Clinical significance of antiphospholipid syndrome nephropathy (APSN) in patients with systemic lupus erythematosus (SLE)

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\textbf{ABSTRACT}

Antiphospholipid syndrome nephropathy (APSN) is now a well recognized vaso-occlusive renal lesion associated with acute thrombosis and chronic arterial and arteriolar lesions, leading to zones of cortical ischemic atrophy. Our objective was to evaluate the prevalence and clinical significance of APSN in patients with Systemic Lupus Erythematosus (SLE).

\textbf{Methods:} Kidney biopsy specimens obtained from 162 patients with lupus glomerulonephritis were retrospectively examined for the presence of APSN. Clinical and laboratory data obtained at the time of kidney biopsy and during a mean follow-up of 7 years were recorded. In cases for which serial kidney biopsy specimens were available, the evolution of APSN was examined.

\textbf{Results:} We found APSN in 17 (10.4\%) patients with lupus glomerulonephritis (GN), 12 with focal or proliferative lesions. Both activity and chronicity indexes were higher in patients with APSN when compared with lupus nephritis without APSN. Patients with APSN had a higher frequency of hypertension and elevated serum creatinine levels at the time or kidney biopsy, as well as a higher frequency of rapidly progressive GN, nephrotic syndrome and death at the end of the follow-up. Anticardiolipin antibodies were found in 52\% of those with APSN and in 27\% of those without APSN. Serial kidney biopsy specimens were available from 18 patients. An increase of glomerular sclerosis was found in the second biopsy particularly in those patients with APSN in the first biopsy.

\textbf{Conclusions:} APSN is a risk factor that contributes to an elevated prevalence of hypertension, elevated serum creatinine, nephrotic syndrome and increased glomerular sclerosis. APSN should be included in the classification criteria of APS, and the use of appropriate anticoagulant therapy should be tested.

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\textbf{Significado clínico de la nefropatía del síndrome antifosfolípido en pacientes con lupus eritematoso sistémico (LES)}

\textbf{RESUMEN}

La nefropatía del síndrome anti fosfolípido (NSAF) es actualmente una alteración patológica bien definida, caracterizada por la presencia de lesiones renales vaso-oclusivas, trombosis aguda arterial y arteriolar, y que ocasiona zonas de atrofia isquémica cortical. El objetivo del presente trabajo fue analizar la prevalencia y el significado clínico de la NSAF en pacientes con glomerulonefríti (GN) secundaria a Lupus Eritematoso Sistémico (LES). Se analizaron retrospectivamente las biopsias renales de 162 pacientes con GN secundaria a LES, buscando intencionadamente los datos histopatológicos de la NSAF. Se registraron los datos clínicos y serológicos al momento de la biopsia renal y durante el período de seguimiento promedio de 7 años. En los casos en que se obtuvo una biopsia renal subsecuente se analizó el desarrollo de la NSAF.

\textbf{Resultados:} Encontramos datos de NSAF en 17 pacientes (10.4\%); 12 de ellos tenían lesiones proliferativas focales o difusas. Los índices histopatológicos de actividad y de cronicidad fueron más altos en los pacientes con la NSAF cuando se compararon con los pacientes sin NSF. Los pacientes con nefropatía anti fosfolípido tuvieron con mayor frecuencia hipertensión arterial, creatinina sérica elevada, síndrome nefrótico, GN rápidamente progresiva y muerte, en comparación con los pacientes con GN lúpica sin NSF. Se detectaron anticuerpos anticoagulantes en 52\% de los pacientes con NSF en quienes se realizó el examen al momento de la biopsia, en comparación con 27\% de los pacientes sin NSF. Se realizó biopsia renal subsecuente en 18 pacientes; quienes tuvieron NSAF en la primera biopsia tuvieron mayor incremento en la esclerosis glomerular en la segunda biopsia, al compararlo con quienes no tuvieron NSF en la biopsia inicial.

