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PAPER

Further description of early clinically silent lupus nephritis

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Thirty silent lupus nephritis (SLN) patients were compared to 16 individuals bearing overt lupus nephritis (OLN). Results included: years of systemic lupus erythematosus (SLE) diagnosis were significantly earlier (4.6 ± 2.8 years) in SLN than in OLN (7.18 ± 3.61) ($P < 0.05$). Neurological compromise, hypertension, normocitic anemia and lymphopenia were significantly prevalent in OLN than in SLN ($P < 0.05$). Beside normal urinary sediment and renal function tests, the SLN group showed a moderate increase of both activity (AI) and chronicity (CI) renal pathology index when compared to highly increased AI and CI in OLN ($P < 0.05$). Seventy percent of SLN patients were ISN/RPS Classes I (6.6%) and II (63.3%) while 81% of OLN cases were Classes III, IV (37.5%) and V. IgG, IgA, IgM, λ chain, C3 and fibrinogen immune deposits were found in 90% or over in both SLN and OLN individuals while in 60% or over, both groups also showed κ chain, C1q and C4 deposits. While prevalence of ANA, anti-dsDNA and anti-C1q antibodies were similar in both groups, anti-histone, anti-RNP, CIC and CH_{50} serum levels were significantly different in OLN versus SLN ($P < 0.05$). We strongly suggest that indeed SLN is the earliest stage in the natural history of lupus nephritis. *Lupus* (2006) **15**, 1–7.

Key words: anti-C1q autoantibodies; overt lupus nephritis; silent lupus nephritis; systemic lupus erythematosus

Introduction

In the last three decades over 200 cases of renal biopsy (RB) proven silent lupus nephritis (SLN) have been published.[1–13] In our previous preliminary report,[13] immunoclinical and histopathological characteristics of a cohort of 42 SLN patients were compared to those of 49 SLE individuals bearers of overt renal disease (OLN). We suggested that renal compromise is seen in all systemic lupus erythematosus (SLE) patients and that SLN may represent an early stage in the natural history of SLE. In 2004, the working group of both the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) reported a new consensus on the classification of the histopathology of lupus nephritis (LN).[14] One of their critical and fundamental modifications of the widely used World Health Organization (WHO) LN classification[15,16] is the identification of Class I as 'minimal mesangial LN' ruling out a complete lack of renal abnormalities in SLE. In the present study, we have employed the ISN/RPS novel approach in the context of an expanded demographic, clinical and immunopathological protocol. The obtained results allowed us to further describe the characteristics of 'early clinically silent lupus nephritis (ESLN)'.

Patients and methods

Forty-six patients were selected from the Institute of Immunology ongoing SLE Outpatient Clinic Database. Forty-five were female and one male. All fulfilled four or more of the American College of Rheumatology (ACR) criteria for SLE.[17] Selected SLN individuals were either already in the database or were new ones. Once these latter patients and the Institute Bioethics Committee gave the respective consent, percutaneous renal biopsy (RB) was performed under local anesthesia and previous localization of left renal pole by ultrasonogram. It should be stated that no clinical complications were detected in any of the patients subjected to RB. We estimated age at disease onset and disease duration according to the first appearance of clinical features of SLE. Patients with LN were divided in two groups: 30 SLN individuals (five new cases and 25 patients from our SLE

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database) as previously described[13] and characterized by normal urinary sediment, creatinine clearance (CrCl) and proteinuria <300 mg/24 hours and those subjects ($n = 16$) with overt renal disease (OLN) showing abnormal urinary sediment, diminished CrCl and proteinuria >300 mg/24 hours.

