients, in whom nephritis is seldom present as a clinical complication; in such patients the presence of immune complex-related glomerulonephritis is quoted as one of the exclusion criteria for the diagnosis of PAPS [21].

Our results indicated that aCL antibodies are more prevalent than anti-β2GPI antibodies in SLE patients with nephritis. We have also found that elevated levels of aCL antibodies in combination with raised anti-dsDNA and anti-C1q antibodies are highly specific markers for nephritis in SLE, whereas anti-β2GPI antibodies are not.

Patients and methods
Assays were performed on stored serum samples (−70 °C) from 25 unselected SLE patients (24 female, 1 male, age range 20–71 years, median 43 years; group I), none of whom had had any evidence of nephritis, and from 31 SLE patients (29 female, 2 male; age range 17–61 years, median 31 years; group II). All samples from group II patients were taken during an active phase of nephritis (proteinuria, impaired renal function, histologically proven WHO GN types I–VI). All 56 SLE patients fulfilled the American College of Rheumatology revised criteria for the classification of SLE [22]. Serum samples were also taken from 36 patients with PAPS, only two of whom had documented evidence of renal disease, and all of whom were aCL-positive and had had at least one of the clinical complications associated with PAPS (29 female, 7 male, age range 20–72 years, median 38 years; group III), and from 88 normal blood donors.

Human β2GPI
The β2GPI preparation used was purified from normal human serum using a modification of the precipitation method of Polz [23] followed by affinity chromatography on a heparin–Sepharose (Hitrap), column and protein G columns (Amersham Pharmacia, St Albans, UK), as described by Matsuura [24]. Purity was assessed on the butanol delipidated preparation by SDS-PAGE, double radial immunodiffusion, and ELISA using a rabbit anti-human β2GPI-specific antibody (Dako, Ely, UK).

Anticardiolipin antibody assays
Estimation of aCL IgG and IgM was performed as described previously [25], with minor modifications. Essentially, plain polystyrene microtitre plates (MP01; Life Sciences International, Basingstoke, UK) were coated with cardiolipin (2 μg/well) in ethanol and allowed to evaporate to dryness at 4 °C. Plates were blocked with a 5% solution of adult bovine serum in phosphate-buffered saline (ABS/PBS), followed by addition of 100 μl diluted serum samples, standards and controls (at 1:100 dilution) in ABS/PBS. Bound IgG and IgM aCL antibodies were detected using alkaline phosphatase-conjugated antibodies against human IgG (γ-chain-specific) or IgM (μ-chain-specific); the concentration of aCL was measured at 405 nm, after addition of p-nitrophenyl phosphate chromogenic substrate, on a microplate spectrophotometer (Titertek Multiscan MCC/340; Life Sciences International); aCL levels were calculated from an eight-point standard curve in arbitrary ELISA units (AEU), and serum internal control samples with low and high aCL levels were placed at the beginning and end of each plate to monitor assay performance.

Anti-β2GPI ELISA
For the IgG and IgM anti-β2GPI assay, duplicate wells on high-binding microtitre plates (Immulon 2; Dynatech Laboratories, Billingshurst, UK), were coated with 50 μl of the purified β2GPI preparation (5 μg/ml) in 0.2 M borate-buffered saline (BBS); single adjacent wells were coated with BBS alone for the estimation of non-specific binding for all serum samples, standards and controls. Plates were subsequently blocked with 0.5% BSA/PBS/0.4% Tween-20 for 2 h at room temperature. Serum samples, standards and controls (at 1:100 dilution), 50 μl/well (all diluted in 0.5% BSA/PBS/0.4% Tween-20), were then added and incubated at room temperature for 1 h; this was followed by addition of alkaline phosphatase-conjugated goat F(ab′)2 fragments of anti-human γ-chain-specific IgG or μ-chain-specific IgM (Sigma, Poole, Dorset, UK), and addition of chromogenic substrate as described above for the aCL assay. All washing steps in the assay were performed with PBS/0.075% Tween-20. The optical absorbance of the appropriate non-specific binding was subtracted from all readings for patient samples, standards and controls before the final levels of IgG and IgM anti-β2GPI in AEU were calculated from an eight-point standard curve. Internal controls were included on each plate to monitor assay performance.

