The importance of serological and histopathological diagnosis of the different forms of systemic lupus erythematosus with and without lupus nephritis

The aim of this study was to compare the serological profiles of 50 systemic lupus erythematosus (SLE) patients with (N+) and 50 without (N-) renal involvement and the histopathology results of their kidney specimens in order to find out whether the presence of specific antibodies correlated with renal involvement. All of the SLE patients had antinuclear antibodies. The mean value for the antinuclear antibodies was 4.9 IU/ml in the N- and 16.3 IU/ml in the N+ group ($p < 0.0321$). A total of 36% of N- patients had dsDNA (mean value 25.5 IU/ml), while, based on biopsy findings, 46% of N+ patients with classes II, III and IV had dsDNA (mean value 25.5 IU/ml; $p < 0.0218$). Lupus anticoagulant was found in 16% of the N- and in 4% of the N+ group ($p < 0.0455$). Arthritis occurred less frequently in the N+ group ($p = 0.0291$), and serositis more frequently in the N+ group ($p = 0.0153$). The serological profile of the SLE patients did not fully reflect the type of renal changes. The gold standard remains the results of histopathological examination.

KEYWORDS: ANA ■ anticardiolipin antibodies ■ dsDNA ■ histopathological examination of kidney ■ lupus anticoagulant ■ lupus nephritis ■ Sm-antibodies ■ SS-A antibodies ■ SS-B antibodies ■ systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is characterized by a breakdown of self-tolerance and production of autoantibodies. Changes in the kidney are estimated to develop in approximately 60–80% of patients with a diagnosis of this disease [1]. The changes observed in the kidney are glomerular, tubular and vascular abnormalities [1]. Antibodies and immune complexes play a key role in the pathogenesis of lupus nephritis by causing an abnormal inflammatory response.

Antinuclear antibodies (ANAs) include a heterogeneous group of antibodies directed against different antigens of both nuclear and cytoplasmic origin. Data indicates that the incidence of ANAs in SLE in the active period of disease is 100% [2].

In SLE patients, the following immunological molecules were observed: anti-DNA (antibody to native DNA in abnormal titer); anti-Smith (presence of antibody to Sm nuclear antigen); or a positive finding for antiphospholipid antibodies (based on abnormal serum concentration of IgG or IgM anticardiolipin antibodies or a positive test result for lupus anticoagulant [LA]).

An increasing concentration of anti-dsDNA antibodies correlates with the deterioration of the patient's condition and inflammation of the kidneys and blood. It is also a good marker to monitor treatment and remission of the disease.

Renal involvement should be considered and can often be found [2]. The huge capillary bed, along with the negatively charged basement membrane, the intricate functional capacity of the cells embedded in the glomerular apparatus and the conducting tubules, creates an environment that is highly susceptible to inflammatory injury, which is caused by autoantibodies [3].

There is a strong connection between mesangial lupus nephritis and antibodies for DNA, and between membranous lupus nephritis and anti-Sm antibodies. Renal involvement in the presence of other autoantibodies, for example the antiphospholipid antibodies, may produce thrombotic microangiopathy [4].

The aim of this study was to compare the serological profile (antibody type) of patients with SLE with and without renal involvement according to their disease activity and histopathology in order to find out whether the possible presence of specific antibodies correlated with renal involvement in SLE patients.

The development of a specific serologic diagnostic marker for lupus nephritis patients would the disease to be diagnosed earlier and help in establishing a correct prognosis. It is possible that such a marker could decrease the need for kidney biopsy.

Materials

■ Characteristics of the SLE patients

A total of 100 SLE patients who gave written consent and were treated between 2012 and 2013 were in the study. SLE patients were diagnosed according to the revised ACR criteria [4,5].

