Association of anticardiolipin antibodies with intraglomerular thrombi and renal dysfunction in lupus nephritis

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Summary

We studied positivity for anti-cardiolipin antibody, intraglomerular capillary thrombi on renal biopsy, and the progression of renal disease in 51 patients (10 male and 41 female), mean age 37 years (range 17–65 years), with a diagnosis of systemic lupus erythematosus and clinically evident nephritis confirmed by renal biopsy. Serum creatinine, serum indicators of disease activity and biopsies were analysed in subgroups according to thrombi and anticardiolipin status. End-points were death or chronic dialysis requirement and survival. Degree of sclerosis, crescent formation and necrosed glomeruli were all greater in those specimens positive for thrombi and in those specimens of patients who were serum ACA-positive, suggesting a relationship to disease activity/severity at presentation. The increase in serum anti-DNA antibodies and ANA and the reduction in C3 and C4 were significant in ACA-positive patients, with a strong relationship to disease activity when compared with changes in the ACA-negative patients (p<0.05 in all cases). There was no significant difference when patients were separated according to the presence or absence of thrombi. Renal function at presentation was worse in patients with intracapillary thrombi and ACA positivity (p=0.085 and p=0.042, respectively). All patients progressed, but only those with intracapillary thrombi or anti-cardiolipin antibody positivity had a significant deterioration in renal function. Twenty-one thrombotic episodes occurred in 14 patients, of whom 13 were ACA-positive. Anti-cardiolipin antibody is a strong predictor of the presence of intraglomerular thrombi in SLE patients with renal involvement. The progression of thrombi and/or anticardiolipin antibodies indicate a worse long-term renal outcome. Anti-cardiolipin antibody positivity is a strong predictor of systemic vascular thrombotic complications.

Introduction

Antiphospholipid antibodies (aPL), namely the lupus anticoagulant (LAC) and the anticardiolipin antibodies (ACA) are a group of antibodies directed against negatively-charged phospholipid antigens (phosphatidyserine), on endothelial cell membranes and platelets. Previously these antibodies were thought to be directed towards portions of the prothrombin activator complex, specifically recognizing epitopes on anionic phospholipids and a complex of lipid-bound human prothrombin. More recently this theory has been surpassed by the identification of beta-2-glycoprotein-1 (β2-GP-I). Several components including high titres of aPL antibodies, β2-GP-I and activation of endothelium or platelets, are now suspected to encompass the antiphospholipid syndrome (APS). It seems likely that binding of aPL antibodies on endothelial cells is mediated through the cofactor β2-GP-I. The target antigen for aPL antibodies could be a complex of β2-GP-I and anionic phospholipids. This increasing constellation of antibodies and cofactors has been eloquently summarized by Alarcon-Segovia et al., and others, who have labelled it the antiphospholipid/cofactor syndromes. The true significance of these antibodies in terms
of clinical implications and pathogenesis is still controversial. Many studies have confirmed that patients positive for ACA are at risk of repeated episodes of thrombosis, fetal loss and thrombocytopenia. Antiphospholipid antibodies were first identified in patients with systemic lupus erythematosis (SLE) and subsequently in other autoimmune disorders,6–10 but can be found in isolation (the primary antiphospholipid syndrome).11 Antiphospholipid antibodies occur in up to 60% of patients with SLE and may be of pathogenic significance in lupus nephritis. The presence of intraglomerular capillary thrombosis has also been described in SLE nephritis. Intraglomerular capillary thrombi in the renal microcirculation12,13 have been associated with aPL antibodies in lupus patients14 in some series while not in others.15 However controversy still remains as to their significance in the progression of renal disease and glomerulosclerosis.16 We therefore carried out a retrospective study in a tertiary care centre to seek correlation between positivity for ACA and the presence of intraglomerular capillary thrombi on renal biopsy and the progression of renal disease in lupus nephritis.

Methods

Fifty-one patients (10 male and 41 female), presenting within a 15-year period satisfied the criteria of the American Rheumatism Association for the diagnosis of SLE, and had clinically evident nephritis, confirmed by at least one renal biopsy. The mean age at the time of renal biopsy was 37 years (range 17–65 years) and the mean duration of follow-up was 41 months (range 4–182 months) with a total of 1190 patient-months elapsing by the end of the follow-up period.