Anti-C1q antibodies
The ELISA for anti-C1q IgG was based on a method described previously [26].

Anti-dsDNA antibodies
Anti-dsDNA antibodies were measured by the Farr assay [27]. Positivity was set as >30% binding.

Normal autoantibody levels
Patient sera were considered positive for IgG and IgM aCL and anti-β2GPI antibodies when autoantibody levels were 4 standard deviations (SDs) above the mean level of the 88 healthy normal blood donors. For aCL this was <14.0 AEU for IgG and <10.0 AEU for IgM; for anti-β2GPI it was <12.6 AEU for IgG and <9.5 for IgM. For IgG anti-C1q IgG antibodies the cutoff point was set at 3 SDs above the mean level of 50 normal controls, which corresponded to <20 AEU.
**Statistical analysis**

Statistical analysis was performed using the Prism v2.0 software package (GraphPad Software, San Diego, CA, USA). Differences between proportions of autoantibodies were assessed using the chi-squared test. The Mann-Whitney U-test and the Spearman’s rank sum test were used to analyse aCL and anti-β2GPI isotypes in the three patient groups.

**Results**

**Prevalence of aCL and anti-β2GPI antibodies**

The prevalence and isotype distribution of aCL and anti-β2GPI antibodies are shown in Table 1. Among the 25 patients without nephritis (group I), a total of 12 (48%) were positive (for IgG and/or IgM aCL), in contrast to 24 (77.4%) of the 31 lupus nephritis patients (group II) and all the PAPS patients (Table 1). The difference in aCL positivity between groups I and II was significant (χ² = 5.2130, P < 0.02). Absolute aCL levels (IgG and/or IgM) were compared in the two SLE patient groups, and were found to be significantly higher in group II than in group I patients (P = 0.02, Mann-Whitney U-test) (Fig. 1).

- Anti-β2GPI antibodies (IgG and/or IgM anti-β2GPI) were positive in seven patients (28%) in group I, in eight of 30 patients (26.7%) in group II and in 16 patients (44.4%) in group III. Anti-β2GPI levels were raised, and were significantly higher in group III patients when compared with group I (P = 0.0004), and group II (P = 0.0002) patients using the Mann-Whitney U-test (Fig. 2).

**Relationship between aCL and anti-β2GPI antibodies**

The relationships between aCL and anti-β2GPI antibodies are shown in Table 1. No significant difference was observed in aCL-positive/anti-β2GPI-negative (β2GPI-independent aCL) patients in the three patient groups. In contrast, for patients who were anti-β2GPI positive/aCL-negative (specific anti-β2GPI antibodies or β2GPI-dependent aCL), a significant difference was seen only between group I and group III patients (χ² = 7.8427, P < 0.01). Both autoantibodies (β2GPI-dependent and β2GPI-independent aCL) were present in two patients (8%) in group I, five patients (16.7%) in group II and 16 patients (44.4%) in group III. There was a significant difference in the number of positive patients between group III and groups I (χ² = 10.58, P < 0.01) and 2 (χ² = 6.87, P = 0.01).

Levels of IgG and IgM aCL and anti-β2GPI antibodies were analysed for possible relationships by Spearman’s rank correlation test (data not shown). Significant positive correlations were found in group I patients between group III and groups 1 (ρ = 0.37, P < 0.01) and 2 (ρ = 0.25, P = 0.01). The relationships between aCL and anti-β2GPI antibodies were significant in two patients (8%) in group I, in five patients (16.7%) in group II and 16 patients (44.4%) in group III. There was a significant difference in the number of positive patients between group III and groups I (χ² = 10.58, P < 0.01) and 2 (χ² = 6.87, P = 0.01).