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The group was divided into two. The first group consisted of 50 patients who were treated in the Clinic of Internal Medicine and Rheumatology at the Central Clinical Hospital of the Ministry of Internal Affairs (Warsaw, Poland), who were hospitalized in 2012–2013 and then treated in the Rheumatology Outpatient Clinic, and were diagnosed with SLE without renal involvement. The next group consisted of 50 patients with SLE with renal involvement who were diagnosed on the basis of the above criteria, and were treated at the same time in the Department of Transplantation Medicine and Nephrology at the Medical University of Warsaw (Warsaw, Poland). These patients had a kidney biopsy and histopathological examination of the biopsied specimen at the same location.

Approval of the local ethics committee to carry out the research was obtained (Ne 79/2012). Demographic and laboratory data of both groups are presented in Table 1.

The vast majority of the population were women (92, 92%). The average age of respondents was 38.9 ± 11.8 years.

Two groups of patients were defined on the basis of changes in the kidneys. Group N- consisted of the 50 SLE patients, in which there was no proteinuria greater than 0.5 g/day. Group N+ consisted of 50 SLE patients who might have renal involvement and proteinuria, which in individual patients ranged from 0.5 to 20 /day, the average value (median value) was 6.0 /day. Hematuria, leukocyturia and cylinduria were also observed in the patients.

Methods

The clinical picture of SLE was assessed according to: the clinical history (taken from patients and the patients' files); physical examination; results of laboratory tests; imaging examinations; and the results of histopathological examination of the kidney from the group with kidney involvement. The following data was analyzed: the patient's age at the onset of the disease; the duration of disease; the presence of malar rash; photosensitivity; oral ulceration; arthritis; serositis; and renal, neurologic and hematologic disorders.

Disease activity was scored using the SLE disease activity index (the Systemic Lupus Erythematosus Disease Activity Index [SLEDAI]) [6]. If the patient has a SLEDAI scale of 0 points, the disease is classified as inactive, 1–5 points indicates mild activity, 6–10 points indicates moderate activity, 11–19 points indicates very active disease and ≥20 points indicates highly active disease.

Disease damage was assessed according to the Systemic Lupus International Collaborating Clinics (SLICC) damage index [6,7].

The indexes of the SLEDAI and the SLICC damage values are presented in Table 1.

In all patients, the erythrocyte sedimentation rate (ESR) was estimated according to Westegren's method. The peripheral blood morphology, total serum protein (and its fraction levels), creatinine, cholesterol (and its fractions), triglycerides and serum concentrations of C-reactive protein (CRP) were measured in all patients. Urine was examined for proteinuria, cells and casts. The glomerular filtration rate (GRF) was calculated from the Cockroft–Goult formula.

We also examined the C3c and C4 components of the complement system, antibody levels of ANA, dsDNA, Sm, SS-A/Ro, SS-B/La, aCL IgM, IgG and LA. The presence of IgG ANAs, dsDNA antibodies and anticardiolipin antibodies in all patients was detected by chemiluminescence

Table 1. Characteristics of the two groups of systemic lupus erythematosus patients with and without kidney involvement.

Parameters	Group N- (n = 50)	Group N+ (n = 50)	p-value
Patient age (years)†	41.8 ± 11.1	34.5 ± 10.6	0.0011
BMI (kg/m^2)†	24.0 ± 3.9	23.1 ± 3.9	0.8954
Disease duration (years)	6 (1–12)	4 (0–9)	0.1654
Female, n (%)	48 (96.0)	44 (88.8)	0.2687
Antiphospholipid syndrome, n (%)	13 (26)	5 (10)	0.0379
Sicca syndrome, n (%)	9 (18)	3 (6)	0.0648
Involvement of CNS, n (%)	13 (26)	9 (18)	0.3343
SLICC†	1.0 ± 1.0	2.9 ± 1.3	<0.0001
SLEDAI†	6.8 ± 3.1	26.6 ± 9.1	<0.0001

†Values are given as mean ± standard deviation.
N+: With renal involvement; N-: Without renal involvement; SLEDAI: Systemic Lupus Erythematosus Disease Activity; SLICC: Systemic Lupus International Collaborating Clinics.

test using Diasorin kit (Diasorin S.p.A., Italy) in LIASION® analizator (HYCOR Biomedical Inc., CA, USA). Antibodies against Sm were detected by the hematoglutinin test according to Sharp's method: the titer of ≥1:160 being considered a positive result. Activation of complement fragment C3c and C4 fragments were measured using the immunoturbidimetric method (**Box 1**). Treatment of SLE patients according to kidney involvement is presented in **Table 2**.