A single pathologist who had no prior knowledge of serum immunological or aPL antibody status examined 56 renal biopsies. Special stains included periodic acid Schiff, the Martius Scarlet Blue (MSB) stain for fibrin and silver methenamine preparations. Immunofluorescence for immunoglobulins and complement was also performed. Renal histology was graded according to the WHO I-V classification of severity for SLE. Numbers of crescents, necrosed and sclerosed glomeruli were noted. Intraglomerular capillary thrombi were identified on light microscopy (see Figure 1). Basic routine clotting studies, the kaolin clotting time (K CCT) and the dilute Russell viper venom test (DRVVT) were used to identify patients with the lupus anticoagulant. Prolongation of the activated partial thromboplastin time (APPT), KCCT and DRVVT and failure of correction by the addition of normal plasma were considered diagnostic of lupus anticoagulant. The presence of antiphospholipid antibodies were detected using the Cambridge Life Science ELISA kit (Anti-CL IgM SELISA S4696) for both IgG and IgM subfractions.17 IgA levels were not recorded. A positive ACA (IgM or IgG) above the normal range for our laboratory on any occasion during presentation or follow-up was considered as significant. Positivity for ACA remained a consistent finding during follow-up. Serum markers of disease activity, including antinuclear antibody (ANA) double-stranded DNA antibodies and complement levels (C3 and C4) were measured using standard laboratory techniques. We reviewed episodes of systemic thrombosis.

Plasma creatinine was used as a measure of renal function, with levels of <130 μmol/l (1.4 mg/dl) being considered as normal. Renal function was compared at the time of biopsy and at the end of follow-up. Doubling of serum creatinine (even within the normal range) was considered as a significant deterioration in renal reserve.

All patients underwent treatment as deemed necessary to potentially conserve renal function and produce remission from disease activity. This included steroids, both oral and intravenous, cyclophosphamide pulse therapy, maintenance azathioprine therapy; cyclosporin in three patients, and in four patients, the use of plasma exchange during episodes of severe relapse. In those patients with episodes of systemic thrombosis anticoagulant therapy was used (warfarin).

Statistics

Both parametric and non-parametric analysis was performed. Correlations between the presence of
thrombi and raised ACA levels, and between ACA positivity/thrombi positivity and function at baseline and at the end of follow-up were analysed. Survival curves were plotted using Kaplan-Meier life table analysis with end points taken as death or commencement of dialysis.

Statistical methods used included the $\chi^2$ test with Yates’ correction, Fisher’s Exact Test, paired and unpaired T-tests with two-tail analysis, and analysis of variance (ANOVA). A $p$ value < 0.05 was considered statistically significant.

**Results**

A total of 55 biopsies produced sufficient samples for analysis. The distribution of WHO categories is given in Table 1. Type IV lupus nephritis was the most common form of nephropathy.

During the follow-up period, two patients became dialysis-dependent, two were transplanted after a period of dialysis dependence, a further seven died, of whom four had been haemodialysis-dependent, and 14 others suffered systemic thrombotic events. In another seven patients, there was a significant deterioration in renal function. Three others required a short period on haemodialysis. Seven patients were lost to follow-up/care transferred and were therefore censored at their most recent follow-up.

A total of 930 glomeruli (median 17, range 1–60) were studied for the presence of intraglomerular capillary thrombi. Of these there were 159 necrosed, 179 sclerosed and 172 glomeruli with crescents. A total of 28 biopsies had intraglomerular thrombi. Of these the ACA status was unknown in five, positive in 17 and negative in six. There were a total of 27 biopsies with no intraglomerular capillary thrombi. Seven were ACA-positive while the other 20 were negative for ACA. The correlation between the presence of intraglomerular thrombi and anticardiolipin antibodies was significant (Table 2).

There was a significant association between disease activity markers and ACA status but not intraglomerular capillary thrombus status (Table 3). The percentage of glomeruli with sclerosis, cellular crescents or necrosis was higher in thrombi-positive and ACA-positive cases (Figure 2). The presence of thrombi in the absence of ACA was associated with sclerosis whereas crescent formation was more common in the ACA-positive patients with no thrombi (Figure 3).

Renal function at presentation was worse in patients with intracapillary thrombi and ACA positivity ($p=0.085$ and $p=0.042$, respectively). All patients progressed, but a significant deterioration in renal function was only observed in those with intracapillary thrombi or ACA positivity (Figures 4 and 5).

However when renal progression was analysed according to reciprocal creatinine plots (Figure 6) the progression of patients with intracapillary thrombi was confirmed, whereas there was no demonstrable deterioration in ACA-positive compared with ACA-negative patients.