**Table 1.** Prevalence of IgG and IgM aCL and anti-β2GPI antibodies in SLE patients with and without nephritis and in PAPS patients

<table>
<thead>
<tr>
<th>Autoantibody isotype</th>
<th>SLE without nephritis (group I, n = 25)</th>
<th>SLE with nephritis (group II, n = 31)</th>
<th>PAPS (group III, n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCL IgG alone</td>
<td>6 (24%)</td>
<td>15 (48%)</td>
<td>13 (36%)</td>
</tr>
<tr>
<td>aCL IgM alone</td>
<td>4 (16%)</td>
<td>0 (0%)</td>
<td>2 (5.6%)</td>
</tr>
<tr>
<td>aCL IgG + IgM</td>
<td>2 (8%)</td>
<td>9 (29%)</td>
<td>21 (58.3%)</td>
</tr>
<tr>
<td>aCL total, any isotype</td>
<td>12 (48%)*</td>
<td>24 (77.4%)*</td>
<td>36 (100%)</td>
</tr>
<tr>
<td>Anti-β2GPI IgG alone</td>
<td>4 (16%)</td>
<td>5 (16%)</td>
<td>14 (35%)</td>
</tr>
<tr>
<td>Anti-β2GPI IgG IgM</td>
<td>2 (8%)</td>
<td>3 (10%)</td>
<td>2 (5.6%)</td>
</tr>
<tr>
<td>Anti-β2GPI IgG + IgM</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Anti-β2GPI total, any isotype</td>
<td>7 (28%)*</td>
<td>8 (26.7%)</td>
<td>16 (44.4%)</td>
</tr>
<tr>
<td>Anti-CL+/anti-β2GPI−</td>
<td>10 (40%)</td>
<td>19 (63.3%)</td>
<td>20 (55.6%)</td>
</tr>
<tr>
<td>Anti-β2GPI+/aCL−</td>
<td>5 (20%)*</td>
<td>3 (10%)</td>
<td>0 (0%)*</td>
</tr>
<tr>
<td>aCL−/anti-β2GPI−</td>
<td>2 (8%)*</td>
<td>5 (16.7%)*</td>
<td>16 (44.4%)*</td>
</tr>
</tbody>
</table>

*P < 0.02; **P < 0.01.

*Only 30 patients were used for comparison of aCL and βGPI positivity.

β2GPI-independent aCL.

Specific anti-β2GPI antibodies or β2GPI-dependent aCL.

β2GPI-dependent and β2GPI-independent aCL.
between IgG and/or IgM aCL and IgG and/or IgM anti-β2GPI levels ($P = 0.05$). In group II, the only correlation seen was between IgM aCL and IgM anti-β2GPI levels ($P = 0.05$). In PAPS patients, positive correlations were seen between IgG aCL and IgG anti-β2GPI ($P = 0.0001$), IgM aCL and IgM anti-β2GPI ($P = 0.005$), and invariably between IgG and/or IgM aCL and IgG and/or IgM anti-β2GPI levels ($P = 0.001$).

**Relationship between aCL, anti-β2GPI anti-C1q and anti-dsDNA antibodies and lupus nephritis**

The relationship between the number of patients with elevated levels of aCL, anti-β2GPI, anti-C1q and anti-dsDNA antibodies and nephritis was investigated, and the results are summarized in Table 2. As none of the anti-β2GPI antibody-positive patients in any of the three groups was also positive for the other three autoantibodies measured, they have been excluded from Table 2.

None of the patients in group I and only two of the 36 PAPS patients in group III had documented evidence of renal disease.