Overall disease activity was assessed by SLEDAI index.[18] Patients were considered to be hypertensive if the blood pressure exceeded 140/90 mmHg. Urinary sediment, quantitative proteinuria and CrCl in 24 hours urine collection were carried out by routine methods. Serum samples were obtained at the time of performing the renal biopsies. Antinuclear antibodies were measured by indirect immunofluorescence on mitotic Hep-2 cells (Fluorescent ANA Test System, Immunoconcepts, Germany). IgG anti-dsDNA was determined by RIA (Diagnostic Products Corporation, Los Angeles). Anti-ENA, anti-Histone and anti-cardiolipin antibodies were detected by ELISA (AESKU, Diagnostics GMBH, Germany). Serum total hemolytic activity (CH_{50}) was measured by the Kent and Fife method.[19] C3 and C4 were determined by nephelometry (Beckman Array 360 System, Miami, USA); CIC were determined by ELISA and by cryoprecipitates following methods previously reported by our laboratory.[20]

RB

Renal tissue was processed for optical and immunofluorescent microscopy. Paraffin sections were stained with Hematoxilin-Eosin, PAS, Gomori trichrome and silver-methenamine-hematoxilin as previously described.[21] Frozen sections were treated with fluoresceinated antiserums to human IgG, IgA, IgM, C1q, C3, C4, κ and λ chains, albumin and fibrinogen and assessed by immunofluorescent microscopy. The new classification for LN proposed by ISN/RPS working group was employed. RB from patients already included in the SLE database were analyzed and independently reclassified by two renal pathologists. Activity and chronicity indexes (AI and CI respectively) were estimated following criteria previously reported.[22]

Anti-C1q autoantibodies

To detect serum IgG anti-C1q, the indirect ELISA method of Siegert *et al.*[23] was applied with minor modifications. Briefly, 96-microwells polystyrene plates were coated with 1 µg/mL of purified C1q (Calbiochem, La Jolla, USA) in carbonate/bicarbonate buffer 0.1 M pH 9.6 overnight at 4°C, blocked with 1% bovine serum albumin (BSA) (Sigma, St Louis, USA) in phosphate buffer saline-Tween-20 0.05% (PBST, Merck, Schuchardt, Germany) pH 7.4 for one hour at 37°C. Serial dilutions of a reference serum and triplicates of serum samples diluted 1:50 in PBST-NaCl 1 M were incubated for one hour at 37°C. Anti-human IgG peroxidase conjugate (Calbiochem, La Jolla, USA) and TMB Substrate Peroxidase (Vector, Burlingame, USA) were added sequentially according to the manufacturer instructions to obtain a colorimetric reaction that was stopped with H_2SO_4 (Merck, Schuchardt, Germany) and read at 450 nm. Results were calculated from a standard curve with reference readings and arbitrary 2500 ELISA Units (EU) for serum lowest dilution. Cut-off point was 60 EU from 120 normal human sera selected.

Statistical analysis

Results in the two groups are shown as mean ± SEM and comparisons between groups were performed employing the Mann–Whitney test. Bivariate correlations were established employing both the Pearson and Spearman correlation coefficients. Histological data in each group were analysed by independent samples Student's t-test. Finally, contingency tables and Fisher's exact test were also employed. Two-tailed $P < 0.05$ were considered statistically significant.

Results

The demographic and clinical characteristics of the patients in the SLN and OLN groups are summarized in Table 1. Mean age was 35 ± 13 and 31 ± 10 years

Table 1 Demographic and clinical characteristics of patients with SLE

Characteristics	*SLN (n = 30) (%)*	*OLN (n = 16) (%)*	*P-value*
Age[a]	35 ± 13	31 ± 10	NS
Years of Dx[a]	4.6 ± 2.8	7.18 ± 3.61	<0.05*†
Male/female patients	1/30	0/16	NS
SLEDAI[a]	7.71 ± 5.1	12.93 ± 6	<0.05*
Malar rash	16 (53.3)	9 (60)	NS
Photosensitivy	15 (50)	10 (66.6)	NS
Oral ulcers	14 (46.6)	8 (53.3)	NS
Arthritis	26 (86.6)	14 (93.3)	NS
Serositis	5 (16.6)	2 (13.3)	NS
Neurological disease	0 (0)	7 (46.6)	<0.05†
Alopecia	20 (66.6)	13 (86.6)	NS
Ecchymosis	11 (36.6)	12 (80)	<0.05†
Hypertension	7 (23.3)	9 (60)	<0.05†
Raynaud's phenomenon	11 (36.6)	1 (6.66)	<0.05†

