The Value of a Panel of Autoantibodies for Predicting the Activity of Lupus Nephritis at Time of Renal Biopsy

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Few studies have correlated serum biomarkers with renal histology, the gold standard for renal activity, in lupus nephritis (LN). We tested a panel of autoantibodies and complement at the time of kidney biopsy and after treatment. Anti-dsDNA, anti-nucleosome, anti-ribosome P, and anti-C1q antibodies and C3/C4 were measured in 107 patients with LN at the time of renal biopsy and after 6–12 months and were correlated with clinical/histological parameters. At multivariate analysis, high titers of anti-C1q antibodies or of anti-dsDNA antibodies ($P = 0.005$, $OR = 8.67$, CI: 2.03–37.3) were the independent predictors that discriminate proliferative from nonproliferative LN. All the immunological parameters, except anti-ribosome, showed a significant correlation with activity index but not with chronicity index. Only anti-C1q showed a significant correlation with the amount of proteinuria ($R = 0.2$, $P = 0.03$). None of the immunological parameters were predictive of remission at 6 and 12 months. We found that anti-C1q alone or in combination with anti-dsDNA emerged as the most reliable test in differentiating proliferative and nonproliferative LN. Anti-C1q was the only test correlated with the clinical presentation of LN. After treatment, the titre of the autoantibodies was significantly reduced, but none was predictive of remission.

1. Introduction

Lupus nephritis (LN) is one of the most frequent manifestations of Systemic Lupus Erythematosus (SLE) and represents a major determinant of disease morbidity and mortality [1]. Its clinical course is often characterized by flares of activity alternated with periods of quiescence, generally induced by therapy [2]. The identification of noninvasive biomarkers may help to predict the renal involvement at diagnosis and monitor relapses of LN during the follow-up. Many studies have tested the value of a number of autoantibodies for predicting or confirming the diagnosis of renal flares with contrasting results. Some [3–5] but not all studies [6] have demonstrated that anti-dsDNA antibodies (anti-dsDNA) and complement fractions may be useful in assessing the disease and the renal activity. One paper [7] and a recent review [8] concluded that anti-nucleosome antibodies have high prevalence in severe LN but are of limited help in differentiating active from inactive LN. A number of cross sectional studies found that antiC1q antibodies (antiC1q) have a significant association with renal involvement [9–15]. In our previous paper on a large cohort of SLE patients evaluated prospectively for 6 years, we demonstrated that renal exacerbations seem to be quite improbable in the presence of normal values of C3, C4, anti-dsDNA, anti-C1q, and that anti-C1q was slightly better than the other tests to confirm the clinical activity of LN [16]. Noteworthy, in the vast majority of studies the diagnosis of LN flares relies on variable clinical definitions based on activity of urine sediment, amount of proteinuria, and deterioration of renal function, whilst the ”gold standard” for the diagnosis of renal activity is represented by renal biopsy. In this prospective study, serum samples at renal biopsy and after the induction therapy of 107 LN patients were tested for a panel of autoantibodies (including anti-dsDNA, anti-C1q,
anti-nucleosome, anti-ribosome antibodies, and C3 and C4 complement fractions) to investigate their association with the clinical and histological data.

2. Patients and Methods

One hundred and seven patients with SLE, diagnosed according to the American College of Rheumatology criteria [17] (94 females, 13 males) at admission in two Italian Renal Units (Fondazione Ospedale Maggiore and Azienda Ospedaliera Ospedale San Carlo Borromeo, Milano) to undergo renal biopsy for assessment of LN, entered the study. The renal biopsies were classified following the ISN/RNP classification [18]. Activity and chronicity indices were calculated according to Austin et al. [19].

Sera at renal biopsy were tested for a panel of autoantibodies including anti-dsDNA and anti-C1q, anti-nucleosome, and anti-ribosome antibodies as well as C3 and C4 complement fractions.

The study does not need an ethical approval. We have obtained an informed consent to participate in the study from all the patients involved.

2.1. Objectives. The aim of this study was to assess the performance of these tests in predicting:

(a) the histological classes of lupus nephritis,
(b) the activity and chronicity index at renal biopsy,
(c) the clinical feature of LN at renal biopsy,
(d) the response of lupus nephritis at 3, 6, and 12 months after the beginning of the induction therapy.