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Introduction

The antiphospholipid syndrome (APS) is defined by the presence of antiphospholipid antibodies (aCL) and/or circulating lupus anticoagulant associated with thrombosis, particularly of the large arteries or veins and/or obstetrical fetal loss (repeated miscarriages or fetal death in utero). New clinical and laboratory insights had been addressed at a workshop in Sidney, Australia, before the Eleven International Congress on antiphospholipid antibodies. Based on this, a recent publication suggests the inclusion of antiphospholipid syndrome in the classification criteria. This syndrome may be primary or secondary, particularly in association with systemic lupus erythematosus (SLE). The frequency of thrombotic complications and the presence of antiphospholipid antibodies was first described in SLE. In a compilation of several series, including more than 1000 lupus patients, a prevalence of 34% for lupus anticoagulant and 44% for aCL was found in these patients. Thrombotic events occurred in nearly 30% of lupus patients demonstrating these antibodies. Although renal involvement is often not prominent, numerous observations show its implication in the course of APS, in which it may worsen the prognosis.

In a retrospective study 16 cases of primary APS were found and followed for at least 5 years. There was a vascular nephropathy in all patients, characterized by small vessel vaso-occlusive lesions associated with fibrous intimal hyperplasia of interlobular arteries, recanalizing thrombi in arteries and arterioles, and focal cortical atrophy. The clinical hallmark of this vascular nephropathy was systemic hypertension, variably associated with renal insufficiency, proteinuria or hematuria. In a subsequent retrospective study of 114 patients, Daugas et al reported the presence of APSN in 32% renal biopsies of lupus patients, independently of lupus nephritis findings; those patients with APSN had association with lupus anticoagulant but not with antiphospholipid antibodies, association with extrarenal APS and with a worse prognosis.

We have previously reported the presence of glomerular thrombosis in 36 of 108 lupus nephritis patients, associated with systemic hypertension, nephrotic syndrome and bad prognosis for both renal function and survival. We are now reporting our studies to evaluate the prevalence and prognostic significance of APSN in lupus nephritis patients.

Patients and methods

The present study included 162 patients followed at the Department of Rheumatology, Centro Médico Nacional La Raza, Mexico City. All patients met the American College of Rheumatology criteria for Systemic Lupus Erythematosus (SLE) and in all cases renal biopsy was performed due to renal function abnormalities. Patients with vascular lesions probably due to other causes, such as systemic sclerosis, malignant hypertension, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, postpartum renal failure, preeclampsia, diabetic nephropathy, human immunodeficiency virus infection, chemotherapy or patients with previous cyclosporine treatment were not included.

For each patient the following demographic data was obtained: age, gender, duration of SLE and duration of lupus nephritis. We also documented clinical and laboratory data relative to SLE: arthritis, malar or discoid rash, mouth ulcers, photosensitivity, serositis, systemic hypertension (systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg), raised sum creatinine levels (> 1.4 mg/dl), proteinuria, nephrotic syndrome (urinary protein concentration > 3 g/24 hrs), leukopenia (white blood cell count < 4000/mm³), thrombocytopenia (platelet count < 100,000/mm³), autoimmune hemolytic anemia, and hyperlipidemia. We also recorded aCL, antinuclear and anti DNA antibodies, and complement levels (C3, C4). Patients were considered to be hypertensive with a systemic blood pressure > 160 mmHg and/or diastolic blood pressure > 95 mmHg and/or if taking antihypertensive medication.

The renal tissues obtained by needle biopsy were fixed in 10% neutral buffered formalin, gradually dehydrated, and embedded in paraffin. Paraffin sections were stained with eosin and hematoxylin, periodic acid Schiff (PAS), silver methenamine, Masson’s trichrome stain, and elastic-Van Gieson stain. In the cases in which TMA was demonstrated on light microscopy, additional paraffin sections were studied for fibrinogen deposits by immunohistochemistry. After dewaxing and dehydration, paraffin sections were transferred to Tris buffered saline (TBS) and subjected to antigen retrieval in a microwave.

The following histologic data were recorded for each renal biopsy: Lupus Nephritis according with the WHO classification. Semiquantitative evaluation of the activity and chronicity indexes. According with previously published reports, APS nephropathy was diagnosed when at least one of the following lesions were detected: thrombotic microangiopathy (TMA), characterized for the presence of fibrin thrombi in arterioles and/or glomeruli (acute lesion), or microlumbrical intimal cellular proliferation leading to intimal thickening of interlobular arteries (FIH), organized thrombi with or without recanalization, fibrous arterial and arteriolar occlusion and subcapsular zone with focal cortical atrophy (FCA) (chronic lesions). In cases of APSN for which serial kidney biopsy specimens were available, the evolution of histologic lesions was examined.

