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Concomitance of IgM and IgG anti-dsDNA Antibodies Does Not Appear to Associate to Active Lupus Nephritis

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Abstract: Previous reports proposed that the IgM anti-dsDNA antibody is protective for lupus nephritis. In this cross-sectional study, we aimed to compare clinical features of systemic lupus erythematosus (SLE) patients positive for IgG anti-dsDNA alone with those presenting both IgG and IgM anti-dsDNA. Anti-dsDNA antibodies, urinary examination and complement levels were assessed in the day of appointment. IgG and IgM anti-dsDNA antibodies were detected by indirect immunofluorescence. Fifty-eight SLE patients (93.1% female, 81% European-derived, mean age 42.8±14.7 years, mean duration of disease 10.9±8 years) positive for IgG anti-dsDNA entered the study. Of those, 15 were also positive for the IgM anti-dsDNA isotype. The group with both isotypes showed significant less frequency of active nephritis (sediment changes and proteinuria) when compared to patients with IgG anti-dsDNA alone (6.7% versus 34.9%, p=0.046). These data suggest a nephroprotective role for IgM anti-dsDNA and a distinct biologic behavior for this isotype in SLE.

Keywords: Systemic lupus erythematosus, lupus nephritis, IgG and IgM anti-dsDNA antibodies.

INTRODUCTION

IgG anti-dsDNA antibodies are usually associated with active lupus disease, particularly nephritis [1]. The pathogenicity of anti-dsDNA antibodies in systemic lupus erythematosus (SLE) is complex. Tissue deposition, isotype, affinity, ability to activate complement and to occupy Fc receptors in cell surfaces all contribute in this scenario [2].

Detected by Crithidia luciliae immunofluorescence (CLIF), Farr assay or immunoenzimatic test, anti-dsDNA antibodies are present in 60 to 80% of SLE patients [3]. Although useful for monitoring disease activity, these autoantibodies can be eventually found in patients under remission. In such circumstance, it is postulated a simultaneous occurrence of the IgM anti-dsDNA isotype (not routinely tested) as a protective antibody [4]. In this study, we set out to compare clinical and laboratory features of SLE patients with IgG anti-dsDNA alone and patients with both isotypes, aiming confirm previous reports from the literature in the Brazilian population.

MATERIALS AND METHODOLOGY

The study, cross-sectional, included SLE patients regularly followed at the Lupus Outpatient Clinic of São Lucas Hospital of PUCRS. Patients with SLE according to the 1997 classification criteria [5], with at least 18 years of age and a recent positive test to IgG anti-dsDNA antibodies in CLIF were included. Lupus nephritis was defined by the presence of at least one of the following: pyuria (leucocytes >5/field 400X, excluding infection); hematuria (red blood cells ≥5/field 400X, excluding infection, lithiasis and other causes); cylindruria (presence of granular or hematic casts); proteinuria (proteins in urine ≥+++ or proteinuria/ creatininuria index ≥0.5) [5]. Parallel occurrence of secondary Sjögren's syndrome (SS) [6] and secondary antiphospholipid syndrome (APS) [7] were admitted in the inclusion criteria. Patients showing any other connective tissue disorder, as well as individuals with mental disease which did not allow free consent, were excluded. The study was approved by the local ethics committee.

Clinical and laboratory data were obtained by using a standardized questionnaire applied in the day of appointment, and also by review of medical records. The questionnaire included demographic findings and the following clinical and laboratory variables: malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, neurologic and hematologic manifestations, antinuclear antibodies (ANA), anti-Sm, anticardiolipin antibodies, lupus anticoagulant, and the VDRL.

At the day of appointment, a fresh sample of urine of each patient was examined as to the presence of protein and sediment changes (pyuria, hematuria, urinary casts); their presence was indicative of active lupus nephritis [5,8]. Anti-dsDNA antibodies and C3 and C4 levels were also searched at the day of appointment. Lupus activity was assessed by the SLEDAI (systemic lupus erythematosus disease activity index); a score above 4 indicated active disease [8]. IgG and IgM anti-dsDNA antibodies were detected using CLIF.

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Samples were considered reagent for IgG or IgM anti-dsDNA if titers were above the 1/10 dilution [9].

