EXTENDED REPORT

Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis

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Background: Lupus nephritis (LN) is a common manifestation in patients with systemic lupus erythematosus (SLE). Autoantibodies and ethnicity have been associated with LN, but the results are controversial.

Objective: To study the immunological and demographic factors associated with the development of LN.

Patients and methods: A retrospective case-control study of 127 patients with biopsy-proven LN, and 206 randomly selected patients with SLE without nephritis as controls was designed. All patients had attended our lupus unit during the past 12 years. Standard methods were used for laboratory testing.

Results: Patients with LN were significantly younger than the controls at the time of SLE diagnosis (mean (SD) 25.6 (8.8) years *v* 33.7 (12.5) years; p<0.0001). The proportion of patients of black ethnic origin was significantly higher in the group with nephritis (p=0.02). There were no differences in sex distribution or duration of follow up. A higher proportion of anti-dsDNA, anti-RNP, anti-Sm, and lupus anticoagulant (LA) was seen in the group with nephritis (p=0.002; p=0.005; p=0.0001; p=0.01, respectively). In univariate, but not in multivariate, analysis male sex and absence of anti-dsDNA were associated with earlier onset of renal disease (p=0.03; p=0.008). In multivariate analysis the only factors associated with nephritis were younger age at diagnosis of SLE, black race, presence of anti-dsDNA, anti-Sm, and LA. No demographic or immunological associations were seen with WHO histological classes.

Conclusions: Young, black patients with anti-dsDNA, anti-Sm antibodies, and positive LA, appear to have a higher risk of renal involvement. These patients should be carefully monitored for the development of LN.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with numerous patterns of clinical and immunological manifestations. The most distinctive laboratory feature of SLE is the presence of autoantibodies to nuclear antigens including double stranded DNA (dsDNA), histones, ribonucleoprotein (RNP), and the Sm antigen.

Renal disease in SLE occurs in 40–75% of patients, most often within five years of disease onset, and is one of the strongest predictors of a poor outcome.[1] Anti-dsDNA antibodies are reported to be more prevalent in patients with SLE who have renal disease. There is evidence supporting a pathogenic role for DNA-anti-DNA immune complexes in lupus nephritis (LN).[2 3] Patients with active renal lupus often have raised levels of anti-dsDNA antibodies and fluctuations in anti-dsDNA antibody levels may reflect global disease activity in some, though not all, patients. Antigen-antibody reactions involving the extractable nuclear antigens (ENA) Ro, RNP, and Sm may also contribute to the pathogenesis of nephritis. Although a number of reports describe associations of anti-ENA with LN, definitive relationships have not been fully established.

It is well recognised that ethnic differences may influence the clinical expression of the disease and the presence of autoantibody profiles.

We have studied the immunological and demographic factors associated with the development of LN.

PATIENTS AND METHODS

We designed a retrospective case-control study comparing 127 patients with biopsy proven LN with 206 patients with SLE without nephritis. All patients were classified as having SLE according to the revised American College of Rheumatology (ACR) classification criteria[4] and all patients had attended the lupus unit over the past 12 years. Patients were divided according to racial origin: white (Caucasian); black (African and Caribbean); and oriental (Chinese, Japanese, Indian).

To avoid left-censorship bias, our starting point was the renal biopsy register, which included all patients undergoing renal biopsy over the past 12 years. Patients who had died or were subsequently lost to follow up were thus included in this study of risk factors for nephritis. We estimated age at disease onset and disease duration according to the first appearance of clinical features of lupus in the medical records. We considered the time at which renal disease developed to be the date of the first biopsy. The time to development of renal disease was ascertained as the difference between time of the diagnosis of SLE and the time of the first renal biopsy.

Antinuclear antibodies were measured by indirect immunofluorescence on rodent liver cells, anti-dsDNA antibodies by radioimmunoassay (Farr assay), and antibodies to ENA by countercurrent immunoelectrophoresis (CIE) using bovine spleen and rabbit thymus extracts.[5 6] Results were considered positive if the assays had ever been positive during the follow

Abbreviations: aCL, anticardiolipin antibodies; CIE, countercurrent immunoelectrophoresis; ELISA, enzyme linked immunosorbent assay; ENA, extractable nuclear antigens; LA, lupus anticoagulant; LN, lupus nephritis; SLE, systemic lupus erythematosus

Table 1 Demographic characteristics of patients with SLE with and without lupus nephritis

	SLE with nephritis (n=127)	SLE without nephritis (n=206)	p Value
Age SLE diagnosis, mean (SD)	25.6 (8.8)	33.7 (12.5)	<0.0001
Sex (female/male)	117/10	196/10	NS
Race (white/black/oriental)*	85/28/13	167/17/11	0.02
Follow up (months), mean (SD)	145.4 (88.4)	122.8 (86.2)	NS

*Details not known for one patient with nephritis and 11 controls without nephritis.