Presence of both intraglomerular thrombi and a positive serum ACA in comparison to their absence was associated with worse renal function at presentation and at the end of follow-up ($p=0.03$ and $p=0.028$, respectively) (Figure 7). Those patients positive for both intraglomerular thrombi and ACA demonstrated a significant deterioration in renal function at the end of the follow-up period in comparison with that at the time of renal biopsy.

Survival analysis, using Kaplan-Meier plots with end-points as death or dialysis, produced a divergence in survival at 2 years which failed to reach significance when separated according to either ACA status or thrombi status (Figures 8 and 9).

Thirteen of the 14 patients who had systemic thrombotic events were ACA+ (54% of the total ACA+ population). (Figure 10). Approximately 22% of these systemic thrombotic events were strokes in relatively young patients. The most frequently occurring thrombotic event was pulmonary emboli, occurring in 50% of the ACA-positive patients with thrombotic episodes (48% of all thrombotic events). Other documented events included deep venous thrombosis and spontaneous abortions. Four patients experienced more than one systemic thromboembolic event, despite anticoagulant therapy.
Table 3  Serum indicators of disease activity in subgroups, comparing ACA+ patients with ACA− patients

<table>
<thead>
<tr>
<th>Serological</th>
<th>DNA (±SEM)</th>
<th>ANA (±SEM)</th>
<th>C3 (±SEM)</th>
<th>C4 (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA+ (n=24)</td>
<td>690.6 ± 171.3</td>
<td>369.7 ± 65.4</td>
<td>0.59 ± 0.053</td>
<td>0.11 ± 0.011</td>
</tr>
<tr>
<td>ACA− (n=26)</td>
<td>178.3 ± 31.8</td>
<td>192 ± 52.1</td>
<td>0.88 ± 0.071</td>
<td>0.2 ± 0.022</td>
</tr>
<tr>
<td>t</td>
<td>2.06</td>
<td>2.01</td>
<td>2.01</td>
<td>2.03</td>
</tr>
<tr>
<td>p</td>
<td>0.007</td>
<td>0.017</td>
<td>0.018</td>
<td>0.001</td>
</tr>
<tr>
<td>Thrombi+ (n=28)</td>
<td>471.9 ± 111.3</td>
<td>274.4 ± 55.3</td>
<td>0.63 ± 0.053</td>
<td>0.15 ± 0.018</td>
</tr>
<tr>
<td>Thrombi+ (n=27)</td>
<td>379.9 ± 128.9</td>
<td>284.2 ± 63.2</td>
<td>0.77 ± 0.078</td>
<td>0.17 ± 0.023</td>
</tr>
<tr>
<td>t</td>
<td>2.01</td>
<td>2.01</td>
<td>2.01</td>
<td>2.01</td>
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<tr>
<td>p</td>
<td>0.59</td>
<td>0.91</td>
<td>0.51</td>
<td>0.44</td>
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</tbody>
</table>

Data are means ± SEM.

Figure 2. Percentage of glomeruli with cellular crescents, sclerosis and necrosis.

Figure 3. Percentage of glomeruli with cellular crescents, necrosis and sclerosis in combined subgroups.

Discussion

Initial identification in 1952 by Conley et al. of the lupus anticoagulant in patients with systemic lupus erythematosus was made by its ability to prolong lipid-dependent coagulation tests. The association between lupus anticoagulant and thrombosis in SLE patients was first described in 1963. About 40% of SLE patients with LAC suffer systemic thrombo-embolic events compared with only 12% of those without LAC. Frampton et al. found the presence of aPL antibodies are known to cause both arterial and venous thrombosis and has been associated with the development of pulmonary emboli, strokes, myocardial infarction, deep venous thrombosis and placental thrombosis. Traditionally antiphospholipid autoantibodies, which having been assayed using phospholipid-dependent tests, are classified as LAC and aCL antibody, based on the method of detection.
8/76 patients with renal lupus and systemic thrombosis. Unfortunately Miranda et al.\textsuperscript{15} did not examine the occurrence of systemic thrombotic events in their large series. In our series, 54% of ACA-positive patients experienced at least one such episode. This high incidence suggests a pathogenic role for these autoantibodies in the development of thrombi. In the earlier series, the low incidence may be related to a failure in clinical detection. Antiphospholipid antibodies may cause end vascular thrombosis in the form of intravascular coagulation with thrombosis of interlobular arteries, arterioles, glomerular capillaries, glomerular subendothelial deposits of granular material and glomerular basement membrane reduplication.\textsuperscript{30}

