ASSOCIATION BETWEEN ANTI-C1q AND ANTI-dsDNA ANTIBODIES IN PATIENTS WITH LUPUS NEPHRITIS


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ABSTRACT

Lupus nephritis (LN) is severe organ manifestation of the systemic lupus erythematosus (SLE). LN usually occurs within 5 years of the onset of disease, but can occur any time throughout the course of the disease. Anti-nuclear antibodies (ANA) and anti-double stranded DNA (anti-dsDNA Abs) antibodies are aiding tools to the kidney biopsy findings in early diagnosis. The aim of this study was to assess the role of anti-C1q Abs, in predicting of renal involvement in comparison with the “gold standard” for SLE anti-dsDNA Abs.

Thirty patients – 23 (77%) women and 7 (23%) men – with biopsy-proven LN were studied. Sera were tested for anti-C1q Abs, anti-dsDNA Abs and complement proteins – C1q, C3 and C4. Association of parameters of renal disease activity and the presence of anti-C1q Abs and ant-dsDNA Abs were further evaluated.

All 30 patients were distributed in three groups according to the degree of the clinical activity of LN: 1) patients with active LN (AN); 2) patients in partial remission (PR) and 3) patients in complete remission (CR). We found significant differences between frequencies of anti-C1q Abs in AN patients and in CR patients (p = 0.007) and between levels of anti-C1q Abs in AN patients and those with PR (p = 0.026). Positive for anti-dsDNA Abs were 56.67% of patients. We found that positive for anti-dsDNA Abs had higher level of anti-C1q Abs in comparison to negative for anti-dsDNA Abs (p = 0.005). 67% of patients with both anti-C1q Abs and anti-dsDNA Abs had high clinical LN activity and all had Class IV LN. Patients who were seropositive for anti-C1q Abs and negative for anti-dsDNA Abs had lower serum C1q, C3 and C4 levels compared with the other patients.

The presence of anti-C1q Abs in serum closely correlated with LN activity. This study indicates the potential superior utility of anti-C1q Abs over anti-dsDNA Abs to track renal disease activity. Systematic detection of anti-C1q and anti-dsDNA Abs should be used in combination to monitor the renal involvement.

Key words: anti-C1q Abs; anti-dsDNA Abs; Lupus nephritis

Introduction

Lupus nephritis (LN) is severe organ manifestation of the systemic lupus erythematosus (SLE). LN almost always occurs within 5 years of the onset of disease, but can occur any time throughout the course of the disease [2, 12]. The identification of non-invasive biomarkers in LN is important for mark renal involvement before clinical manifestation and for reflecting clinical and pathological disease activity, guiding treatment [16]. Among intensely studied biomarkers, a significant place is reserved for anti-nucleosome and anti-C1q antibodies. Anti-nucleosome antibodies are highly correlated with anti-double stranded DNA (anti-dsDNA Abs) and are considered sensitive SLE markers, but their correlation with renal involvement remains controversial [8, 17]. In contrast to anti-dsDNA Abs, for anti-C1q Abs is clearly demonstrated that their presence has been closely correlated with renal disease activity. The measurement of anti-C1q Abs are important in the clinical monitoring of SLE patients, as a marker of renal involvement - for
early detection of nephritis and for prediction of exacerbations. In some recent reports, the authors have stated that anti-C1q Abs in combination with anti-dsDNA Abs and low complement components (C3 and C4) are the strongest serological association with renal involvement [3, 7, 9, 17].

The aim of this study was to assess the role of anti-C1q Abs, in predicting of renal involvement in comparison with the „gold standard“ for SLE anti-dsDNA Abs.

**Material and Methods**

**Study subjects**

30 serum samples from patients with systemic lupus erythematosus (SLE) and biopsy-proven lupus nephritis (World Health Organization Class I, II, III, IV and V) were collected in the Clinics of Nephrology, University Hospital “Tzaritza Ioanna – ISUL” - Sofia, and Clinics of Nephrology, Dialysis and Transplantation, University Hospital – “St. Marina” - Varna, Bulgaria. The lupus nephritis group included 23 (77%) women and 7 (23%) men, average age 35.4 ± 9.6 years (ranging from 23 to 58). The duration of LN lasted from 0.33 to 32 years, with an average of 11.75 ± 7.94 years. Diagnosis of LN was based on clinical and laboratory parameters including proteinuria, urinary sediment, creatinine level, and erythrocyte sedimentation rate. Inclusion criteria were defined as proteinuria 500 mg/24 hr or higher in the last 10 days, erythrocyturia as 8 RBC per microliter or higher, renal dysfunction as any increase in creatinine value at any time of the history of the disease, and renal involvement as any of the two above variables. Each patient signed a consent form on enrolment.