2.2. Laboratory Investigations. Anti-dsDNA antibodies were measured by a commercial quantitative ELISA (Varelisa anti-dsDNA Antibodies, Phadia GmbH, Freiburg, Germany) and C3 and C4 plasma levels by nephelometry (Nephelometer Analyser II, Behring, Marburg GmbH, Germany).

Anti-C1q antibodies were detected using a home-made ELISA as described by Sinico et al. [11].

Anti-nucleosome antibodies were measured by ELISA according to manufacturer instructions using Quanta Lite Chromatin assay (INOVA diagnostics, Inc., San Diego, CA, USA) [20].

Anti-ribosome P antibodies were measured by ELISA according to manufacturer instructions using Quanta Lite Ribosomal P assay (INOVA diagnostics, Inc., San Diego, CA, USA).

2.3. Definition of Activity. At each clinical examination the activity of LN was classified as follows [2]:

0 = complete renal remission: normal renal function for at least 6 months, proteinuria <0.5 g/24 h, and urinary red blood cells <5/hpf;
1 = partial renal remission: for nephritic flare: improvement of at least 30% of serum creatinine but persistence of active urinary sediment; for proteinuric flares improvement of 50% of proteinuria;
2 = nephritic flare: increase of 30% of serum creatinine over the basal value and active urinary sediment (>10 red blood cells/hpf, cellular casts) with or without an increase in proteinuria;
3 = proteinuric flare: increase of proteinuria of at least 2 g/day in patients with non nephrotic syndrome or the doubling of nephrotic proteinuria with stable renal function;
4 = persistent renal activity: the lack of achievement of remission after induction therapy.

3. Statistical Analysis

Mean and standard deviation, together with median and interquartile (IQ) range (25°–75° percentile) were used as descriptive statistics. For continuous variables, the nonparametric Wilcoxon test was used for assessing any difference between the two groups of patients, while the chi-square test was used for dichotomized variables. The Spearman correlation was used to analyse correlation.

Multivariate logistic regression analysis has been used to find predictors of histological classes of lupus nephritis and for the predictors of complete renal response after the beginning of induction therapy. Odds ratios (OR) and their 95% confidence interval (CI) for the covariates were derived as the antilogarithm of the regression coefficients. Multivariate linear regression analysis was used to predict the activity index at renal biopsy.

The statistical package S-Plus (MathSoft Inc.) was used for all the analyses and plots.

4. Results

The characteristics of the patients at renal biopsy are reported in Table 1. The 107 patients received 111 renal biopsies (4 patients, 2 biopsies). The mean age at diagnosis of SLE was 35.3 ± 14.2 years, (median 34) and that at renal biopsy was 36.4 ± 13.9 years (median 36). The mean time between the diagnosis of SLE and that of renal involvement was 5.1 ± 6.5 years, (median 3 years). In 45 patients, renal involvement was present at diagnosis of SLE.

Considering that a preliminary analysis demonstrated no significant differences in the mean values of C3, C4, anti-dsDNA, anti-C1q, anti-nucleosome, and anti-ribosome antibodies between class II and class V and between class III and Class IV LN (data not shown), the subsequent analysis was performed comparing Class II plus class V (nonproliferative forms; 26 patients) versus class III plus class IV (proliferative forms; 85 patients).

4.1. Prevalence of Autoantibodies and Histopathological Associations. At renal biopsy, high titers of anti-dsDNA were present in 77.5% of cases, high titers of anti-C1q in 70.5% of cases, high titers of anti-nucleosome antibodies in 80.3% of cases, and high titers of anti-ribosome antibodies in 14% of cases; C3 were low in 82% of cases and C4 in 74% of cases. Table 2 reports the comparison at time of renal biopsy of
clinical data and of the panel of autoantibodies between proliferative forms (class III plus class IV) and nonproliferative forms (class II plus class V) of LN. At univariate analysis, among the clinical parameters, proteinuria ($P = 0.008$, OR = 0.68, CI: 0.52–0.9) and anti-C1q antibodies ($P = 0.03$, OR = 1.004, CI: 1.0003–1.007) were the independent predictors to discriminate between proliferative versus nonproliferative lupus nephritis. Excluding clinical parameters, at multivariate analysis, logarithm of erythrocyte sedimentation rate (ESR) ($P = 0.03$, OR = 1.9, CI: 1.08–3.42) and high titers of anti-C1q antibodies or of anti-dsDNA antibodies ($P = 0.005$, OR = 8.67, CI: 2.03–37.3) are the independent predictors which are able to discriminate proliferative from nonproliferative lupus nephritis. Among patients with proliferative forms of lupus nephritis, 95% have high titers of anti Clq or of anti-dsDNA (66.2% have high titers of both anti-C1q and of anti-dsDNA) while 5% have the results of both tests in a normal range. Among patients with nonproliferative forms, 64% have high titers of anti Clq or of anti DNA while 36% have the results of both tests in a normal range ($P = 0.000$).

