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

P Alba, L Bento, M J Cuadrado, Y Karim, M F Tungekar, I Abbs, M A Khamashta, D D’Cruz, G R V Hughes

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.

Abbreviations: aCL, anticardiolipin antibodies; CIE, counter-current immunoelectrophoresis; ELISA, enzyme linked immunosorbent assay; ENA, extractable nuclear antigens; LA, lupus anticoagulant; LN, lupus nephritis; SLE, systemic lupus erythematosus
up period. Anticardiolipin antibodies (aCL) were measured by enzyme linked immunosorbent assay (ELISA), using standardised methods. 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. 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.

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. The individual components of the renal pathology were classified and scored according to previously published activity and chronicity scores.

**Statistical analysis**
Associations between demographic and immunological profiles and presence of nephritis, time to renal disease and class of nephritis were analysed by $\chi^2$ 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 vs 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.

| 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 (0.7)                | 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)                   | 23 (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 | 0.00001                        | 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] |

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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 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 earlier nephritis than white though the difference was not significant. Male sex and absence of anti-dsDNA antibodies seems to have an important role in disease expression.

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. Although 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. 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, we did not find significant differences in sex between the two groups.

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. 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.

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### DISCUSSION

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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.24-25 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-ENA is present in association with anti-Ro 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.29,30 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.31-33 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.34 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.35 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.36 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.37 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.38-40 Loizou et al found that raised levels of aCL were associated with LN but this did not reach significance.41 We also found that raised levels of aCL were associated with the absence of aCL in our SLE patients.42-44 However, this may not be the case when anti-dsDNA is present.45,46

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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