Descriptive analysis was done using mean and standard deviation (SD) for quantitative variables and frequency and percentage for categorical variables. Median and interquartil intervals were used to calculate variables with asymmetric distribution. The Chi-square test was used for analysis of categorical variables. Student's t test was applied for quantitative variables with symmetric distribution, while the Mann-Whitney U test was utilized for variables of asymmetric distribution. Data were analyzed using SPSS version 17.0, and a p value <0.05 was considered statistically significant.

RESULTS

Fifty-eight SLE patients selected by the presence of IgG anti-dsDNA antibodies entered the study. Of these, 54 (93.2%) were female and 47 (81%) were European-derived. This classification was based on physical appearance, as judged by the researcher at the time of blood collection, and data about the ethnicity of parents/grandparents reported by the participants. This classification criteria that is used in Brazil is well documented and has been already assessed in previous studies [10]. Also, a recent study assessing individual interethnic admixture and population substructure using a panel of 48-insertion-deletion ancestry-informative markers validated this classification in European-derived individuals from our geographic region [11]. In southern Brazil, where this study was conducted, there is a defined predominance of European-derived individuals due to the massive immigration occurred in the past. The mean age was 42.8±14.7 years, and the mean duration of disease was 10.9±8.0 years. Among the 58 patients, 31 (53.4%) had previous lupus nephritis. Median SLEDAI was 4 [2-8].

Out of the 58 IgG anti-dsDNA positive patients, 15 (25.8%) also tested positive for IgM anti-dsDNA antibodies. The comparison of the two groups (43 patients with IgG anti-dsDNA alone, 15 patients with both isotypes) in the context of clinical and laboratory variables is seen in Table **1**. Gender, age, age at diagnosis and disease duration did not significantly differ between groups. Patients with both

Table 1. Demographic, Clinical and Laboratory Findings of 43 Patients Positive for IgG Anti-dsDNA Alone and 15 Patients with Both IgG and IgM Anti-dsDNA Isotypes

Characteristics	IgG Anti-dsDNA(+) n=43	IgG+IgM Anti-dsDNA(+) n=15	*p* Value[a]
Female gender (%)	40 (93)	13 (86.7)	0.596
Age (years±SD)	40.6±13.3	43.1±12.0	0.518
Age at diagnosis (years±SD)	29.7±12.4	32.1±10.2	0.554
Disease duration (years±SD)	10.5±87.6	11.3±9.1	0.913
Malar rash (%)	28 (65.1)	9 (60)	0.966
Discoid rash (%)	1 (2.3)	1 (6.7)	0.454
Photosensitivity (%)	32 (74.4)	13 (86.7)	0.480
Nasal/oral ulcers (%)	17 (39.5)	7 (46.7)	0.858
Arthritis (%)	31 (72.1)	12 (80)	0.736
Serositis (%)	14 (32.6)	5 (33.3)	0.999
Neurologic manifestations (%)	9 (20.9)	1 (6.7)	0.427
Hematologic manifestations (%)	29 (67.4)	10 (66.7)	0.999
ANA (%)	43 (100)	15 (100)	-
Anti-Sm (%)	12 (28.6)	3 (20)	0.693
Anticardiolipin antibodies (%)	18 (41.9)	6 (40)	0.999
Lupus anticoagulant (%)	7 (17.1)	3 (20)	0.999
False-positive VDRL (%)	5 (11.6)	1 (6.7)	0.999
SLEDAI[b]	4 (2-6)	2 (1-8)	0.361
Active nephritis[c] (%)	15 (34.9)	1 (6.7)	**0.046**
C3 (mg/dL±SD)[d]	99.6±29.3	96.2±29.6	0.706
C4 (mg/dL±SD)[d]	16±8.9	16.67±8.7	0.894
Sjögren's syndrome (%)	4 (9.5)	0 (0)	0.564
Antiphospholipid syndrome (%)	4 (9.5)	0 (0)	0.564

ANA: antinuclear antibody; SD: standard deviation; SLEDAI: systemic lupus erythematosus disease activity index; VDRL: venereal disease research laboratory test; (+): positive.
[a]Chi-square test for qualitative variables and Mann-Whitney U test for asymmetric quantitative variables or Student's t test for symmetric quantitative variables.
[b]Median (interquartile interval).
[c]As defined in references 5 and 8, using fresh urine analysis in the day of appointment.
[d]Normal values for C3 and C4 were 88-165mg/dL and 14-44 mg/dL, respectively.

isotypes had significantly lower frequency of active lupus nephritis as compared to the other group (6.7% versus 34.9%, p=0.046). For the other clinical and laboratory variables, including SLEDAI and complement levels, there were no significant differences between the two groups.

