Silent nephritis in systemic lupus erythematosus

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Silent lupus nephritis (SLN) was investigated in 42 renal asymptomatic patients and compared with 49 untreated patients with overt lupus nephropathy (OLN). Urinary sediment, quantitative proteinuria, creatinine clearance, antinuclear antibodies (ANA), complement, circulating immune complexes (CIC) and renal biopsies were evaluated in all of the patients. Forty-one out of the 42 (97.6%) patients had SLN according to histopathological findings. Results showed that the mean age, female/male ratio and the clinical activity index (SLEDAI) were similar in both groups (P > 0.05). The prevalence of ANA, anti-ds DNA, anti-ENA autoantibodies and C4 serum levels showed no statistical differences between the two groups (P > 0.05). Conversely, in the OLN group, elevated CIC and diminished CH50 and C3 serum levels were significantly different (P < 0.01). WHO class II was the predominant renal lesion in the group with SLN (P < 0.0001), whereas class IV was in the OLN patients (P < 0.0001).

We conclude that, in our series, SLN was highly prevalent in renal asymptomatic patients with otherwise systemic lupus erythematosus. Furthermore, abnormal levels of CIC, CH50 and C3 associated with WHO class II suggest a moderate but ongoing activation of immune-mediated renal injury mechanisms. Lupus (2003) 12, 26–30.

Key words: lupus nephritis; overt lupus nephropathy; systemic lupus erythematosus; silent lupus nephritis

Introduction

The renal manifestations (LN) of systemic lupus erythematosus (SLE) are highly pleomorphic with respect to their clinical and morphologic expressions.1 Clinical involvement is expressed in about two-thirds of patients,2,3 but several studies suggest that a much higher percentage would have morphologic evidence of renal disease without clinical manifestations.4–7 This condition has been referred as silent lupus nephritis (SLN) and may only be diagnosed if renal biopsy is performed systematically. From studies carried out in the 1970s, we know that LN is not a static entity and it has a high capacity of transformation from one histological class to another.8–12 Therefore, a precise histologic diagnosis is required for a rational management and follow-up of the glomerular lesion in SLN. In the present investigation, clinical, immunological and histological data is presented from two different groups of SLE patients: group I, without clinical evidence of LN (42 patients); and group II, with SLE overt nephropathy (OLN, 49 patients). The study was designed to further characterize not only the prevalence and the diversity of glomerular lesions in SLN but the behavior of clinical and immunopathological parameters in these patients when compared with patients with OLN.

Patients and methods

Patients

One-hundred and forty-seven patients were studied at the SLE Clinic of the Institute of Institute of Immunology from April 1990 to November 2000. All fulfilled four or more of the American College of Rheumatology criteria for classification of SLE.13 A total of 91 patients, 84 females and seven males from 12 to 65 years of age, were included once the patients and the Institute of Immunology Bioethics Committee gave their respective consent for renal
biopsy. The remaining 56 patients were excluded because either were reluctant to undergo renal biopsy ($n = 46$) or for medical reasons ($n = 10$) such as advanced renal failure, severe arterial hypertension or abnormalities in the coagulation profile. Clinical, immunopathological and histological data allowed us to differentiate two groups of patients: group I, without clinical evidence of LN (42 patients); and group II, with SLE overt nephropathy (OLN, 49 patients).

In all the 147 SLE patients, BUN, plasma creatinine and examination of spot urine among other laboratory tests were carried out during the first consultation. In addition, quantitative urinary sediment and proteinuria and creatinine clearance were practiced in the 91 selected patients in each clinic visit and at least 4 days before performing the renal biopsy.

**Methods**

Overall disease activity was assessed by the SLEDAI index.$^{14}$ Urinary sediment, quantitative proteinuria, creatinine clearance (CrCl), autoantibodies to ds-DNA, RNP, SSA, SSB, Sm and Scl-70, total hemolytic activity (CH$_{50}$), C4 and C3 serum levels were evaluated in all of the patients. In addition, circulating immune complexes (CIC) were also investigated.