*Mann-Whitney test.
†Fisher's exact test.
[a](mean ± SD).

for SLN and OLN respectively. The range of years of initial diagnosis was 1–10 (mean 4.6 ± 2.8) in SLN and 1–12 (mean 7.18 ± 3.61) years in OLN. Arthritis was the most frequently observed clinical finding in both groups followed by alopecia, malar rash, photosensitivity, oral ulcers and serositis. Neurological disease, ecchymosis and hypertension were significantly greater in OLN that SLN ($P < 0.05$) while the prevalence of Raynaud phenomena was significantly higher in SLN patients ($P < 0.05$). The SLEDAI index showed a significant difference ($P < 0.01$) between the OLN (12.93 ± 6) and the SLN individuals (7.71 ± 5.1). Hematological profile in both SLN and OLN are depicted in Table 2. Normocitic anemia and lymphopenia were significantly different in OLN when compared to SLN.

Renal profiles

Renal function tests, histopathological classes and AI and CI indexes are shown in Table 3. Proteinuria in SLN was within normal limits (mean value: 140 ± 80.7 mg/24 hours) when compared to 2147.6 ± 1607.8 mg/24 hours in the OLN individuals ($P < 0.01$). Similarly, CrCl in the SLN was 96.08 ± 17.78 mL/min compared to 65.75 ± 28.83 ml/min in the OLN group ($P < 0.05$). Urinary sediments in the SLN patients were unremarkable while in all OLN cases were abnormal. In relation to the histopathology assessment, in the SLN group, 69.9% of the patients belonged to Class I ($n = 2$) and Class II ($n = 19$) while 13 (81.2%) out of the 16 OLN individuals showed renal lesions compatible with Classes III (18.7%), IV (37.5%) and V (25%) while only three patients were found to be class II. AI and CI index were significantly higher in the OLN group (AI: 5.81 ± 2.8; CI: 3.37 ± 1.85) in comparison with the SLN group (AI: 2.96 ± 1.29; CI: 1.93 ± 1.04) ($P < 0.05$). Immune deposits (IgG, IgA, IgM, C1q, C4, C3, κ, λ, albumin and fibrinogen) were searched in all 46 RB (Table 4). Patients with SLN showed an almost similar pattern and prevalence of deposits of immunoglobulins, light chains, complement components and fibrinogen as observed in the OLN group.

Table 2 Hematologic findings in SLN and OLN

	SLN (n = 30)	*OLN* (n = 16)	P-*value*
WBC × mm³	5520.68 ± 2510	5731.33 ±	NS
Platelets count × mm³	236.181.5 ± 98.532	285.666.6 ± 85.909	NS
Hemoglobin (g/DL)[c]	11.75 ±1.47	10.3 ± 1.64	NS
False positive VDRL	2/30 (6.6)	1/16 (6.2)	NS
Hemolytic anemia	2/30 (6.6)	1/16 (6.2)	NS
Normocitic anemia	7/30 (23.3)	12/16 (75)	<0.05[d]
Thrombocytopenia[a]	4/30 (13.3)	2/16 (12.5)	NS
Lymphocytopenia[b]	12/30 (40)	12/16 (75)	<0.05[d]

[a]Platelet: <150 000 × mm³;
[b]White blood cells: < 4500 × mm³ . normal values: white blood cells: 4.5–11.0 × 10³/mm³ platelet: 150–350 × 10³mm³;
[c]Hemoglobin: male: 13.5–17.5, female: 12.0–16.0 g/dL.
[d]Fisher's exact test.