Table 2 Immunological profile in patients with SLE with and without lupus nephritis

	SLE with nephritis +ve (%)/–ve (%)	SLE without nephritis +ve (%)/–ve (%)	p Value	OR	95% CI
ANA	126 (99.2)/1 (0.8)	203 (99)/3 (1)	NS	1.86	0.19 to 18.10
DNA	86 (68)/41 (32)	104 (50)/102 (50)	0.002	2.06	1.30 to 3.26
RNP	43 (34)/82 (66)	42 (20)/164 (80)	0.005	2.05	1.24 to 3.38
Sm	31 (25)/94 (75)	19 (9)/187 (91)	0.0001	3.25	1.74 to 6.05
Ro	47 (38)/78 (62)	76 (37)/130 (63)	NS	1.03	0.65 to 1.63
La	11 (9)/114 (91)	34 (17)/172 (83)	0.047	0.49	0.24 to 1.00
aCL IgG	38 (31)/83 (69)	47 (24)/146 (76)	NS	1.42	0.86 to 2.36
aCL IgM	11 (9)/110 (91)	25 (13)/168 (87)	NS	0.67	0.32 to 1.42
LA	46 (38)/75 (62)	48 (25)/145 (75)	0.01	1.85	1.13 to 3.03

Table 3 Demographic and immunological data—univariate and multivariate analysis

	p Value (univariate analysis)	p Value (multivariate analysis)	Odds ratio (95% CI)
Age at diagnosis	<0.00001	<0.000001	
Race	0.001	0.04	
Anti-dsDNA	0.002	0.002	2.35 (1.38 to 4.03)
Anti-RNP	0.0057	NS	
Anti-Sm	0.0001	0.01	3.27 (1.30 to 8.23)
Anti-La	0.04	NS	
Lupus anticoagulant (LA)	0.01	0.02	1.98 (1.13 to 3.48)

up period. Anticardiolipin antibodies (aCL) were measured by enzyme linked immunosorbent assay (ELISA), using standardised methods.[7] The presence of lupus anticoagulant (LA) was assessed by measurement of the activated partial thromboplastin time and the dilute Russell viper venom time and confirmatory correction tests.[8] Patients were considered positive for aCL/LA when the results of these tests were positive on at least two occasions, at least six weeks apart.[9]

Renal biopsies were assessed by a histopathologist specialising in renal pathology. The renal biopsy specimens were classified according to the World Health Organisation (WHO) criteria: minimal changes (class I), mesangial alterations (class II), focal proliferative (III), diffuse proliferative (IV), membranous (V) glomerulonephritis.[10] The individual components of the renal pathology were classified and scored according to previously published activity and chronicity scores.[11]

Statistical analysis

Associations between demographic and immunological profiles and presence of nephritis, time to renal disease and class of nephritis were analysed by χ^2 test, Aspin-Welch unequal variance T-test, Kolmogorov-Smirnov test, or Kruskal-Wallis one-way analysis of variance by ranks when appropriate. To verify if an association exists between age of SLE diagnosis and time of renal disease, the Pearson correlation was used. Variables significantly associated with LN ($p<0.05$) were entered into a logistic regression model. Multivariate analysis for time to renal disease was performed using multiple regression. The results were expressed as mean (SD) and as p value. When appropriate, the results were expressed as an odds ratio with 95% confidence limits. A value of $p<0.05$ (two tailed) was considered significant. All analyses were performed with the NCSS statistical software.

RESULTS

The group of patients with nephritis was significantly younger than the control group at the time of SLE diagnosis (25.6 (8.8) years *v* 33.7 (12.5) years; $p<0.0001$). There were no differences in sex distribution or duration of disease between the groups. The proportion of black patients was significantly higher in the group with nephritis than in the control group ($p=0.02$), although in controls, race was known only in 195 out of 206. Table 1 shows more detail of the demographic data.

The immunological profile differed between the groups. A higher frequency of anti-dsDNA, anti-RNP, anti-Sm and LA was observed in the nephritis group. Table 2 lists these results and expresses them as p value and odds ratio (OR) with 95% confidence limits (CI). In multivariate analysis, the following parameters correlated with the presence of nephritis: younger age at SLE diagnosis, black race, presence of anti-dsDNA, anti-Sm, and LA (table 3).