Urinary sediment, quantitative proteinuria and CrCl in 24-hour urine collection were examined by routine methods. Antinuclear antibodies were studied by indirect immunofluorescence in Hep-2 cells (Kallestad Diagnostic, MN, USA). Anti-DNA autoantibodies were determined by RIA (Diagnostic Product Corporation, USA)$^{15}$ and anti-ENA autoantibodies (RNP, SSA, SSB, Sm and Scl-70) were determined in Hep-2 cellular extracts by Western Blot and ELISA (Sanofi Diagnostic Pasteur Inc., Paris) methods.$^{16,17}$ CIC were searched by ELISA.$^{18}$ Total hemolytic complement activity in serum (CH$_{50}$) was measured as described by Kent and Fife.$^{19}$ Serum levels of C3 and C4 were determined by nephelometry (QM-300 Automated Analyzer, Kallestad Diagnostic MN, USA).

Percutaneous renal biopsy was performed by the classical method under local anesthesia and previous localization of left renal pole by renal ultrasonogram. Renal tissue was processed by routine methods for optical and immunofluorescent microscopy.$^{20}$ Paraffin sections were stained with Hematoxylin–Eosin, PAS, Gomori thiorheme and silver-methenamine-hematoxilin (SMH) as previously described. Frozen sections were treated by fluoresceinated antisera to IgG, IgA, IgM, C3, C4, k and $\lambda$ chains, properdin and Fibrinogen and assessed by immunofluorescent microscopy. Glomerular lesions were classified according to WHO criteria for lupus nephritis in classes I–VI.$^{21}$ Activity and chronicity indexes were estimated following criteria previously published by D’Agati et al in 1998.$^{6}$

**Patient characterization**

Forty-two patients with normal urinary sediment, proteinuria less than 300 mg per day and CrCl greater than $70 \text{ ml/min per 1.73 m}^2$ of body surface area were considered free of clinical findings of renal involvement and were included in group I. Forty-nine patients with abnormal urinary sediment (presence of more than five leukocytes and/or five red cells per 40× power field and granules cylinders), proteinuria greater than 300 mg per day and CrCl lower than $70 \text{ ml/min per 1.73 m}^2$ were considered as having OLN and were included in group II.

**Statistical analysis**

Histological and immunological data from each group were reported in absolute numbers and percentages in a descriptive form for analysis of frequencies. Statistical inferences between the two groups in relation to prevalence of histological classes and autoantibodies were assessed by Fisher’s exact test and Student’s $t$-test methods. The analyses of CH$_{50}$, C4, C3 and CIC were done by Mann–Whitney test for unpaired samples.$^{22}$

**Results**

Forty-one patients from group I (97.6%) showed glomerular lesions on renal biopsy (SLN). This group included 38 females and three males with an age at onset of $33 \pm 22$ years, a clinical activity index of the disease (SLEDAI) of $19 \pm 12$ points and were clinically followed between 2 and 5 years with a mean of 3.1 years. The 49 OLN patients were 45 females and four males with an age at onset of $30 \pm 10$ years and a SLEDAI of $21 \pm 16$ points. According to these results no differences in age, gender and clinical activity were found between the two groups (Table 1).

Comparison of the selected immunopathological parameters were carried out between the two groups of SLE patients. It is important to keep in mind that both sets of results were significantly abnormal when compared with control values. Thus, elevated ANA titers were found in more than 90% of patients with either SLN or OLN with no statistical differences ($P > 0.05$); similarly, no differences were encountered in serum levels of Anti-DNA or Anti-ENA autoantibodies between the two groups ($P > 0.05$). Conversely, elevated CIC ($P < 0.004$), diminished CH$_{50}$ ($P < 0.01$) and C3 ($P < 0.003$) serum levels were significantly different in the OLN group when
compared with the SLN group (Table 1). WHO class II was the predominant renal lesion in the group with SLN ($P < 0.0001$), whereas class IV was in OLN ($P < 0.0001$; Figure 1). Both activity and chronicity indexes were significantly lower in SLN in absence of necrosis or crescents (Table 1).

**Discussion**

Lupus nephritis is one of the most frequent and serious complications of SLE, although long-term prognosis may be dramatically improved by current therapeutic protocols. Clinical involvement is expressed in about two-thirds of patients (overt nephritis); however, several studies have suggested that the prevalence of SLE renal disease is probably higher due to the existence of SLN. Moreover, SLN may only be diagnosed if renal biopsy is performed systematically.4-7 Our study was carried out based not only on the premise that histological assessment is required for diagnosis and rational management of the glomerular lesion in SLE but to further delineate the immunoclinical and pathological characteristics of SLN. The study evaluated two different groups of SLE patients: group I, without clinical evidence of LN (42 patients); and group II, with OLN (49 patients). The prevalence of histological classes and severity, as well as the behavior of immunopathological parameters were compared between the two groups.