Table 3 Renal profile in SLN and OLN

	SLN (n = 30) (%)	*OLN* (n = 16) (%)	P-*value*
Proteinuria/mg/24 h[a]	140 ± 80.7	2147.6 ± 1607.8	<0.05 *
CrCl[b] (mL/min)	96.08 ± 17.78	65.75 ± 28.83	<0.05 *
Urinary sediment	Normal	Abnormal	
RB (ISN/RPS)			
I	2 (6.66)	0 (0.0)	NS
II	19 (63.3)	3 (18.7)	<0.05†
III	6 (20)	3 (18.7)	NS
IV	1 (3.33)	6 (37.5)	<0.05†
V	2 (6.66)	4 (25)	NS
VI	0	0	
Activity Index (AI)	2.96 ± 1.29	5.81 ± 2.8	<0.05*
Chronicity Index (CI)	1.93 ± 1.04	3.37 ± 1.85	<0.05*

*Mann–Whitney test.
†Fisher's exact test.
[a]≤300 mg/24 hours in SLN.
[b]Creatinine clearance (CrCl): 80–120 mL/min.

Immunopathological profiles

The investigated parameters were subclassified in autoantibodies, CIC and the complement system (Table 5). The prevalence of both ANA and anti-dsDNA antibodies were basically similar in both groups. While detectable anti-C1q antibodies were higher in SLN patients when compared to OLN, the difference did not reach statistical significance. anti-histone and anti-RNP showed the opposite trend being significantly higher in the OLN patients. Otherwise, no significant differences were found between both groups in relation to the rest of the evaluated autoantibodies.

Table 4 Immune deposits in LN

	*SLN**	(n = 30) (%)	*OLN**	(n = 16) (%)
IgG+	28	(93.3)	15	(93.7)
IgM+	28	(93.3)	15	(93.7)
IgA+	28	(93.3)	16	(100)
K+	21	(70)	15	(93.7)
λ+	27	(90)	15	(93.7)
C1q+	18	(60)	12	(75)
C3+	28	(93.3)	16	(100)
C4+	19	(63.3)	13	(81.3)
Fib[a]+	27	(90)	15	(93.7)
Alb[b]+	26	(86)	15	(93.7)

*Fisher's tests were not significant among the different groups.
[a]Fibrinogen.
[b]Albumin.

Table 5 Immunopathological parameters in SLN and OLN

Parameters	*SLN (n = 30) (%)*	*OLN (n = 16) (%)*	P-*value*
Auto-antibodies			
ANA	30 (100)	16 (100)	NS
Anti-dsDNA	29 (96.6)	14 (93.3)	NS
Anti-C1q	19 (63.3)	7 (44)	NS
Anti-Histone	10 (37)	12 (80)	<0.05£
Anti-Sm	8 (26.6)	5 (31.2)	NS
Anti-RNP	6 (20)	8 (50)	<0.05£
Anti-SSA	12 (40)	6 (37.5)	NS
Anti-SSB	4 (13.3)	5 (31.2)	NS
Anti-Scl-70	1 (3.3)	1 (6.2)	NS
Anti-Jo1	0 (0)	0 (0)	
aCL IgG	15 (50)	10 (62.5)	NS
aCL IgM	4 (13.3)	4 (25)	NS
Immune complexes			
CIC (high)	10 (33.3)	12 (75)	<0.05[a]
Cryoprecipitates (high)	10/21 (47.6)	6/8 (75)	<0.05[a]
Complement			
C3 (low)	14 (46.6)	9 (56)	NS
C4 (low)	20 (66.6)	11 (68.7)	NS
CH_{50} (low)	23 (76.6)	15 (93.7)	<0.05[a]

Normal values: anti-C1q: <60 EU/ml; anti-histone: <15 UI/mL; anti-dsDNA: <6 UI/mL; CIC: ≥10 µeq/mL, C3 = 90–150 UI/mL, C4 = 20–40 UI/mL; CH_{50} = 150–250 UI/mL, a CL IgG <10 GPL, a CL IgM <11 MPL, Cryoglobulins: <16–30 years = 0.092–0.115 mg/mL, 31–92 years: 0.436–0.520 mg/dL. Anti-ENA (Sm, RNP, SS-A. SS-B, Scl-70, Jo1) = 0–15 U/mL.
[a]Fisher's exact test.

CIC screened either by ELISA or by cryoprecipitates were detectable in both groups. Levels in the OLN patients were significantly higher than those detected in SLN ($P < 0.05$).