Table 4 Demographic, immunological profile, and time of renal disease (months). The results are expressed as mean (SD)

		p Value
Univariate analysis		
Race		NS
White	65.0 (71.0)	
Black	52.5 (87.3)	
Oriental	43.6 (50.2)	
Sex		0.03
Female	62.1 (72.9)	
Male	32.2 (69.8)	
dsDNA		0.008
Positive	68.5 (72.1)	
Negative	41.5 (71.7)	
RNP		NS
Positive	49.5 (72.7)	
Negative	66.3 (73.1)	
Sm		NS
Positive	49.0 (58.3)	
Negative	64.3 (77.3)	
Ro		NS
Positive	60.8 (79.0)	
Negative	60.3 (69.9)	
La		NS
Positive	59.3 (61.9)	
Negative	60.6 (74.4)	
aCL IgG		NS
Positive	68.3 (76.7)	
Negative	57.2 (73.1)	
aCL IgM		NS
Positive	54.7 (64.4)	
Negative	61.3 (75.2)	
LA		NS
Positive	70.2 (86.4)	
Negative	54.9 (65.4)	
Multivariate analysis		
Sex		NS
dsDNA		NS

We analysed whether demographic and immunological factors influenced time to development of renal disease. There was no association between the age at SLE diagnosis and time to development of renal disease (r=0.09). In univariate analysis, we observed that oriental and black patients developed earlier nephritis than white though the difference was not significant. Male sex and absence of anti-dsDNA antibodies were associated with earlier onset of renal disease (p=0.03; p=0.008 respectively). However, in multivariate analyses these associations were not significant (table 4). No demographic or immunological factors were associated with any histological class of nephritis (table 5).

DISCUSSION

We observed that patients were younger at the time of SLE diagnosis in the group with nephritis than in the controls. Previous reports have noted that nephropathy is less common in older onset (>50 years) SLE than in adult onset (18–50 years) disease.[12 13] Although the explanation for this apparent age related variability in the disease expression remains unclear, differences in demographic factors and responsiveness of an aging immune system have been implicated. It has been speculated that older and younger onset patients may vary in genetic predisposition and respond to different triggering mechanisms.[14 15]

We found more black patients in the group with nephritis (22%) than in the controls (8.7%). Black race was a factor significantly influencing the development of LN in univariate and multivariate analysis, consistent with previous studies from the United States.[16 17] Isenberg *et al* did not observe any ethnic influence in the development of LN in their prospective study of black patients from a cohort of 200 patients with SLE. Fourteen black patients were included and there was no significant difference in renal disease between the black subjects and the white and oriental patients. The age at disease onset and follow up period was similar.[18] A possible explanation for the differences found between American and European studies may be the role of geographical and ethnic differences—that is, the difference between African and Caribbean black patients.

Although other studies have found an increased prevalence of renal disease in male patients with SLE,[16 19 20] we did not find significant differences in sex between the two groups.

The clinical significance of autoantibodies and their relationships to disease subsets in rheumatic diseases has been the subject of extensive study and discussion. Since their discovery in 1957, attention has focused on anti-dsDNA antibodies in an attempt to determine their role in disease pathogenesis. Anti-dsDNA antibodies can be isolated from lupus kidneys in both humans and mice. High titres of anti-dsDNA antibodies have been identified in LN and their levels tend to rise and fall with disease activity.[2 3 6] Several lines of experimental evidence have demonstrated a more direct link between anti-dsDNA and nephritis. Different authors have indicated that some but not all monoclonal anti-dsDNA can induce glomerular immune deposits and nephritis in non-autoimmune mice.[21] It has been shown that the antibodies which can initiate immune deposits, are of IgG class.[22] In addition, it has been demonstrated that immunoglobulin deposition is intimately related to DNA binding. Decreasing the affinity of this antibody for DNA can eliminate glomerular deposition and nephritis.[23] Not all patients with antibodies to dsDNA develop nephritis and the avidity of the antibodies for DNA seems to have an important role in disease expression. However, we found that the presence of anti-dsDNA was a

Table 5 Demographic, immunological profile, and type of nephritis. Number of patients (%) is given for all parameters except age in years

	Class II No (%)	Class III No (%)	Class IV No (%)	Class V No (%)	p Value
Age at SLE diagnosis (years), mean (SD)	21.6 (5.8)	25.5 (9.9)	25.2 (9.3)	27.3 (7.8)	NS
Sex (F/M)	10 (100)/0 (0)	29 (91)/3 (9)	45 (96)/2 (4)	33 (87)/5 (13)	NS
Race (W/B/O)	9 (90)/1 (10)/0 (0)	18 (56)/9 (28)/5 (16)	31 (67)/10 (22)/5 (11)	27 (71)/8 (21)/3 (8)	NS
DsDNA (+ve/–ve)	8 (80)/2 (20)	25 (78)/7 (22)	33 (70)/14 (30)	20 (53)/18 (47)	NS
RNP (+ve/–ve)	2 (20)/8 (80)	9 (28)/23 (72)	15 (33)/30 (67)	17 (45)/21 (55)	NS
Sm (+ve/–ve)	1 (10)/9 (90)	6 (19)/26 (81)	11 (24)/34 (76)	13 (34)/25 (66)	NS
Ro (+ve/–ve)	2 (20)/8 (80)	18 (56)/14 (44)	14 (31)/31 (69)	13 (34)/25 (66)	NS
La (+ve/–ve)	1 (10)/9 (90)	4 (13)/28 (88)	4 (9)/41 (91)	2 (5)/36 (95)	NS
aCL IgG (+ve/–ve)	4 (40)/6 (60)	11 (35)/20 (65)	8 (19)/35 (81)	15 (41)/22 (59)	NS
aCL IgM (+ve/–ve)	1 (10)/9 (90)	4 (13)/27 (87)	2 (5)/41 (95)	4 (11)/33 (89)	NS
LA (+ve/–ve)	4 (40)/6 (60)	12 (39)/19 (61)	16 (37)/27 (63)	14 (38)/23 (62)	NS