We are aware that few clinicians would advocate baseline renal biopsy in a newly diagnosed patient with SLE, while most specialists would agree that the appearance of any marker of renal disease such as hematuria, proteinuria, nephritic syndrome or renal insufficiency at any time in the course of SLE is a
clear indication for renal biopsy. Our results offer new and solid evidence in favor of the rationale of performing renal biopsy in patients with SLE; moreover, this procedure may provide valuable information about the class, severity, activity and chronicity index of the renal disease when SLN is present which cannot be predicted on the basis of only extra-renal clinical manifestations.

In our series, SLN was present in the 97.6% of the patients with absence of clinical manifestations. This is coincident with previous studies which have clearly shown that lesions of varying severity may occur in almost all SLE patients without clinical manifestations of renal involvement. Traditionally, only 25–50% of unselected patients with SLE have abnormalities of urine or renal function early in their course, although up to 60% of adults and 80% of children may later develop overt renal abnormalities. It is also important to stress that renal lesions were found in our patients without clinical renal manifestations, regardless of time of evolution from apparent onset (data not shown), age of the patient, gender or degree of extra-renal clinical activity of the disease as measured by the SLEDAI scale.

However, in spite of the fact that no statistical differences were found between the groups of patients with SLN and OLN regarding extrarenal clinical activity and in the prevalence of ANA and anti-DNA autoantibodies, high levels of CIC and diminished CH50 and C3 levels were significantly different in OLN when compared with SLN.

The presence of possible pathogenic autoantibodies, capable of structuring complement activating immune complexes in our SLN patients, allows us to emphasize their possible participation in the induction of early silent glomerular lesions. Among them, cationic anti-DNA antibodies which are able to interact with heparin sulfate, heavily present in the glomerular basement membrane, would facilitate the complexes deposition and/or the in situ immune complexes formation and the local activation of the complement cascade. In this context, it is also pertinent to mention that recently, Arbuckle et al. examining sera stored for over 10 years, reported the detection of anti-DNA antibodies many years before the clinical onset of overt SLE. Furthermore, we have previously reported, following a cluster analysis approach, that the absence of antibodies against extractable nuclear antigens (anti-ENA) increased 11-fold the odds ratio of developing SLE nephritis. In the present investigation, we found the same trend, although the differences between the two groups of SLE patients in anti-ENA antibodies levels did not reach statistical significance. The histological data that emerged from our study deserve some comments. WHO class II was present in 64% of patients with SLN while class IV was observed in only 7.7% of the cases. WHO class II and less frequently class V may be found in early stages of the disease, before overt extra-renal manifestations of SLE and serologic markers are detectable, and months or years before the American College of Rheumatology criteria for SLE classification are fulfilled. These findings and those encountered in our investigation tend to confirm the idea that SLE is in fact a polymorphic clinical syndrome with a wide range of immunoclinical expressions even in the early course of the disease, that goes from high levels of anti-DNA antibodies prior to clinical diagnosis to tissue damage, ie OLN with WHO class II or V without extra-renal manifestations of SLE and absence of serologic markers. On the other hand, as in previously published series, WHO class IV was the most prevalent histological form (51%) found in the group of SLE patients with OLN, while WHO class II was found in only 14% of these cases.

Although genetic and environmental factors which influence the evolution of the disease have been identified, the reason why some patients have mild renal lesions and others have fulminant or rapidly evolving renal injury remains a mystery. Moreover, we still do not have definite clinical or histological predictors with high specificity and sensitivity of the natural history of LN. We also do not know the prognostic significance of renal changes in SLN, a matter that also remains controversial.

In conclusion, in our series, the prevalence of SLE renal involvement in patients without renal clinical manifestations was basically universal. Elevated CIC and diminished CH50 and C3 levels with WHO class II in the majority of SLN patients suggest a moderate but ongoing activation of immune-based mechanisms promoters of renal damage. These results tend to favor the notion that SLN may represent an early stage in the natural history of SLE nephritis. Prospective studies are required to further seek for eventual immunopathological markers to assess not only the possible patterns of SLN progression but also its response to comprehensive therapeutic protocols.

References