Levels of aCL were raised in 12 patients (48%) in group I, in 24 patients (77.4%) in group II and in all 36 patients (100%) in group III, in which all patients were chosen for their aCL positivity. The difference in the number of positive patients between groups I and II ($\chi^2 = 5.2167$, $P < 0.02$) was significant. For anti-C1q antibodies, there were five positive patients (20%) in group I, 20 (64.5%) in group II and none in group III. The differences between groups I and II ($\chi^2 = 11.097$, $P < 0.001$), groups I and III ($\chi^2 = 7.9853$, $P < 0.01$) and groups II and III ($\chi^2 = 33.109$, $P < 0.001$) were highly significant; anti-C1q antibody levels were significantly higher in group II patients than in groups I ($P = 0.0002$) and III ($P = 0.0001$, Mann–Whitney $U$-test) (data not shown). For anti-dsDNA antibodies, levels were raised in nine (36%) group I patients, in 26 (83.9%) group II patients, and in 3 (13%) of the 35 group III patients in whom anti-dsDNA was measured; these differences were highly significant between groups I and II ($\chi^2 = 13.531$, $P < 0.001$), between groups I and III ($\chi^2 = 6.8571$) and between groups II and III ($\chi^2 = 37.840$, $P < 0.001$). Anti-dsDNA levels varied between the patient groups and were significantly higher in group II than in groups I ($P < 0.001$) and III ($P < 0.0001$, Mann–Whitney $U$-test) (data not shown).

When possible correlations (Spearman’s rank sum test) between pairs of individual autoantibodies were investigated in the three different patient groups, the only correlation found was between aCL and anti-dsDNA antibody levels in group II lupus nephritis patients ($P < 0.005$). The relationship between anti-C1q and aCL levels in lupus nephritis did not reach statistical significance ($P = 0.07$) (data not shown). When the number of patients positive for all three autoantibodies shown in Table 2 was investigated, only one patient (4%) was positive in group I, 16 patients (51.6%) were positive in group II and none was positive in group III. Highly significant differences were found in the number of patients positive for all three autoantibodies between groups II and I ($\chi^2 = 14.839$, $P < 0.001$) and between groups II and III ($\chi^2 = 23.845$, $P < 0.001$).

**Table 2.** Comparison of aCL, aClq and anti-dsDNA autoantibody profiles in SLE patients with and without nephritis and PAPS patients

<table>
<thead>
<tr>
<th>Autoantibody positivity</th>
<th>SLE without nephritis (group I, $n = 25$)</th>
<th>SLE with nephritis (group II, $n = 31$)</th>
<th>PAPS (group III, $n = 36$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCL-positive (IgG, IgM)</td>
<td>12 (48)</td>
<td>$\chi^2 = 5.2167$, $P &lt; 0.02$</td>
<td>24 (77.4)</td>
</tr>
<tr>
<td>aClq-positive</td>
<td>5 (20)</td>
<td>$\chi^2 = 11.097$, $P &lt; 0.001$</td>
<td>20 (64.5)</td>
</tr>
<tr>
<td>Anti-dsDNA-positive</td>
<td>9 (36)</td>
<td>$\chi^2 = 13.531$, $P &lt; 0.001$</td>
<td>26 (83.9)</td>
</tr>
<tr>
<td>Positive for all three autoantibodies</td>
<td>1 (4)</td>
<td>$\chi^2 = 14.839$, $P &lt; 0.001$</td>
<td>16 (51.6)</td>
</tr>
</tbody>
</table>
Relationship between aCL and anti-β₂GPI antibodies and aPL-associated clinical features

We examined the relationship between aCL and anti-β₂GPI antibodies and the aPL-associated clinical manifestations of thrombosis (venous and arterial), thrombocytopenia (platelet count <120,000/ml) and fetal loss (more than one fetal loss) in our three groups of patients, and our findings are summarized in Table 3. The number of patients positive for aCL or anti-β₂GPI with these complications was often too low for meaningful statistical evaluation. For thrombotic complications, we found that patients with these complications in group II (85.7%) were more likely to be positive for aCL than group I patients (60%). The same was true for thrombocytopenia (100 vs 66.7%) and fetal loss (100 vs 50%); these relationships could not be assessed in our PAPS patients, who by definition were all chosen for their aCL positivity and the presence of at least one of these clinical complications. For PAPS patients on the whole, patients who were anti-β₂GPI-positive were much more likely to suffer one of the above clinical complications than anti-β₂GPI-positive SLE patients.

