Antiphospholipid Antibodies and Renal Outcomes in Patients with Lupus Nephritis

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Abstract

Objective Previous studies have documented a high frequency of thrombotic events in lupus nephritis patients with antiphospholipid (aPL) antibodies in the serum, but there is little information on the impact of serum aPL antibodies on the clinical outcome of lupus nephritis. The aims of this study were to evaluate the seroprevalence of aPL antibodies in patients with lupus nephritis and assess their prognostic value in relation to long-term renal outcomes.

Patients and Methods A retrospective analysis was undertaken in 49 patients with lupus nephritis who underwent renal biopsy. The serum aPL antibodies were monitored regularly in the patients who were followed up for a mean of 76.4±47.2 months, and possible factors associated with the long-term renal outcomes in these patients were analyzed.

Results The overall seroprevalence of aPL antibodies was 41%. During the follow-up, 40% of aPL antibody-positive patients experienced thrombotic events. The frequency of class V lupus nephritis was lower in the aPL antibody-positive patients (6 out of the 20 aPL antibody-positive vs. 14 out of the 29 patients aPL antibody-negative patients; p=0.03). A multivariate analysis identified age (p=0.0001), eGFR at presentation (p=0.0015) and presence of hypertension (p=0.0025) as independent risk factors for the development of chronic kidney disease (CKD) with eGFR less than 60 ml/min/1.73 m².

Conclusion Detection of aPL antibodies in the serum of patients with lupus nephritis is useful to identify patients at risk of thrombotic events. Hypertension is associated with the probability of CKD with eGFR less than 60 ml/min/1.73 m².

Key words: lupus nephritis, antiphospholipid antibody, prognostic indicator, renal biopsy, thrombosis

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Introduction

Antiphospholipid syndrome (APS) is a disorder characterized by recurrent arterial or venous thrombosis and/or pregnancy morbidity, along with the sustained presence of antiphospholipid (aPL) antibodies in the serum (1). aPL antibodies are a heterogeneous family of pathogenic autoantibodies that act against various phospholipid-binding proteins, and their presence in the serum has been demonstrated to be associated with recurrent thrombosis and fetal loss (2). APS can occur alone as a primary disease or can be secondary to systemic lupus erythematosus (SLE), some infections and reaction to drug use. These antibodies can be detected in 26-40% of SLE patients (3, 4).

The kidney is one of the major organs affected in APS. A wide spectrum of renal manifestations has been described in association with the presence of aPL antibodies in the serum, including renal artery stenosis, renal infarction, renal vein thrombosis, acute or chronic thrombotic microangiopathy (5), and the more recently reported so-called APS nephropathy (6, 7).

Although there is evidence that the presence of aPL antibodies in the serum may place patients with SLE at an increased risk of vascular thrombotic events and pregnancy morbidity (5), data on the impact of seropositivity for aPL...
antibodies on the outcomes of lupus nephritis are conflicting, mainly because they are based on studies with short follow-up periods (8-12).

In the present study, we retrospectively studied 49 patients with lupus nephritis to evaluate: 1) the seroprevalence of aPL antibodies in patients with lupus nephritis, 2) the clinical and laboratory associations of aPL antibody seropositivity, and 3) possible factors associated with the long-term renal outcomes in these patients.

Patients and Methods

Patients

We performed this retrospective study of the prevalence/associations of aPL antibodies in consecutive patients with lupus nephritis seen at our renal unit, between January 1995 and June 2006. By the time of the start of the study, 49 patients had already been tested for the presence/absence of aPL antibodies, and these patients were followed up for a mean duration of 76.4 months. All of the patients enrolled into the study satisfied more than 4 of the diagnostic criteria for SLE established by the American College of Rheumatology (13), and showed the clinical manifestations of lupus nephritis. A renal biopsy had been undertaken at the time of diagnosis of lupus nephritis in all the patients. For each patient, the following data were obtained: demographic information, and the clinical and laboratory data at each visit. The study was performed in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice. Approval was obtained from the local ethic committee, and informed consent was obtained from each subject at the time of the renal biopsy.

Definitions

Thrombotic events refer to arterial and venous thrombosis defined on clinical grounds and confirmed by Doppler study, angiography and/or imaging scans, as required.

Estimated glomerular filtration rate (eGFR) was estimated by the formula described previously (14). The stage of chronic kidney disease (CKD) was defined based on the eGFR.

Hypertension was defined as a supine diastolic blood pressure of greater than 90 mmHg and/or systolic blood pressure of greater than 140 mmHg in 3 consecutive measurements.