DISCUSSION

SLE is a disease of high complexity, and a variety of autoantibodies can be detected during the course of disease. The IgG anti-dsDNA isotype is largely studied in SLE patients, and its clinical association with active nephritis is well known [12]. Differently, the biological behaviour of the IgM anti-dsDNA isotype has been a matter of polemic. We here address the question whether the occurrence of IgM anti-dsDNA determines any peculiarity in the clinical and laboratory context of SLE.

In this cross-sectional study carried out in a tertiary center from southern Brazil, our IgG anti-dsDNA positive SLE survey showed a strong predominance of European-derived females. While the absolute female predominance is according to the literature, the strong predominance of European-derived our survey differed from previous data [13]. Corroborating our results, Chahade *et al.* documented higher incidence of SLE in European-derived from Brazilian Southeast [14]. The mean age of our SLE population (approximately 43 years) was similar to previously reported [15]. Overall, disease duration was of approximately a decade.

When we compared clinical and laboratory findings of patients with IgG anti-dsDNA alone (43 individuals) with those of patients with both anti-dsDNA isotypes (15 cases), there was no significant differences as to demographic and laboratory findings, as well as to the majority of clinical manifestations.

Of importance, the concomitance of IgM and IgG anti-dsDNA in our survey associated to a significantly lower frequency of active lupus nephritis; the latter was evaluated cross-sectionally in a fresh urine sample, and concomitantly to the anti-DNA and complement assays. Unexpectedly, medium SLEDAI and complement levels were not discriminative between groups.

From these data, we could infer that the parallel presence of IgM anti-dsDNA may be somehow nephroprotective. Moreover, this could explain why, in clinical practice, some SLE patients with a positive IgG anti-dsDNA test do not present renal abnormalities, once the IgM isotype is not routinely searched.

IgM anti-dsDNA antibodies showed a negative association with nephritis in a study published yet in 1998 [16]. In other report, an eventual increase in IgM anti-dsDNA levels were not predictive for lupus flares, neither associated to specific manifestations [17]. In Brazilian SLE patients of mainly African descent, no association of the IgG, IgM and IgA anti-dsDNA isotypes with renal lupus was seen [18]. Other group of authors reported that the presence of IgA (but not IgM) anti-dsDNA was concomitant to the IgG isotype in active SLE including nephropathy [19]. Recently, Villalta *et al.* suggested that the presence of IgA anti-dsDNA autoantibodies improved the ability to diagnose SLE and to define lupus nephritis phenotype and active disease. By contrast, IgM anti-dsDNA antibodies would be protective for renal involvement [20].

As far as we are aware, only one study has evaluated the IgG/IgM anti-dsDNA ratio in SLE so far: in 2004, Forger *et al.* demonstrated that an IgG/IgM anti-dsDNA ratio under 0.8 in an ELISA was protective for nephropathy in a longitudinal analysis [21]. Also of interest, IgM anti-dsDNA treatment inhibited glomerular deposition of immune complexes in (NZB x NZW)F1 mice [22].

Some limitations of our study must be brought about, starting by the cross-sectional design. A cohort study with longitudinal assessment would generate consistent findings. Our data were collected in a tertiary center, so that there was a trend for patients with active disease (our mean SLEDAI was of 4 in the global population). Also, we selected positive IgG anti-dsDNA patients only. Testing of both isotypes in a larger and unselected SLE population could have provided more accurate results, allowing multivariate analysis. The small sample made not possible the utilization of a regression model to access the influence of gender and ethnicity (two potencial confounders in this study). Besides, our IgM positive population was small, limiting the statistical analysis. Consequently, our study lost statistical power to find other possible clinical and laboratory associations with the proportion of IgG and IgM anti-dsDNA isotypes. Thus, our findings can not be extrapolated to other populations. Apart from it, our results might reopen a field of interest in isotypes anti-dsDNA and their clinical associations in SLE.

CONCLUSION

The presence of both IgG and IgM anti-dsDNA did not associate with active lupus nephritis in our SLE survey. These data appear to indicate a distinct biological behaviour for the IgM anti-dsDNA isotype in SLE patients. An eventual nephroprotective role for IgM anti-dsDNA antibodies warrants further elucidation in longitudinal studies.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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