Results

We studied 162 patients, 144 female, with mean age of 27.6±8.1 years and mean disease evolution of 3.5±1.9 years. The mean follow up of these patients was 7.0±4.4 years. The kidney biopsy specimens obtained from patients with lupus nephritis were classified according to WHO criteria as follows: 13 patients with mesangial lupus nephritis, 30 with focal proliferative, 86 with diffuse proliferative, 22 with membranous and 11 with the sclerosing form.

We searched for vascular lesions in all biopsies (n = 162) and found vascular abnormalities in 132 cases (81.4%). The most frequent alteration in vesseles was fibrosis in 93 patients (70.4%). Necrosis was noted in 6 patients (4.5%), leukocytoclastic vasculitis in 4 (4.0%), thrombosis in 43 (32.5%) and necrotizing vasculitis in 5 (5).
Table 1
Vascular abnormalities distribution by GN class

<table>
<thead>
<tr>
<th>GN class (n = 162)</th>
<th>Necrosis</th>
<th>Vascular thrombosis</th>
<th>Glomerular thrombosis</th>
<th>Leukocytoclastic vasculitis</th>
<th>Fibrosis</th>
<th>APSN</th>
</tr>
</thead>
<tbody>
<tr>
<td>II (13)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>III (30)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>IV (86)</td>
<td>4</td>
<td>6</td>
<td>26</td>
<td>2</td>
<td>39</td>
<td>8</td>
</tr>
<tr>
<td>V (22)</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>VI (11)</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>6</td>
<td>11</td>
<td>32</td>
<td>4</td>
<td>93</td>
<td>17</td>
</tr>
</tbody>
</table>

APSN: antiphospholipid syndrome nephropathy; GN: glomerulonephritis.

Table 2
Histologic, clinical manifestations and outcome comparing patients with and without Anti Phospholipid Syndrome Nephropathy

<table>
<thead>
<tr>
<th>Findings</th>
<th>APSN (n = 17)</th>
<th>Without APSN (n = 145)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity Index</td>
<td>12.1 ± 4.5</td>
<td>8.7 ± 3.2</td>
</tr>
<tr>
<td>Chronicity Index</td>
<td>6.5 ± 1.6</td>
<td>4.9 ± 2.2</td>
</tr>
<tr>
<td>Nephrotic Syndrome</td>
<td>12 (70.5)*</td>
<td>23 (15.8)</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>15 (88.2)</td>
<td>88 (60.6)</td>
</tr>
<tr>
<td>Rapidly progressive GN</td>
<td>6 (35.2)</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td>aCL antibodies</td>
<td>9 (52.9)</td>
<td>21/76 (27)</td>
</tr>
<tr>
<td>Death</td>
<td>9 (52.9)*</td>
<td>6 (4)</td>
</tr>
</tbody>
</table>

APSN: antiphospholipid syndrome nephropathy. *p < 0.05.

Table 3
Development of glomerular sclerosis in a subsequent biopsy according to the histologic abnormalities in the initial biopsy: APSN as a predictor of glomerular sclerosis

<table>
<thead>
<tr>
<th>Findings in the initial biopsy (number with abnormality)</th>
<th>Glomerular sclerosis. Initial biopsy</th>
<th>Glomerular sclerosis. Subsequent biopsy (n = 18)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APSN (4)</td>
<td>1.3 ± 1.0</td>
<td>4.5 ± 1.5</td>
<td>ns</td>
</tr>
<tr>
<td>Fibrinoid necrosis (11)</td>
<td>1.2 ± 1.1</td>
<td>3.7 ± 1.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Extracapilar proliferation (13)</td>
<td>1.3 ± 1.1</td>
<td>3.2 ± 1.6</td>
<td>ns</td>
</tr>
<tr>
<td>Glomerular proliferation (16)</td>
<td>1.2 ± 1.1</td>
<td>2.8 ± 1.7</td>
<td>ns</td>
</tr>
<tr>
<td>Hialine deposits (13)</td>
<td>1.3 ± 1.0</td>
<td>3.1 ± 1.8</td>
<td>ns</td>
</tr>
<tr>
<td>Leukocytes infiltrate (15)</td>
<td>1.0 ± 1.0</td>
<td>2.0 ± 1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Interstitial inflammation (16)</td>
<td>1.3 ± 1.0</td>
<td>2.8 ± 1.7</td>
<td>ns</td>
</tr>
</tbody>
</table>

APSN: antiphospholipid syndrome nephropathy.