Renal biopsy

The renal biopsies were performed under ultrasound-guided needle biopsy. The specimens were fixed in 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, and cut into 4-μm sections. The sections were stained with hematoxylin and eosin, periodic acid Schiff, silver methenamine, and masson trichrome stains for light microscopy to evaluate the glomerular, interstitial, and vascular changes. The changes were scored semiquantitatively by two independent observers who were blinded to the clinical data as previously described (15). The World Health Organization (WHO) originally graded the histological features of LN in 1982 and this classification was revised in 2003 by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) (16).

Laboratory measurements

Retrospective data on the renal response to therapy and disease activity were collected. The severity of the proteinuria was estimated by measuring the 24-h urinary protein excretion, and the peripheral blood total leukocyte, erythrocyte and platelet counts, serum levels of complement hemolytic activity (CH50), serum C3 and C4, and the serum titers of anti-ds DNA antibody were measured in all of the patients. Serum creatinine was determined by the standard enzymatic method. The serum titers of aCL antibodies (IgG/IgM) were measured by a standard ELISA method (17). The cut-off value for assay positivity was set at the 99th percentile for 134 healthy controls, according to the laboratory criteria for the diagnosis of APS (18).

Statistical analysis

The data were expressed as mean ± SD. The Student’s t-test was used to compare continuous variables between the two groups. The chi-square or Fisher’s exact probability test was applied for the categorical data. For the multivariate analysis conducted to examine the factors that might be independently associated with the prevalence of CKD at the final observation, the following variables were tested: age, sex, serum creatinine, eGFR, proteinuria, serum ds-DNA levels at presentation, presence of aPL antibody, presence of hypertension, diabetes mellitus and/or thrombosis, treatment by plasma exchange, use of administration of heparin and warfarin, prescription of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), and the duration of follow-up. Probability values of less than 0.05 were considered to denote significance. All statistical calculations were performed with the JMP 5.1 software (SAS for Windows, Car! NC, USA).

Results

Clinical and biochemical characteristics at diagnosis of lupus nephritis

Of the 49 patients, 44 patients were women and 5 were men, with a mean age of 40.1±14.3 years (Table 1). At entry, the mean eGFR was 70.8±34.8 ml/min/1.73 m² and 19 patients had CKD with a mean eGFR value of lower than 60 ml/min/1.73 m². Of the 49 patients, 13 had CKD stage 1, 15 had CKD stage 2, 16 had CKD stage 3, 3 had CKD stage 4 and 2 had CKD stage 5. The CKD patients who had nephrotic syndrome were 15 of the 49 patients. Of the 49 patients, 24 (49%) had arterial hypertension at presentation. Only 3 patients had diabetes mellitus. The mean serum anti-
Table 1. Characteristics of the Patients with Lupus Nephritis Studied

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (rate or range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (men/women)</td>
<td>49 (5/44)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.1±14.3 (16.0-71.0)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.93±0.67 (0.32-4.33)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>70.8±34.8 (8.7-183.5)</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>2.98±2.92 (0.09-9.46)</td>
</tr>
<tr>
<td>ds-DNA (IU/mL)</td>
<td>6.8±8.6 (0.2-35.0)</td>
</tr>
<tr>
<td>aPL antibody-positive (%)</td>
<td>20 (40.8)</td>
</tr>
<tr>
<td>Thrombosis (%)</td>
<td>8 (16.3)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>4 (8.1)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>24 (49.0)</td>
</tr>
<tr>
<td>The use of ACE inhibitors (%)</td>
<td>10 (20.4)</td>
</tr>
<tr>
<td>The use of ARB (%)</td>
<td>18 (36.7)</td>
</tr>
<tr>
<td>The use of heparin (%)</td>
<td>13 (26.5)</td>
</tr>
<tr>
<td>The use of warfarin (%)</td>
<td>10 (20.4)</td>
</tr>
<tr>
<td>MP pulse therapy (%)</td>
<td>23 (47.0)</td>
</tr>
<tr>
<td>Plasma exchange (%)</td>
<td>9 (18.4)</td>
</tr>
<tr>
<td>Follow-up period (months)</td>
<td>76.4±47.2 (8-166)</td>
</tr>
</tbody>
</table>


ds-DNA titers were 6.8±8.6 IU/mL. According to the results of the renal biopsy, 6 patients had class II, 2 patients had class III, 17 patients had class IV, 19 patients had class V, 3 patients had III+V, and 2 patients had class IV+V lupus nephritis.

Homogeneous treatment was administered to the entire patient series. For patients with class III and class IV lupus nephritis, treatment consisted of high-dose intravenous steroidal pulses for 3 days, followed by oral prednisolone and oral cyclophosphamide for induction and oral prednisolone with or without azathioprine for maintenance. Patients with class V lupus nephritis and nephrotic syndrome were treated with steroids and mizoribine alternately every other month for a total treatment period of 6 months. Twenty-three patients (47%) received methylprednisolone pulse therapy.