Kidney biopsy

Kidney biopsy was taken and assessed at the Department of Transplantation Medicine and Nephrology at the Medical University of Warsaw.

Histopathological analysis of renal biopsy

Histological classification of renal biopsy involved changes in the glomeruli, which are characteristic to a particular class [8]. Immunofluorescence microscopy was used to analyze the specimen.

Results of renal biopsy

The WHO classification of lupus nephritis is based on biopsy finding: class I (normal finding on biopsy); class II (mesangial hypertrophy and mesangial immune deposits); class III (mesangial and endothelial proliferation with immune deposits along capillaries but less than 50% of glomeruli involved); class IV (diffuse proliferative glomerulonephritis with greater than 50% of glomeruli involved and cell proliferation resulting in crescent formation); class V (membranous glomerulonephritis with subepithelial granular immune deposits); and class VI (sclerosing changes with fibrous crescents and vascular sclerosis). Designation of class V is associated with nephrotic range proteinuria in two-thirds of patients, but patients often maintained normal creatinine clearance. Class VI designation is an ominous sign that there are few reversible elements to the kidney involvement [8].

Statistical calculations

Statistical analysis was performed using a statistical software package SPSS/PC+. Continuous parameters were shown as mean ± SD or median. Wilcoxon, Kruskal–Wallis and median tests were used to compare continuous variables, for which the distribution of the sample was not Gaussian, and the paired t-test was used for the comparison of two population means, for which distribution was normal [9]. Correlation analysis was performed using Pearson's correlation coefficient after application of the logarithmic transformation. All reported p-values were two-sided and a type 1 error level of 0.05 was used [9].

Results

Table 1 shows the characteristics of the patients. Since these groups differed in terms of age, the difference between the average duration of disease did not show statistical significance. In addition, the age range of the patients in both groups was similar. The age of the patients at the time of the study ranged from 18 to 57 years in the N- group and from 16 to 60 years in the N+ group.

Antiphospholipid syndrome syndrome was observed more frequently in SLE patients without renal involvement ($p = 0.0379$). Sicca syndrome and involvement of the CNS was also observed, but rarely seen in patients with SLE without renal involvement compared with those with renal involvement. This difference did not show statistical significance.

The N+ group in the study showed a significantly higher value of disease activity characterized by SLEDAI and higher chronic damage characterized by the SLICC damage scale (**Table 1**). The size of both of these indicators turned out to be approximately three-times greater in the group with renal involvement than that observed in those without renal involvement.

Table 3 shows a comparison of the clinical picture of lupus and frequency of specific criteria for lupus in the analyzed groups.

All examined patients with SLE had 100% presence of antinuclear antibodies, regardless of renal involvement. In patients with SLE without renal involvement, the incidence of LA was four-times higher (16% in the N+ group and 4% in the N- group; $p = 0.0455$).

Patients without renal involvement also showed dsDNA antibodies (n = 18, 36%), and SS-A/Ro antibodies (n = 9, 18%). SS-B/La, aCL

Box 1. Normal value of serological examinations.

- Antinuclear antibodies (chemiluminescence method): <1.5 IU/ml
- Anti-dsDNA antibodies (chemiluminescence method): <20 IU/ml
- Anti-Sm antibodies (ELISA method): <10 IU/ml
- Anti SS-A (Ro) antibodies (ELISA method): <10 IU/ml
- Anti SS-B (La) antibodies (ELISA method): <10 IU/ml
- Anticardiolipin antibodies IgG (chemiluminescence method): <20 IU/ml
- Anticardiolipin antibodies IgM (chemiluminescence method): <13 IU/ml
- Lupus anticoagulant (colorimetry method): <1.20 ratio
- C3 components of the complement system (immunoturbidymetric method): 90–180 ng/ml
- C4 components of the complement system (immunoturbidymetric method): 10–40 ng/ml

Table 2. Treatment of systemic lupus erythematosus patients according to kidney involvement.