The correlations among the basal clinical and the immunological data and the activity and the chronicity index at renal biopsy are reported in Table 3. All the clinical and immunological parameters evaluated with the exception for C reactive protein (CRP) and anti-ribosome antibodies showed a significant correlation with activity index. At multivariate analysis proteinuria ($P = 0.0013$), low C4 ($P = 0.0010$), and high ESR ($P = 0.037$) were the independent predictors of the activity index. Excluding the clinical variables, low C4 ($P = 0.0004$) and high ESR ($P = 0.0025$) were the independent predictors of activity index.

In contrast, serum creatinine was the only parameter among those evaluated that showed a direct correlation with the chronicity index ($R = 0.4$, $P = 0.0000$).

### 4.2. Correlations between Autoantibodies and Clinical Parameters

No correlation was found at time of renal biopsy between serum creatinine and the panel of autoantibodies, C3 and C4 complement fractions, and ESR and CRP. Among these tests, anti-C1q only showed a significant direct correlation with the amount of proteinuria ($R = 0.2$, $P = 0.03$). In addition, patients with high titers of anti-C1q or of anti-dsDNA had significant higher proteinuria (median range (1.8 g/day, IQ 1.0–2.2, $P = 0.05$). Anti-C1q, anti-dsDNA, and ESR were inversely correlated with hemoglobin ($R = 0.02$, $P = 0.02$, $R = 0.24$, $P = 0.01$, $R = 0.32$, $P = 0.002$, resp.), while C3 and C4 were correlated with hemoglobin ($R = 0.36$, $P = 0.0002$ and $R = 0.25$, $P = 0.01$).

### 4.3. Clinical and Immunological Outcome after the Beginning of Induction Therapy

After renal biopsy and the beginning of induction therapy, 104 patients had a second evaluation between 3 and 12 months. Table 4 reported the results of clinical and immunological tests in patients reevaluated at 3 months, at 6 months, and at 12 months. At 3 months, serum creatinine was unchanged and proteinuria did not show a significant improvement, while ESR, C3, anti-DNA, anti-C1q, and anti-nucleosome antibodies showed a significant improvement in the median values. At 6 and at 12 months, proteinuria significantly improved together with all
immunological tests with the exception of ESR at 6 months and anti-ribosome antibodies at 12 months.

Altogether, during the observation period, 39 patients (37.5%) achieved and 65 (62.5%) did not achieve complete renal remission (46 were in partial remission, 13 had persistent renal activity, and 6 had persistent nephrotic syndrome). Clinical and immunological tests at the time of renal biopsy have been tested as predictors of complete renal remission (Table 5). At univariate analysis, none of the immunological tests were predictive of complete remission. At multivariate analysis, proteinuria \((P = 0.015, \text{OR: 0.76 CI 0.62–0.95})\) and the duration of therapy \((P = 0.03 \text{ OR: 1.19 CI 1.017–1.39})\) were the independent predictors of complete renal remission.