Discussion

Raised levels of circulating antiphospholipid antibodies (anticardiolipin) have been strongly associated with arterial and venous thrombosis, thrombocytopenia and recurrent fetal loss in SLE and PAPS patients [1–6]; more recently, thrombosis has also been associated with raised levels of anti-β₂GPI antibodies in both these patient groups, especially in PAPS patients [13–16]. Despite the numerous reports that have appeared describing clinical complications and their association with these two autoantibodies, very little has been reported regarding the clinical significance of raised levels of aCL and/or anti-β₂GPI antibodies and SLE nephritis [14, 17, 18], a common clinical manifestation of lupus [21, 28]. Although the pathogenetic role of aCL in lupus nephritis is still not clear [21], recent evidence has suggested that ‘hidden’ β₂GPI-independent antibodies to negatively charged phospholipids may circulate as immune complexes in normal human serum, and that the relevant antigen can be removed by heat treatment, acid treatment, hypermolar buffers or phospholipase enzymes [29].

In the present study, using validated ELISA assays for the measurement of IgG and IgM aCL and anti-β₂GPI antibody levels, we have examined the relationship between these two autoantibodies in SLE patients with and without nephritis, and compared them with those found in PAPS patients, only two of whom had documented evidence of nephritis (Table 1). We noted a much higher prevalence and significantly higher levels for IgG and/or IgM aCL in SLE patients with active nephritis (group I) than in patients without nephritis (group II, \( P < 0.02 \)), and, as previously described [13], a larger but not statistically significant difference in the prevalence of anti-β₂GPI between group III and groups I and II. Anti-β₂GPI levels were significantly higher in group III than in groups I (\( P < 0.004 \)) and II (\( P < 0.002 \)) (Fig. 2). Differences in isotype distribution and positivity were also seen between our three patient groups for both autoantibodies (Table 1; Figs 1 and 2).

Results of previous studies have been conflicting regarding the association of raised antiphospholipid and/or aCL levels with renal disease in SLE patients; one study reported associations between IgG aCL and SLE nephritis [30] similar to those we found. In contrast, another study, which appeared at the same time, found an inverse (negative) correlation between antiphospholipid antibodies and SLE nephritis [19], and a later study reported no association at all and a lower prevalence (43%) than ours (77.4%) for aCL [17]; this difference could be due to differences in the methods used and patient selection. In our ELISA for aCL we incorporate adult bovine serum (containing β₂GPI) in both blocking and diluent solutions, whereas the earlier study used a blocking solution containing purified BSA (with very little or no β₂GPI) in their aCL assay. It could be, therefore, that we measured both β₂GPI-dependent and β₂GPI-independent aCL (Table 1), whereas the earlier study had measured only β₂GPI-independent aCL [31]. Our patient samples were collected from SLE patients during an active phase of nephritis, whereas both the earlier study [17] and a more recent study, which

<table>
<thead>
<tr>
<th>Patient group or autoantibody positivity</th>
<th>Thrombotic complications</th>
<th>Thrombocytopenia</th>
<th>Fetal loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE without nephritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I (n = 25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCL+</td>
<td>3 (60%)</td>
<td>4 (66.7%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>Anti-β₂GPI+</td>
<td>1 (20%)</td>
<td>1 (16.7%)</td>
<td>0</td>
</tr>
<tr>
<td>SLE with nephritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II (n = 30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCL+</td>
<td>6 (85.7%)</td>
<td>3 (100%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Anti-β₂GPI+</td>
<td>3 (42.9%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PAPS patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III (n = 36)</td>
<td>9 (40%)</td>
<td>4 (66.7%)</td>
<td>4 (33.3%)</td>
</tr>
</tbody>
</table>

*Anti-β₂GPI+ was measured in only 30 patients.