In keeping with this, we have demonstrated that 71% of the ACA-positive SLE patients had glomerular capillary thrombi on biopsy, as previously shown by others\textsuperscript{12,13} compared with 23% of ACA-negative patients. The absence of demonstrable thrombi in the remainder may be attributable to the timing of the biopsy and/or biopsy sampling errors. Glomerular thrombi may also occur in the absence of SLE and thus may be directly related to the presence of circulating aPL antibodies.\textsuperscript{31} Intraglomerular capillary thrombi were also associated with glomerulosclerosis, as previously shown by others.\textsuperscript{12} This may be due to progressive loss of glomerular function secondary to the obstruction of the capillaries followed by collapse of affected tufts with subsequent sclerosis.

Viard and colleagues found that 36% of their
lupus patients had \( \beta_2 \)-GP-I antibodies; in these patients there was a strong association with thrombosis, and this association has been verified by others. However, multivariate analysis has shown that aPL antibodies are the strongest risk factor for thrombosis, with the presence of \( \beta_2 \)-GP-I having no added significance. The picture remains confusing and complex. As yet, with the rapidly increasing number of described antigens the area remains undefined. Possibly aPL antibodies and \( \beta_2 \)-GP-I are distinct entities. In our study, \( \beta_2 \)-GP-I antibodies were not evaluated (test unavailable) but further study of this may help to clarify their significance.

In contrast with other, smaller series, we have found an association between the presence of ACA and serum markers of disease activity, as well as pathological markers of activity such as crescents. Our data do not of course prove that the association is causal. However, the lupus anticoagulant is known to impair prostacyclin production, inhibit protein activation by endothelial cells, increase platelet production of thromboxane A2 and increase pro-coagulant activity, and may therefore cause glomerular injury. Since immunosuppressive therapy inhibits the synthesis of antiphospholipid antibodies, this may be another mechanism through which this therapy is effective. Possibly use of an antithrombotic drug regimen may be beneficial in patients with ACA and thrombosis.

Miranda et al., in their study of 108 lupus patients,
confirmed the association of glomerular thrombi with disease severity, activity of nephritis and also to glomerulosclerosis. They failed to find any relationship with aPL antibodies; however, only nine of their patients were positive for aPL antibodies.

The presence of thrombi was associated with a worse outcome in our series despite aggressive treatment. Further, patients who were positive for both thrombi and ACA had more active SLE and more active nephritis. The independent association between crescents and ACA suggests that both are immune-mediated, and that ACA positivity is strongly associated with disease activity. Since ACA appears to be related to serological markers of disease activity, perhaps the other markers, or more active disease in general, may account for any apparent effect of ACA.

Patients with both ACA and intraglomerular thrombi had worse renal function at presentation than those without either feature alone. This could be due to a longer period of disease activity and/or the treatment regimen used prior to renal biopsy. However, only those patients with both characteristics demonstrated a significant increase in serum creatinine during the study period, suggesting a possible synergistic effect of ACA and intraglomerular thrombi. Clarification of this remains difficult, as it is dependent on when patients were first identified with SLE and renal involvement, and when appropriate treatment was first instigated. Therefore, the
Figure 10. Thromboembolic events: comparison of their prevalence in patients according to the presence of antiphospholipid antibodies.

prognostic value of ACA on renal function remains to be further clarified.

One can postulate that the development of intraglomerular capillary thrombi leads to glomerular ischaemia and resultant sclerosis with inevitable progressive renal deterioration. In those lupus patients with an associated circulating anticoagulant there may be a tendency to develop thrombi and crescents, leading to further sclerosis and more aggressive deterioration in glomerular function. However, a complex interrelationship between intracapillary glomerular thrombi, circulating anticoagu-

lants and lupus nephritis probably exists.

In conclusion, anti-cardiolipin antibodies are associated with the presence of intraglomerular capillary thrombi, disease activity and systemic vascular thrombotic complications in SLE patients with renal involvement. The presence of antiphospholipid antibodies may prove to be a significant predictor of the course of lupus nephritis. The independent predictive value of the presence of thrombi or ACA positivity needs to be tested on a larger patient population, and compared with known clinical and pathological indicators of progression.43–46

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References

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