### 5. Discussion

In this study, we have investigated the prevalence and the value of a panel of autoantibodies (anti-dsDNA, anti-C1q, anti-nucleosome, and anti-ribosome antibodies) as well as C3 and C4 complement fractions in predicting the activity of LN at the time of renal biopsy. The most important difference of our study compared to many previous studies is the timing of blood sampling in relation to renal activity. As a matter of fact, in the majority of the studies evaluating the predictive values of autoantibodies \([11, 15, 21–23]\), the renal activity of LN at the time of blood sampling was judged by clinical parameters but not confirmed by renal biopsy. To the best of our knowledge, only a few studies have evaluated the association of some autoantibodies with activity of LN at the time of renal biopsy \([24–27]\). Trendelenburg et al. \([24]\) reported that all but one out of 36 patients with proliferative lupus were positive for anti-C1q at the time of renal biopsy compared with 35% of patients with inactive LN. In 136 Chinese patients \([25]\), anti-C1q and anti-dsDNA were more closely correlated with histological activity of LN at the time of renal biopsy than anti-extractable nuclear antigen antibodies, anti-C protein antibodies, anti cardiolipin, and anti Beta2 glycoprotein antibodies. The combination of anti-C1q and anti-dsDNA indicates higher renal disease activity and predicts poor long term renal outcome. Another paper \([26]\) investigated the clinical and pathological association of anti-C1q in LN and found a higher prevalence of the autoantibody in class IV than in the other histological classes. Among the clinical variables low haemoglobin was associated with anti-C1q positivity.
In this paper, we have shown that there was a significant difference in the autoantibodies profile between proliferative forms (class III plus IV) and the other forms of LN (class V and class II). All the autoantibodies evaluated, with the exception of anti-ribosome antibodies, had significant higher prevalence and higher titres in proliferative than in non-proliferative forms of LN. C3 and C4 complement fractions too were significantly lower in proliferative than in non-proliferative LN. At multivariate analysis, considering clinical and immunological tests, only low haemoglobin and high anti-C1q were the independent predictors of proliferative LN. Excluding the clinical variables, high ESR and positive anti-C1q, anti-dsDNA, and anti-nucleosome antibody were the independent predictors of a high activity index at multivariate analysis while none of the tests of the panel correlated with chronicity index.

This discrepancy could be due to the fact that in these studies the diagnosis of renal activity was done on clinical grounds and not confirmed by renal biopsy. Again, anti-C1q, alone or associated with anti-dsDNA, was the only test among the immunological parameters that significantly correlated with the amount of proteinuria. None of the immunological tests correlated with serum creatinine but the majority of our patients had normal renal function, and many tests correlated with hemoglobin, a manifestation not specific for LN but an expression of the general activity of SLE. Low C4 and high ESR were the independent predictors of a high activity index at multivariate analysis while none of the tests of the panel correlated with chronicity index.

In contrast to what was reported by Yang et al. [25], in our cohort, none of the immunological tests at the time of renal biopsy was predictive of the renal response, at least in the short term. However, we have shown that, three months after the start of the induction therapy and prior to the improvement of proteinuria, a significant reduction of the mean value of anti-C1q, anti-dsDNA, and anti-nucleosome antibody occurred. The progressive and significant drop in autoantibodies titres continued at 6 and at 12 months together with a clinical improvement as reported in other studies [24, 26, 31].

Anti-C1q antibodies can be detected by different methods (reviewed in [32, 33]). In the early 1980s, a solid-phase assay using purified C1q, immobilized on plastic assay plates, was used for the detection of circulating immune complexes in SLE patients. To differentiate between immune complexes and anti-C1q antibodies, high-salt concentrations (0.5–1.0 M sodium chloride) were used. Using this method, the binding of the globular heads of C1q to immune complexes is prevented, whereas anti-C1q antibodies can still interact with the coated C1q. Subsequently, to eliminate the need to use high-ionic strength buffer, assays have been developed that utilize only the C1q collagen-like region. The use of the purified collagen-like region may potentially be more reliable. However, additional exposed epitopes, by cleaving of the C1q molecule, might interfere with the results obtained with this assay. More recently, peptides derived from C1q that have the properties to detect a major linear epitope in a high percentage of the patients in the absence of high-ionic strength buffer has been proposed [33]. Unfortunately, systematic studies comparing anti-C1q antibody detected by different assays are not available and different studies have used different methods. In our study, we have used the “classic” assay which has been used in the majority of published clinical studies because it is more readily available.