Ten patients were treated with angiotensin-converting enzyme (ACE) inhibitors and 18 patients were treated with angiotensin receptor blockers (ARB) for the control of arterial hypertension or proteinuria. The mean duration of follow-up of the patients was 76.4±47.2 months (range, 8 to 166 months). As an anticoagulant, 13 patients (26.5%) received heparin and 10 patients (20.4%) received warfarin.

At presentation, 20 patients (41.0%) were found to be aPL antibody positive, whereas only 16.3% of these patients suffered from thrombotic events. Table 2 shows the clinical characteristics of the patients that were positive and negative for aPL antibodies. As shown in Fig. 1, there was a significant difference in the frequency of thrombotic events between aPL antibody-positive and aPL antibody-negative patients (40% vs. 10.4%; p=0.015). Conversely, there was no significant difference between the two groups in terms of the age (40.3±12.7 vs. 40.0±15.5 years; p=0.387), sex distribution (16 women, 4 men vs. 28 women, 1 men; p=0.186), serum creatinine level (1.15±0.95 vs. 0.78±0.33 mg/dL; p=0.053), eGFR (63.7±33.1 vs. 75.7±35.6 ml/min/1.73 m²; p=0.05), proteinuria (3.02±3.18 g/day vs. 3.06±2.77 g/day; p=0.702), or frequency of hypertension (50.0% vs. 48.3%; p=0.214). In regard to the results of the renal biopsy, the frequency of class V nephritis was lower in the aPL antibody-positive group (6 of the 20 aPL antibody-positive patients vs. 14 of the 29 aPL antibody-negative patients; p=0.03). Both the aPL antibody-positive and aPL antibody-negative patients were followed up for a similar duration (mean, 69.9±44.6 months for the aPL antibody-positive group vs. 80.9±49.3 months for the aPL antibody-negative group; p=0.055).

Renal outcome

At the end of the mean follow-up duration of 76.4±47.2 months, 16 of the 49 patients (32.7%) were diagnosed to have developed CKD with eGFR less than 60 ml/min/1.73 m². The advanced CKD patients had a mean eGFR of 44.8±12.3 ml/min/1.73 m² at the last observation. None of the patients required long-term maintenance dialysis therapy. Thirty-three patients continued to show normal renal function until the last observation (mean eGFR, 83.3±19.1 ml/
Table 2. Comparison of Clinical and Biochemical Profiles between Presence and Absence of Antiphospholipid (aPL) Antibodies in Study Population

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>aPL(+)</th>
<th>aPL(-)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.3±12.7</td>
<td>40.0±15.5</td>
<td>0.387</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>4/16</td>
<td>1/28</td>
<td>0.186</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.15±0.95</td>
<td>0.78±0.33</td>
<td>0.053</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>63.70±33.09</td>
<td>75.72±35.60</td>
<td>0.049</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>3.02±3.18</td>
<td>3.06±2.77</td>
<td>0.702</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>10 (50.0)</td>
<td>14 (48.3)</td>
<td>0.214</td>
</tr>
<tr>
<td>ISN/RPS classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>3</td>
<td>0.625</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>3</td>
<td>0.969</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>9</td>
<td>0.319</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>14</td>
<td>0.03</td>
</tr>
</tbody>
</table>

eGFR: estimated glomerular filtration rate, ISN/RPS: WHO: renal biopsy findings according to the International Society of Nephrology/Renal Pathology Society.

As shown in Fig. 2, the prevalence of CKD less than 60 mL/min/1.73 m² tended to be greater in the aPL antibody-positive patients, but did not reach statistical significance (p=0.27). The advanced CKD was detected in 9 of the 20 aPL antibody-positive patients (45%) (6 patients with class IV, 2 patients with class V, and 1 patient with class III lupus nephritis). None of these patients with CKD required long-term maintenance dialysis therapy, and had a mean eGFR of 43.9±11.3 mL/min/1.73 m² at the last observation. Eleven of the 20 aPL antibody-positive patients continued to show normal renal function. On the other hand, 8 of the 29 aPL antibody-negative patients (27.6%) developed CKD including 3 patients with class IV, 2 patients with class V, 2 patients with class III+V, and 1 patient with class III lupus nephritis. Of these patients, none required long-term maintenance dialysis therapy.