1 (0.7%) were also documented (Table 1). APSN, according with the presence of thrombotic microangiopathy, organized thrombi, fibrous artery and arteriolar occlusion, and focal cortical atrophy, was found in 17 patients (10.4%).

Vascular lesions were present in all lupus nephritis classes, but there were a strong association with class IV (64.3%). In class II there were 11 cases (8.3%), in class III and V 25 cases (18.9% each) and 17 (12.8%) in class VI. APSN was also prevalent in class IV (8/17 cases).

Table 2 shows the comparative clinical and laboratory characteristics of SLE patients with or without APS nephropathy at the time of kidney biopsies. Patients were more frequently hypertensive in the APSN group (88 vs 60%) and it was difficult to control, in spite of intensive anti hypertensive drugs, in 5 cases (33.3%). A significant association between APSN and nephrotic syndrome was found. Anticardiolipin antibodies were found in 9 out of 17 (52.9%) with APSN and in 21 of 76 (27%) patients without the syndrome in whom the test was done at the time of biopsy. An association between APS nephropathy and class IV lupus nephritis was detected. The WHO activity and chronicity index scores were not different between the two groups (12.1 ± 4.5 vs 8.7 ± 3.2 and 6.5 ± 1.6 vs 4.9 ± 2.2 respectively). A clear association was found between APSN nephropathy and survival during the 7 years follow-up: 9 (52.9%) patients died, due to end stage renal failure in 3, cardiovascular complications in 2 and in the other 4 cause of death could not be established. In contrast, there were only 6 deaths (4%) in the control group: due to end stage renal failure in 2, septicemia in 1 and it was not established in 3.

In 18 patients we had a subsequent renal biopsy (Table 3). In these cases we analyzed the impact of the histologic findings in the first biopsy as predictors of glomerular sclerosis in the second biopsy. The histopathologic changes that were first analyzed included fibrinoid necrosis, extracapilar proliferation, glomerular proliferation, hyaline deposits, leukocyte infiltration, interstitial inflammation and APSN. As expected, in all cases there were an increase in glomerular sclerosis in the subsequent biopsy. However, there were a significant association between fibrinoid necrosis and the increase in the semiquantitative index of glomerular sclerosis (1.2 ± 1.1 to 3.7 ± 1.4, p < 0.01) and between leukocyte infiltrate with glomerular sclerosis (1.0 ± 1.0 to 2.0 ± 1.7, p < 0.01). The presence of glomerular sclerosis was even higher in patients whom initial renal biopsy showed APSN (1.3 ± 1.0 to 4.5 ± 1.5) but due to the number of patients this difference was not significant.

Discussion

In the present study, we examined the prevalence and clinical implications of APS nephropathy in 162 SLE patients. The vascular lesions have been the subject of a renewal interest, particularly arteriolar or glomerular thrombotic microangiopathy (TAM) and chronic vascular lesions similar to those described in the APS nephropathy of primary antiphospholipid syndrome.9 We found high blood pressure in 88% of APSN patients. Others9,10,16–18,24 have found similar results, with frequencies varying from 60 to 93%. Hypertension was sometimes the prevalent clinical sign suggestive of nephropathy. It was often difficult to control, with diastolic pressure >110 mmHg in 5 patients (33.3%). The association between hypertension and APSN leads to the question of whether the hypertension is the cause or the consequence of APSN. Previous studies9 of APSN in primary APS supported the idea in favor of the secondary nature of the hypertension, due to the strong stimulation of the renin-angiotensin system. Our data show that APSN accounts for the excess of hypertension in patients with lupus nephritis plus APSN over those with lupus nephritis alone. Regarding the possibility that features of APSN are secondary to hypertension, as during the course of nephroangi-sclerosis, it could be argued that the histologic lesions described above developed in several patients without hypertension. The severity of vascular lesions in two patients in our series who were normotensive would seem to favor the first hypothesis. We propose, therefore, that at least initially, the intrarrenal vascular...
lesions related to the APSN cause the hypertension, which may secondarily worsen and extend the lesions.