Table 3. Comparison of the prevalence of aCL and anti-β₂GPI1 antibodies with clinical complications in SLE patients with and without nephritis and PAPS patients.
reported a lower aCL prevalence of 38% in SLE nephritis patients [28], did not indicate whether aCL was measured during an active phase of nephritis in their SLE patients. Our findings for anti-β2-GPI antibodies are in agreement with those reported in previous studies that had also failed to find an association between anti-β2-GPI levels and renal disease in SLE patients [14, 18].

On examining the relationship between aCL and anti-β2-GPI levels, the only significant correlations seen using the Spearman’s rank correlation test were between aCL and anti-β2-GPI IgG and/or IgM in patients with nephritis (P < 0.05) and between aCL and anti-β2-GPI IgM in patients without nephritis (P < 0.05). In contrast, correlations were found in PAPS patients between the IgG (P < 0.001) and IgM isotypes (P < 0.005) for both autoantibodies, a strong correlation was also found between aCL and anti-β2-GPI IgG and/or IgM (P < 0.005) and in PAPS patients (Table 2). Our findings are similar to those reported in two previous studies, which had also found significant correlations between aCL and anti-β2-GPI antibody levels [12, 14].

We also measured anti-dsDNA and anti-C1q antibodies in all but one of our PAPS patients, and examined the relationship of the raised levels of these autoantibodies with the levels found for aCL, and their relationship to lupus nephritis. It is well documented that SLE patients have a higher prevalence of many autoantibodies, including anti-C1q and anti-dsDNA antibodies. For example, we have demonstrated recently an association between lupus nephritis, anti-C1q (CLR), and the presence of the R151 allele of receptor FcγRIIA [26], which suggests a pathogenetic role for IgG2 anti-C1q antibodies in nephritis. However, not all patients with raised levels of antibodies to C1q and DNA develop nephritis [32]; the data in the present study (Table 2) confirm this. We did show, however, a significant correlation between aCL and anti-dsDNA levels and significantly higher numbers of patients positive for all three autoantibodies in SLE patients with nephritis than in those without this complication (χ² = 14.839, P < 0.001), and in PAPS patients (χ² = 23.845, P < 0.001).

We have found positive correlations between aCL and anti-β2-GPI antibodies in SLE and PAPS patients, and an association between both the prevalence and levels of aCL in patients with lupus nephritis, when compared with the other two patient groups (Figs 1 and 2); for anti-β2-GPI, no association was found, except that, as expected, levels were significantly higher in PAPS patients than in either of the two SLE groups. It is well known that, in some patients with SLE, renal manifestations, such as intrarenal thromboses [33], systemic hypertension, proteinuria, thrombotic microangiopathy and progressive renal failure, have been associated with raised levels of antiphospholipid antibodies [21], but their exact role in the pathogenesis of lupus nephritis is still obscure. The higher prevalence seen here for aCL in lupus nephritis was further strengthened by the strong association seen when aCL positivity was examined in conjunction with positivity for anti-dsDNA and anti-C1q antibodies; patients with lupus nephritis were much more likely to be positive for all three autoantibodies than non-nephritis SLE or PAPS patients. We feel that, from the results shown in Table 1, we can infer that many more of our SLE patients (groups I and II) have β2-GPI-independent than β2-GPI-dependent aCL antibodies.

The association found for aCL, but not for anti-β2-GPI, with lupus nephritis was further strengthened by the strong association seen when aCL positivity in conjunction with positivity for anti-dsDNA and anti-C1q antibodies was examined; their presence and levels in serum could act as useful markers of the severity of renal disease, as we found that lupus nephritis patients were much more likely to be positive for all three autoantibodies than non-nephritis SLE or PAPS patients. Further prospective studies monitoring the levels of these three autoantibodies could be very useful in determining disease activity and in predicting the development of nephritis in SLE.

Acknowledgements

We acknowledge the help of Dr P Athanassiou in the collection of patient data. The work was performed with financial support from the Arthritis Research Campaign of Great Britain.

References