**Measurement of anti-C1q and anti-dsDNA antibodies and complement proteins**

Anti-C1q autoantibody levels were measured in human serum samples by ELISA under 0.75 M NaCl conditions as described previously [10, 15]. The presence of anti-dsDNA antibodies were detected by indirect immunofluorescence in University Hospital “Tzaritza Ioanna – ISUL” - Sofia. The concentrations of C1q, C4 and C3 antigens in serum were measured by means of a double-ligand ELISA [11].

**Statistical Analysis**

Statistical analysis was carried out using software GraphPad Prism 5.01. Quantitative data were expressed as mean ± SD. For comparison between groups, the two-tailed Student’s t-test for unpaired samples with Welch’s correction was used; for comparison between more than two groups, ANOVA was used. Statistical significance was considered as P < 0.05.

**Results**

**Characterization of investigated subjects**

All 30 patients were distributed in three groups according to the degree of the clinical activity of LN: 1) 10 patients (33.33%) (all women) with active LN (AN); 2) 5 patients (16.67%) (3 women and 2 men) in partial remission (PR) and 3) 15 patients (50.00%) (10 women and 5 men) in complete remission (CR). The partial remission (PR) was defined as having an improvement in any of the following items: a decrease of serum creatinine to <130 μmol/l for patients with a baseline serum creatinine level ≥130 μmol/l and ≤260 μmol/l; a decrease of serum creatinine by >50% for patients with a baseline serum creatinine level >260 μmol/l; a decrease of urinary protein excretion by >50% and <3.0 g/day with a serum albumin level ≥30 g/l and stable renal function. Complete remission (CR) was defined as urinary protein excretion <0.5 g/day, normal urinary sediment (<8 RBC/μl, <8 WBC/μl, absence of casts other than hyaline), serum creatinine and albumin concentrations in reference ranges.

**Levels of anti-C1q autoantibodies in sera of LN patients**

We analyzed by ELISA 30 serum samples from LN patients for autoantibodies against C1q molecule. 43.33% of patients were seropositive for anti-C1q Abs. The levels of anti-C1q Abs in investigated three groups patients – patients in CR, patients in PR and patients with active Lupus
nephritis (AN) – were presented in Figure 1. We found significant differences between frequencies of anti-C1q Abs in lupus nephritis patients with active disease and in patients with complete remission (p = 0.007) and between levels of anti-C1q Abs in AN patients and those with partial remission (p = 0.026) (Fig. 1).

In LN patients above 40 years only 4/21 (19.05%) patients were seropositive for anti-C1q Abs, but in the group of older patients – below 40 years – 55.56% (5/9 patients) were seropositive for anti-C1q Abs.

**Levels of anti-dsDNA autoantibodies in sera of LN patients**

56.67% of patients were positive for anti-dsDNA Abs. In group of patients in CR positive for anti-dsDNA Ab were 46.67%, in group of patients in PR – 20% and in group of patients in AN – 90%. In group of LN patients above 40 years 12/21 (57.14%) were seropositive for anti-dsDNA Abs. In the group of older patients – below 40 years – the rates were similar 55.56% (5/9 patients). We compared positive with negative for anti-dsDNA Abs patients to the levels of anti-C1q Abs. We found that positive for anti-dsDNA Abs had higher level of anti-C1q Abs in comparison to negative for anti-dsDNA Abs (p = 0.005) (Fig. 2).

**Association of LN activity and the presence of anti-C1q and anti-dsDNA antibodies**

We stratified the patients into four groups according to the presence of anti-C1q Abs and anti-dsDNA Abs to evaluate the role of these antibodies together to the activity of LN. Some immunological parameters of LN activity were further analyzed in the groups (Table 1). Among the groups, patients who were + anti-C1q/ + anti-dsDNA Abs had the highest median age of 52 ± 10.12 compared with the others groups. 67% of these patients had high LN activity and all had Class IV LN. The group who was + anti-C1q/ – anti-dsDNA Abs had lower serum C1q, C3 and C4 levels and all had Class IV LN.

**Discussion**

Anti-dsDNA Abs were considered a reliable marker of disease activity in SLE and implicated in the pathogenesis of LN. Anti-C1q Abs have been associated with renal involvement and with LN activity [1, 13, 14]. The present study showed that a positive for anti-dsDNA Abs patients had higher level of anti-C1q Abs in comparison to negative for anti-dsDNA Abs patients. Positive for anti-dsDNA and anti-C1q Abs patients had more severe LN – Class IV. The patients positive for anti-C1q Abs had lower levels of C1q, C3 and C4, than negative for anti-C1q Abs patients. This could be explained with complement consumption because of local renal complement activation. The patients positive for anti-C1q Abs but negative for anti-dsDNA Abs all had clinically active LN and Class IV LN. Julkunen, et al. 2012 found that anti-C1q Abs and complement C3 and C4 were better markers for LN activity than anti-dsDNA Abs, and that anti-dsDNA Abs and complement C3 and C4 were better than anti-C1q Abs to evaluate the overall and nonrenal activity of SLE [5]. We also found that anti-C1q Abs were more closely associated with LN activity and severity than the anti-dsDNA Abs. The explanation for these findings is connected to the important role of complement in the onset as well as throughout the course of SLE - is relevant to delayed renal clearance of immune complexes and apoptotic cells. A low C1q level is related to the presence of anti-C1q Abs with the formation of C1q/anti-C1q immune complexes with subsequent development of glomerulonephritis. According to Yang, et al. 2012 neither the presence of anti-C1q Abs nor the presence of anti-dsDNA Abs at renal biopsy was a risk factor for renal outcome [16]. However, the combination of the two Abs could predict renal prognosis and evaluate the renal disease activity best [4, 6]. Probably the anti-dsDNA Abs and anti-C1q Abs have an additive effect in lupus nephritis pathogenesis.