Serum total hemolytic activity was diminished in 15 out of 16 OLN cases and in 23 out of 30 SLN patients. The difference was statistically significant ($P < 0.05$). C4 and C3 serum levels were similarly low in both groups.

Bivariate correlations between parameters were established in both SLN and OLN (Table 6). In SLN, positive correlations were found between anti-C1q antibodies and both AI and CI index, C3 and C4, C4 and CH_{50} and AI and CI while inverse correlations were detected between anti-dsDNA antibodies, C3 and C4. In OLN, positive correlations were encountered between anti-C1q and anti-histone, CIC and SLEDAI index, anti-histone and CIC, CIC and SLEDAI index, C3 and C4, C3 and CH_{50}, SLEDAI index and CI and also between AI and CI while inverse correlations were demonstrable between anti-dsDNA and CI and C3 and C4 with SLEDAI index.

Discussion

In 2003 our institute reported the immunoclinical and histopathological characteristics of 42 patients bearing the diagnosis of SLN compared with 49 SLE individuals with OLN. We advanced the hypothesis that renal involvement is universal among SLE patients and that RB proven SLN may represent an early stage in the natural history of LN.[13]

Shortly after, both the International Society of Nephrology and the Renal Pathology Society (ISN/RPS) proposed a new classification for LN. The exhaustive report[14] described an overall critical assessment of the 1982 WHO classification, based upon 'the need for clarification of the different categories and the

Table 6 Bivariate Correlations in both SLN and OLN

Groups	*Variables*	*Anti-C1q*	*Anti- histone*	*Anti-dsDNA*	*CIC*	*C3*	*C4*	*SLEDAI*	*CH_{50}*	*AI*	*CI*
SLN	Anti-C1q	1.000	0.222	−0.131	−0.004	−0.129	−0.165	−0.145	0.318	**0.403***	**0.596***
	Anti- histone		1.000	−0.024	−0.050	0.033	−0.142	−0.002	0.147	−0.003	0.011
	Anti-dsDNA			1.000	0.105	**−0.444***	**−0.375***	0.130	−0.152	0.160	− 0.184
	CIC				1.000	−0.146	−0.193	0.102	−0.040	0.170	0.069
	C3					1.000	**0.676***	−0.101	0.098	−0.061	− 0.120
	C4						1.000	−0.150	**0.436***	−0.287	− 0.259
	SLEDAI							1.000	−0.232	−0.089	0.113
	CH_{50}								1.000	0.154	0.087
	AI									1.000	**0.625***
	CI										1.000
OLN	Anti-C1q	1.000	**0.568***	−0.318	**0.695***	−0.008	−0.124	**0.579***	0.294	−0.102	0.308
	Anti- histone		1.000	−0.276	**0.579***	−0.266	−0.369	0.367	0.148	0.400	0.278
	Anti-dsDNA			1.000	−0.126	0.115	0.016	−0.358	−0.477	−0.257	**− 0.514***
	CIC				1.000	−0.156	−0.174	**0.430***	0.033	0.135	0.172
	C3					1.000	**0.799***	**−0.511***	**0.566***	−0.476	- 0.303
	C4						1.000	**−0.536***	0.439	−0.422	− 0.540
	SLEDAI							1.000	0.021	0.346	**0.576***
	CH_{50}								1.000	−0.061	0.104
	AI									1.000	**0.680***
	CI										1.000

*$P < 0.05$.

diagnostic terminology and the fact that classification of renal involvement in SLE is critical in terms of patient care and therapeutic trials'.

Their proposal includes the 'minimal mesangial lupus nephritis' as Class I characterized by normal glomeruli by light microscopy but mesangial immune deposits by immunofluorescence. Class I as now defined by the group of experts strengthen the concept that in fact all SLE patients shows renal lesions and that the indication of performing a renal biopsy at the moment of definitely establishing the diagnosis of SLE is essential for diagnostic, therapeutic and prognostic purposes.[24] Class II have been defined as mesangial proliferative lupus nephritis with purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy associated to immune deposits or few isolated subepithelial or subendothelial deposits visible only by immunofluorescence or electron microscopy.