6. Conclusions

We found a significantly different autoantibodies profile between proliferative and nonproliferative forms of LN at the time of renal biopsy. Among the panel of autoantibodies evaluated in this study, anti-C1q alone or in combination with anti-dsDNA emerged as the most reliable in differentiating proliferative and nonproliferative LN and anti-C1q is the
Table 4: Outcome of clinical and immunological parameters at 3, 6, and 12 months after the beginning of induction therapy.

| Parameter                  | Basal 16 pts | At 3 months | P     | Basal 46 pts | At 6 months | P     | Basal 42 pts | At 12 months | P     |
|----------------------------|--------------|-------------|-------|--------------|-------------|-------|--------------|--------------|-------|
| S. Creat. mg/dL            | 0.76         | 0.76        | 0.68–1.6 | 0.76         | 0.85        | 0.7–1 | 0.74         | 0.82        | 0.67–0.96 | 0.74 |
| Proteinuria g/day          | 2.5          | 0.94        | 1.8–4  | 1.3–3.9      | 3.0000      | 1.92  | 0.92         | 0.0000       | 2.05–4.9 | 0.51 |
| ESR mm                     | 65           | 36.5        | 25.7–82 | 29.5         | 33          | 0.04  | 0.21         | 0.0000       | 20–67   | 13.5 |
| CRP mg/dL                  | 0.47         | 0.30        | 0.26–11 | 0.29         | 0.31        | 0.09–0.7| 0.1      | 0.0005       | 0.04–0.2 | 0.005 |
| C3 mg/dL                   | 67           | 83          | 60–87  | 73           | 63          | 0.03  | 92          | 0.0000       | 48–81.2 | 86.5 |
| C4 mg/dL                   | 10           | 13          | 8–13–17| 17           | 9           | 0.0000| 11–21       | 13.5         | 5–14.7  | 10–20|
| Anti-DNA Ab UmL            | 79.5         | 38          | 10–291 | 173          | 124         | 0.01  | 73          | 0.0000       | 83.7–345 | 34–104|
| Anti-C1q Ab AU             | 120.5        | 52.5        | 52–315 | 94           | 147         | 0.002 | 50          | 0.0003       | 56–320   | 35.7–122|
| Anti-nucleosome Ab         | 66           | 58          | 42.7–110| 97.5         | 88          | 0.04  | 41          | 0.0001       | 51–109   | 16–70 |
| Anti-ribosome Ab           | 2.5          | 2           | 1.75–4 | 3.5          | 2           | 0.0007| 2          | 2           | 2–10    | 2–3  |

The data are reported as Median and interquartile ranges: S. = serum, Creat. = creatinine, ESR = erythrocyte sedimentation rate, CPR = C reactive protein, and Ab = antibodies.

Table 5: Predictors of complete renal remissions among the clinical and the immunological tests at renal biopsy.

| Parameter                  | Complete remission | No complete remission | P     |
|----------------------------|---------------------|-----------------------|-------|
| Months of therapy          | 6.0 (5.2–7.4)       | 5.2 (3.5–6.8)         | 0.02  |
| Serum creatinine mg/dL     | 0.83 (0.64–0.95)    | 0.85 (0.7–1.26)       | 0.15  |
| Proteinuria g/day          | 1.96 (12–2.76)      | 3.45 (2.1–5)          | 0.0009|
| Hemoglobin g/dL            | 12.0 (11.0–12.9)    | 11.6 (10–12.7)        | 0.12  |
| Erythrocyte sedimentation  | 33 (175–612)        | 39 (16–73)            | 0.7   |
| rate                      | C reactive protein mg/dL | 0.3 (0.09–1.22)  | 0.3 (0.14–0.67) | 0.6 |
| C3 mg/dL                  | 66 (48–82.2)        | 70 (48–88)            | 0.5   |
| C4 mg/dL                  | 9 (5.2–115.7)       | 10 (5–14)             | 0.7   |
| Anti DNA antibodies U/mL   | 147 (83.5–380)      | 132 (43–400)          | 0.5   |
| Anti C1q antibodies AU     | 120 (35–320)        | 128 (42.5–320)        | 0.5   |
| Anti-nucleosome antibodies U | 88 (57–111)       | 92.5 (33.2–115.7)     | 0.8   |
| Anti-DNA >55 U/mL or anti-C1q >50 AU antibodies | 34 (87%) | 58 (89.2%) | 1 |
| Anti-ribosome antibodies   | 2 (2–5.5)           | 2 (2–5.7)             | 0.1   |

The data are reported as Median and interquartile ranges.

only test correlated with the clinical presentation of LN. After the beginning of therapy, the titer of the autoantibodies progressively and significantly reduced, but none of them was predictive of complete renal remission. The results of this work, which outlines the role of autoantibodies and in particular of anti-C1q, in defining the activity of lupus nephritis at the time of renal biopsy, confirm their utility in diagnosing the acute exacerbations of LN made on clinical grounds only.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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