Alternatively, previous studies have demonstrated a critical role for activation of the classical pathway of complement that leads to thrombotic injury in the presence of Antiphospholipid antibodies. A recent study has shown a strong relationship between the intensity of glomerular C4d staining and the presence of microthrombi in 7 of 8 biopsy samples, suggesting that immunodetection of glomerular C4d deposition on renal biopsy samples could be a convenient method of identifying patients at risk of thrombotic microangiopathy.

In previous studies, the prevalence of proteinuria, nephrotic syndrome, chronic renal failure or elevated serum creatinine levels in patients with SLE and/or APSN and renal vascular lesions was variable. Tektodinou et al. found that elevated creatinine levels at the time of kidney biopsy were associated with chronic APS nephropathy, and the occurrence of proteinuria or nephritic syndrome was not different between patients with and those without APS nephropathy. Daugas et al. reported a significantly higher serum creatinine among patients with APSN in comparison with patients without APSN. Only interstitial fibrosis was independently and significantly associated with the creatinine level. They did not find difference among patients in terms of hematuria, proteinuria or nephritic syndrome.

Our series reveal that activity and chronicity indexes are higher in APSN patients compared with patients without APSN, although this association is not statistically significative. In contrast with previous reports, the components of these histopathologic data, including fibrosis, were not associated with the presence of APSN. On the other hand, in our series APSN is a risk factor for the prevalence of hypertension, nephritic syndrome and more severely altered renal function, including death during the follow-up period. We have been able to demonstrate more rapid loss of renal function in cases with both APSN and lupus nephritis. ESRD related solely to the vascular lesions of APS in the absence of lupus nephritis has indeed been described, as well as APSN in catastrophic primary and SLE-related APS. In addition, these results are consistent with those described by Banfi et al., who demonstrated that renal vascular involvement in lupus must be considered a pejorative prognostic factor. Thus, APSN may well participate in the progression of renal insufficiency in SLE and must be sought on renal biopsy to direct therapy.

A strong association between APSN and APS-related manifestations such as arterial thromboses and livedo reticularis was found in the Tektodinou et al. series. It was observed that among SLE patients with aPL, those with APSN developed thromboses more frequently than did those without APSN. Moreover, patients who had this characteristic renal small-artery vasculopathy developed arterial thromboses, while those without APSN developed venous thromboses. Due to the retrospective nature of this study, we were not able to search for an association between APSN and APS-related manifestations such as arterial or venous thromboses, or lupus anticoagulant (LAC). We found a higher frequency of aCL in patients with APSN (52.9 vs 27%) but this difference was not statistically significant.

Due to the fact that renal histologic findings in patients with SLE has been correlated with a poor renal prognosis, the evolution of APSN on repeated kidney biopsy specimens has been examined. A progression of the thrombotic lesions to chronic and fibrotic forms was reported, and the presence of APSN in the first biopsy was usually followed by the appearance of chronic, sclerotic lesions in the second biopsy. Kincaid-Smith reported biopsy specimens showing fibrinoid thrombi in glomerular arterioles and interlobular arteries at the time of acute renal episodes, and ischemic glomeruli and cellular or fibroelastic intimal proliferation in specimens obtained at repeated biopsies. Results of second biopsies showed that evolution toward glomerular sclerosis was significantly more frequent when capillary thrombosis had been present initially. Our results show fibrinoid necrosis and leukocytes infiltrate significantly associated with the glomerular sclerosis increase. However, as previously reported, the presence of APSN in the first biopsy was associated with the major increase of sclerotic lesions in the repeated biopsy, although not significant in this study due to the small number of patients.

In conclusion, APSN has to be specifically sought in lupus nephritis patients. These patients develop hypertension, raised serum creatinine levels, nephrotic syndrome and progression of histologic lesions in serial kidney biopsy specimens. These features are associated with a worse renal prognosis. These conclusions demonstrate the necessity of a long-term prospective study to characterize the contribution of APSN to the renal evolution of affected patients. Treatment options should be evaluated beyond the usual immunosuppressive therapy and the potential role of anticoagulant therapy and/or vasoprotective agents on renal prognosis should be evaluated.

References