In addition, review of the literature revealed thirteen published series[1–13] (Table 7) comprising 204 renal biopsy proven cases of SLN with a predominant prevalence of 122 patients (59.8%) classified following the former WHO classification for LN in Class I ($n = 38$) and Class II ($n = 84$) and 82 individuals distributed in 32 Class III (16%), 40 Class IV (20%), nine Class V (4%) and one Class VI (0.4%).

In the present study, we expanded our investigations on the demographic, clinical, renal and immunopathological characteristics of 30 SLN patients from our SLE database, employing the ISN/RPS classification and focusing on the search for possible serum markers in this particular stage of LN.

As shown in Tables 1 and 2, these two groups of SLE patients differed significantly (OLN versus SLN) in terms of the SLEDAI index, neurological involvement, lymphopenia, impaired renal function and the most severe histopathological classes. In addition, in the prevalence of ecchymosis, hypertension and normocitic anemia. Most of these latter parameters reflect chronicity of the disease.

Within the context of the ISN/RPS new classification and of the revised series, our new set of findings of the renal compromise in the SLN patients deserve further comments. Two new cases were found to show 'minimal mesangial LN' (Class I). In one of the two patients, all the selected immune deposits were found including C1q deposit while in the other Class I individual IgM, C3, λ chain and fibrinogen deposits were detected. Nineteen patients were classified as bearers of 'minimal proliferative mesangial LN' (Class II). All showed IgG, IgA, IgM, λ chain, C3 and fibrinogen while in decreasing frequency, κ chain, C4 and C1q deposits were also found (data not shown). The whole SLN group exhibited moderate increase of AI and CI indexes and unremarkable urinary sediment and kidney function.

Twelve immunopathological parameters were assessed in both groups (Table 4). Within the autoantibodies group, antinuclear and anti-dsDNA antibodies prevalence were identical in both sets of SLE patients. The anti-C1q antibodies were detected more frequently in the SLN group while anti-histone and anti-RNP prevalence in the OLN individuals were significantly different than in SLN.

Anti-C1q antibodies have been associated with the clinical presence of LN, as predictor of nephritis flares and may be found deposited in the kidneys.[23,25–28] Marto and co-workers compared patients with RB proven OLN with SLE individuals that clinically showed no evidences of renal disease. In addition, a second group of 83 SLE cases with median disease duration of nine years and absence of clinical renal disease were also evaluated. A strong association between high titers of anti-C1q antibodies and active glomerulonephritis was found. Moreover, the retrospective analysis of the 83 consecutive patients showed that anti-C1q antibodies had a very high sensitivity and negative predictive values for the occurrence. of renal disease These investigators stressed the possibility of the potential pathogenic effect of C1q-anti-C1q complexes in all types of LN including the mesangial compromise.[29] In 2005, we reported the presence of anti-C1q antibodies in 66.6% of SLN individuals.[30] In the present study, we further confirm the remarkable prevalence of anti-C1q antibodies in the early stages of LN. Moreover, a positive correlation between anti-C1q antibodies and both AI and CI in SLN was found, further confirming Marto *et al.* proposal of a pathogenic role of C1q-anti-C1q complexes in early LN.[29] Trouw *et al.* recently reported in the mouse model,[31,32] that the amount of C1q present in the glomerulus seems critical for renal damage to occur. The C1q deposits

Table 7 Silent lupus nephritis: reported series

Authors	*Total*	*RB*[b]	*I*[a]	*II*[a]	*III*[a]	*IV*[a]	*V*[a]	*VI*[a]
Cruchaud *et al.*, 1975[1]	6		0	5	0	1	0	0
Hollcraft *et al.*, 1976[2]	10		0	0	0	10	0	0
Mahajan *et al.*, 1977[3]	15		0	3	12	0	0	0
Cavallo *et al.*, 1977[4]	8		0	4	0	4	0	0
Eiser *et al.*, 1979[5]	13		3	4	3	3	0	0
Woolf *et al.*, 1979[6]	8		0	2	2	4	0	1
Bennet *et al.*, 1982[7]	20		3	4	9	3	0	0
Roujeau *et al.*, 1984[8]	7		5	0	0	2	0	0
Stamenkovic *et al.*, 1986[9]	24		11	7	0	5	1	0
Font et al: 1987[10]	15		6	7	2	0	0	0
Miyata, 1993[11]	16		0	16	0	0	0	0
González-Crespo *et al.*, 1996[12]	20		9	6	0	3	2	0
Zabaleta-Lanz *et al.*, 2003[13]	42		1	26	4	5	6	0
Total	204		38	84	32	40	9	1