**Conclusions**

This study confirmed that, although the anti-C1q Abs have a lower sensitivity compared to anti-dsDNA Abs, the positive predictive value of both antibodies could help in LN diagnosis. We consider that the anti-C1q Abs have potential superior utility over anti-dsDNA Abs to track renal disease activity,
especially in LN. Systematic detection of anti-C1q, anti-dsDNA Abs and low complement should be used in combination to monitor the renal involvement.

Acknowledgements
MR and KB conceived and designed the study; MR performed the experiments, analyzed the data and wrote the manuscript; VV, BD, VL and VI referred patients to the study, VI and TA edited the manuscript. We thank Prof. Lubka Roumenina, for providing the conditions for complement analyses.

References
1. Akhter, E., R.W. Burlingame, A.L. Seaman, L. Magder, M. Petri, 2011. Anti-C1q antibodies have higher correlation with flares of lupus nephritis than other serum markers, Lupus, 20, 1267-1274


16. Yang, X., Y. Tan, F. Yul, M. Zhao, 2012. Combination of anti-C1q and anti-dsDNA antibodies is associated with higher renal disease activity and predicts renal prognosis of patients with lupus nephritis, Nephrol Dial Transplant, 27, 3552-3559


Figure 1. ELISA for anti-C1q autoantibodies in lupus nephritis sera (30) divided in three groups – AN patients with active LN; PR – patients with partial remission and CR – patients with complete remission. ELISA plates coated with 20µg/ml human C1q in sodium carbonate buffer were blocked with 1% BSA and incubated with LN sera (1:100 in PBS/0.75 M NaCl) o/n at 4°C. The immobilized anti-C1q autoantibodies were detected by HRP-conjugated goat anti-human IgG (1/1000) and o-phenylenediamine (OPD). Values were expressed in fold increase compared to pooled normal human serum (norm = 1).

Figure 2. Comparison between positive and negative for anti-dsDNA antibodies patients to the level of anti-C1q antibodies in serum.
Table 1. Association of some parameters of renal disease activity and the presence of anti-C1q and anti-dsDNA antibodies

<table>
<thead>
<tr>
<th></th>
<th>+ anti-C1q/+ anti-dsDNA</th>
<th>+ anti-C1q/- anti-dsDNA</th>
<th>- anti-C1q/+ anti-dsDNA</th>
<th>- anti-C1q/- anti-dsDNA</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD, years)</td>
<td>52 ± 10.12</td>
<td>29 ± 3.10</td>
<td>29 ± 6.11</td>
<td>37 ± 8.54</td>
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<td>LN duration (mean ± SD, years)</td>
<td>10 ± 0.70</td>
<td>1 ± 0.07</td>
<td>7 ± 6.85</td>
<td>12 ± 8.45</td>
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<tr>
<td>C1q (mean ± SD, µg/L)</td>
<td>61 ± 28.28</td>
<td>55 ± 0.71</td>
<td>73 ± 28.90</td>
<td>71 ± 17.10</td>
<td>0.731</td>
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<tr>
<td>C3 (mean ± SD, µg/L)</td>
<td>795 ± 282.10</td>
<td>644 ± 0.72</td>
<td>866 ± 169.76</td>
<td>988 ± 157.20</td>
<td>0.098</td>
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<tr>
<td>C4 (mean ± SD, µg/L)</td>
<td>127 ± 46.67</td>
<td>80 ± 0.65</td>
<td>142 ± 43.59</td>
<td>135 ± 25.40</td>
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<td>Histopathological type of LN</td>
<td></td>
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<tr>
<td>Class II (%)</td>
<td>-</td>
<td>-</td>
<td>20%</td>
<td>22%</td>
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<tr>
<td>Class III + IV (%)</td>
<td>100%</td>
<td>100%</td>
<td>80%</td>
<td>56%</td>
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<td>Class V (%)</td>
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<td>22%</td>
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<td>Clinical activity of LN</td>
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<tr>
<td>Active (AN)</td>
<td>67%</td>
<td>100%</td>
<td>60%</td>
<td>11%</td>
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<tr>
<td>Remission (PR + CR)</td>
<td>33%</td>
<td>-</td>
<td>40%</td>
<td>89%</td>
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