Finally, the possible factors associated with the long-term renal outcomes in these patients were analyzed. By stepwise multivariate analysis, risk factors for CKD were age (F=17.638, p=0.0001), eGFR at presentation (F=11.333, p=0.0015) and presence of hypertension (F=10.233, p=0.0025). Other factors were not significantly associated with the probability of CKD.

Discussion

We have shown that positivity for aPL antibodies in patients with lupus nephritis represents a risk factor for thrombotic events, and also we found an association between the presence of aPL and probability of CKD on long-term follow-up. The prevalence of aPL antibody was similar to that reported in many other studies of patients with SLE.
(19), it was also apparently similar to that reported from previous studies of patients with lupus nephritis (7, 8, 12, 20). Forty-one percent of our patients developed vascular thrombosis, similar to that reported recently for a lupus nephritis series (21). Because all our patients had nephritis and were followed up for a relatively long period, it is possible that hypertension and atherosclerosis, which are frequent in patients with lupus nephritis, act synergistically with aPL antibodies to produce arterial vasculopathy (22).

Arterial hypertension is frequent in patients with renal SLE (23) and may be associated with the presence of aPL antibodies (7). In the present patients, 49% of all patients had persistent arterial hypertension. This is not surprising because aPL antibodies may cause acute or chronic lesions of preglomerular, arcuate, interlobular, or even the main renal arteries and also may upregulate endothelial expression of prepro-endothelin-1 messenger RNA, the most potent endothelium-derived vasoconstrictive factor (24). In the present study, 10 patients were treated with ACE inhibitors and 18 with ARB for the control of arterial hypertension or proteinuria. Korkmaz et al (25) previously suggested the beneficial effects of immunosuppressive therapy with warfarin and ACE inhibitors in patients with aPL antibodies. However, further research is warranted on alternative therapies such as ACE inhibitors and/or ARBs.

It is still not clear if aPL antibodies might have an impact on the renal outcomes in patients of lupus nephritis. Some investigators have reported an association between high aPL antibody titers and high serum creatinine levels (11) or severe proteinuria at presentation (26), however, these data remain to be confirmed (9, 27). In the present study, there were no significant differences in the age, sex distribution, eGFR or severity of proteinuria between the aPL antibody-positive and aPL antibody-negative patients at presentation. We found a significant lower prevalence of class V lupus nephritis in aPL antibody-positive patients, although additional studies are needed to confirm this association. Advanced CKD was detected in 9 of the 20 aPL antibody-positive patients (45%) and 6 patients of the 20 patients were classified with class IV, suggesting that class IV may be associated with the progression of CKD. In previous studies, a number of patients with aPL antibody positivity and nephrotic syndrome showed membranous nephropathy at renal biopsy (28, 29). This discrepancy is thought to be possibly explained by the differences in the characteristics of the study population.

The present study showed the presence of influence of aPL antibodies on the long-term outcomes of lupus nephritis. Moroni et al (30) reported that the probability of developing chronic renal failure was significantly greater in aPL antibody-positive patients, and that the difference increased after year 15 of follow-up. In our study, age, eGFR at presentation and presence of hypertension were identified by multivariate analysis as independent risk factors for the development of CKD. In line with previous reports, additional parameters affecting the renal function were confirmed by univariate analysis, such as the sex, platelet count, serum creatinine level, chronicity index, severity of tubular atrophy and interstitial fibrosis (31-35). However, multivariate analysis identified only aPL antibody positivity, the chronicity index, and a high plasma creatinine level at presentation as independent predictors of chronic renal function deterioration (30). Hypertension at presentation was an independent variable associated with the development of CKD. However, considered as a time-dependent factor, persistent arterial hypertension, particularly uncontrolled hypertension, was strongly correlated with the development of CKD. Taken together, it is noteworthy that blood pressure control is important to increase the likelihood of a good prognosis in patients with lupus nephritis.

In conclusion, our results suggest the usefulness of the monitoring of all patients with lupus nephritis for the presence of aPL antibodies in the serum to determine the risk of thrombotic events in these patients. Our data also emphasize the prognostic importance of blood pressure control. Specific studies with antiplatelet or anticoagulant therapies would be useful to evaluate the potential protective effects of these agents against the development of aPL antibody-associated vascular renal lesions in lupus nephritis.

**Limitations**

The present study was limited to SLE patients who underwent renal biopsy, therefore, the sample size was small. In addition, measurements of lupus anticoagulant and anti-β2 GPI antibodies were not conducted in all of the patients studied. Finally, the therapeutic regimens adopted for hypertension and thrombosis were not identical. Therefore, studies with longer durations of follow-up and use of homogeneous therapeutic regimens are necessary to evaluate the renal outcomes in lupus nephritis patients with and without aPL antibodies in the serum.

**References**