[a]WHO classes (I–VI).
[b]RB: renal biopsies.

both in Class I and II, the significant correlation of both activity and chronicity index with serum levels of anti-C1q antibodies in SLN and the altered renal histopathology in all SLN studied individuals are strong evidences suggesting that in human SLE, C1q-anti-C1q immune complexes may indeed participate very early in the induction of renal damage.

We also report herein for the first time detectable anticardiolipin antibodies (ACAs) in patients with SLN. The prevalence (including all isotypes) of these autoantibodies varies from 16% to 60% in the different reported series.[33] IgG ACAs have been identified as possible risk factors in the development of thrombosis and the antiphospholipid syndrome (APS). Whether their presence is related to LN is still controversial.[34, 35] In our SLN patients, ACAs were predominantly IgG as in OLN. The increased serum levels were moderate and in no instance we found among the SLN individuals evidences of either APS or renal thrombotic changes.

The data on the prevalence of CIC and on complement activation and consumption in the SLN patients, further support the possibility that a 'pool' of complement activating circulating immune complexes may be operative before the renal lesion is installed and becomes clinical. Since Schur and Sandson initial report in 1968,[36] dsDNA have been a leading antigenic system. Progressively, ribosomal P protein,[37] Ro/SSA,[38] histone,[39] C1q[26] and nucleosome[40] have been added to the pool. More recently, Reichlin reported that lipoprotein lipase (LPL) may also be involved in SLE patients with OLN.[41]

The recent results obtained by Cortes-Hernandez *et al.*[42] and by Mok and Tang[43] from two large SLE cohorts which addressed the possible role of some of these immune complexes either as predictors or as part of the pathogenesis of SLE renal involvement added valuable information to the possible participation of antibodies against dsDNA, histone, nucleosome and C1q in LN. Thus, in the former cohort, the OLN group showed that anti-histone and to a lesser extent anti-dsDNA were associated to a greater risk of developing Class IV (diffuse) LN. In the latter cohort, the observations in newly diagnosed SLE in a Chinese population led to find a 60% prevalence of OLN. anti-dsDNA and anti-Ro antibodies were not independent predictors of development of renal disease. Moreover, on the basis of microscopic hematuria and proteinuria <0.5 g/24 hours, these investigators concluded that 'subclinical renal disease' at the onset of the disease heralded the subsequent appearance of OLN.

Within this context, employing cluster analysis instead of assessing individual antibodies systems, Tapanes *et al.* originally described the Sm/RNP cluster associated with absence and/or the most benign form of SLE nephropathy.[44] These observations have been now confirmed by To and Petri in the largest cohort of SLE individuals studied thus far by cluster analysis.[45]

As encountered in our previous report,[13] the majority of our SLN patients showed low total hemolytic activity and C4 serum levels and almost half also showed low C3. This complement profile is usually the case for SLE individuals with clinically evident renal disease. Moreover, as shown above, C1q, C4 and C3 deposits were frequently detectable in the renal specimens of the SLN individuals.

Thus, the integration of the three main components of a complement activating immune complex mediated systemic vascular disease and the presence of RB proven initial renal histopathological changes are readily demonstrable in SLE patients characterized by an unremarkable kidney functional status.

Therefore, supported by our new set of findings and by the ISN/RPS classification which ruled out that the former WHO Class I is a normal histological stage in SLE kidney involvement, we confirmed our hypothesis on the universality of renal compromise in SLE and propose to identify RB proven SLN as the earliest stage (ESLN) in the natural history of LN.

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