F , female; M, male; W, white; B, black; O, oriental.

factor which was independently associated with the presence of nephritis in both uni- and multivariate analyses.

The presence of LN has been found to be uncommon in patients with both anti-Ro/SSA and anti-La/SSB antibodies and with anti-La/SSB antibodies alone.[24–26] Conversely, anti-Ro/SSA antibodies alone were associated with a higher prevalence of nephritis.[25 26] Although we found a negative association between the presence of anti-La and nephritis, after multivariate analysis it was no longer significant as an independent factor. We did not find any correlation between anti-Ro/SSA antibodies and nephritis.

Autoantibodies to RNP have been reported to occur at a lower frequency in LN.[27] However, this may not be the case when anti-RNP occurs in association with anti-Sm and anti-Ro autoantibodies. McCarty *et al* described a distinctive serological profile characterised by the presence of anti-Sm, RNP, and Ro in eight black women with LN.[28] Other studies did not provide evidence to support this distinctive profile.[13 29] Although there was a higher proportion of RNP-positive patients in our LN group, it was not significant.

The presence of anti-Sm has been reported to be related to renal disease and this association was more common when anti-Sm was found together with anti-dsDNA.[30–32] We also found anti-Sm to be an important factor in the development of nephritis.

A group from Venezuela analysed the possible role of anti-ENA autoantibodies in the pathogenesis of LN. They found that anti-ENA positivity was associated with the absence of a more benign form of SLE nephropathy.[33] In our study the presence of antibodies to ENA was assessed by CIE. This was the standard technique in use for anti-ENA detection in the patients studied earlier.[6] It was decided that to maintain consistency the same technique should be used throughout the study period. A number of other techniques are now available for the detection of anti-ENA, including ELISA and immunoblotting. CIE and ELISA are now in widespread use in laboratories in the United Kingdom. ELISA is reported to be more sensitive for the detection of anti-ENA antibodies.[6] However, the clinical significance of this increased sensitivity has not been fully established, particularly as many of the known disease associations with ENA were established using older techniques such as CIE and double diffusion. Lopez-Longo *et al* studied the clinical manifestations associated with anti-Sm and RNP antibodies identified by different techniques.[32] They found that anti-Sm antibodies were associated with Raynaud's phenomenon and renal disease when measured by CIE, while results measured by ELISA showed associations with arthritis and a lower incidence of chronic renal insufficiency. This fact might explain some differences between the results.

The role of antiphospholipid antibodies in the pathogenesis of LN is not clear, with reports often showing contradictory results.[34 35] Loizou *et al* found that raised levels of aCL were associated with LN but were unable to show an association with anti-β_2-glycoprotein I and did not look for the presence of LA. Moreover, they found that the presence of aCL in conjunction with raised levels of anti-dsDNA and anti-C1q antibodies is highly specific for LN.[36] We found that only the presence of LA was a significant independent factor for the development of nephritis. The presence of LA has been associated with certain clinical features, in particular, a predisposition to venous and arterial thrombotic vascular disorders in multiple organ systems. The thrombotic effects may also extend to the renal circulation, resulting in renal thrombotic microangiopathy or renal artery stenosis.[37]

Our analysis of the time to develop renal disease showed a tendency for black patients to develop nephritis earlier than white and oriental patients, but this did not reach significance. Bastian *et al* examined the time at which renal disease occurred in different ethnic groups in America and found that two thirds of Hispanic patients had evidence of renal disease at SLE diagnosis.[38] We found that men and patients without anti-dsDNA antibodies developed significantly earlier nephritis in the univariate analysis, though after multivariate analysis these were not significant factors. The presence of other autoantibodies, including ENA, was not associated with earlier nephritis.

The immunological profile was not associated with any histological class of nephritis, confirming the earlier work of Garcia *et al*.[13]

In summary, our results suggest that factors associated with LN in our group were black race, younger age at SLE diagnosis and the presence of anti-dsDNA, anti-Sm, and LA. This group of patients should be carefully monitored for the development of renal disease.

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