Drugs	Group N- (n = 50); n (%)	Group N+ (n = 50); n (%)	p-value
Glucocorticosteroids pulses	0	49 (98)	<0.0001
Glucocorticosteroids	50 (100)	50 (100)	NS
Cyclophosphamide	7 (14)	37 (74)	<0.0001
Azathioprine	12 (24)	30 (60)	0.0002
Chloroquine	30 (60)	11 (22)	0.0001
Metothrexate	7 (14)	3 (6)	0.3178
Tacrolimus	0	3(6)	NS

The patients in group N+ more frequently received cyclophosphamide (p < 0.0001) and azathioprine (p = 0.0002), and patients in group N- received chloroquine (p = 0.0001). Glucocorticosteroids pulses were only given to patients from group N+ (p < 0.0001).
N+: With renal involvement; N-: Without renal involvement; NS: Nonsignificant.

IgM, IgG aCL and Sm antibodies were seen in individual cases (in one in five people). A decrease of complement component C3c (16 cases, which represents 34.8%) compared with complement component C4 (n = 10, 21.7%) was frequently observed. In patients with renal involvement, dsDNA antibodies were found in close to half of the patients (46%). Sm, SS-A/Ro and SS-B/La antibodies were only observed in some individuals. Anticardiolipin antibodies aCL IgM and aCL IgG were observed in this group of patients; however, only in a few individual cases. The decrease of the complement components C3c and C4 were more frequently found in this group of patients than in those without renal involvement (91.8% for C3c and 95.9% for C4).

Table 3. Characteristics of systemic lupus diagnostic criteria, antibodies SS-A/Ro, SS-B/La and complement proteins C3c and C4 in systemic lupus erythematosus patients with and without renal involvement.

Criteria	Group N- (n = 50); n (%)	Group N+ (n = 50); n (%)	p-value
Malar rash	30 (60)	33 (66)	0.5344
Discoid rash	3 (6)	6 (12)	0.4870
Photosensitivity	31 (62)	30 (60)	0.8376
Oral ulcers	8 (16)	5 (10)	0.3724
Arthritis	46 (92)	38 (76)	0.0291
Serositis	9 (18)	20 (40)	0.0153
Renal disorders	0	50 (100)	
Neurologic disorders	10 (20)	9 (18)	0.7988
Hematologic disorders	27 (54)	32 (64)	0.3093
Immunologic disorders	50 (100)	50 (100)	1.000
Presence of antibodies			
Anti dsDNA antibody	18 (36)	23 (46)	0.3093
Anti-Sm antibody	2 (4)	2 (4)	1.000
IgG aCL	1 (2)	3 (6)	0.6173
IgM aCL	4 (8)	1 (2)	0.1175
Lupus anticoagulant	8 (16)	2 (4)	0.0455
Anti SS-A/Ro antibody	9 (18)	3 (6)	0.0648
Anti SS-B/La antibody	5 (10)	2 (4)	0.4360
Low C3c complement level	16 (34.8)	45 (91.8)	<0.0001
Low C4 complement level	10 (21.7)	47 (95.9)	<0.0001
Antinuclear antibody	50 (100)	50 (100)	1.0000

N+: With renal involvement; N-: Without renal involvement.

The numerical values of the antibodies and complement components are shown in Table 4. Statistical differences were found in the following antibodies: ANA, dsDNA and Sm. These antibodies were found in higher concentrations in the patient group with renal involvement. In addition to immunological disorders occurring in all patients in the study population, the most common symptoms observed in patients of the N- group was arthritis, which occurred in almost all patients (92%). The next most common symptoms were photosensitivity (62%) and malar rash (60%). Much less frequently diagnosed were neurological disorders (20%), serositis (18%) and oral ulcers (16%). Discoid rash occurred in this group; however, only occasionally (in three patients, 6%).

In N+ patients, in addition to immunological disorders and changes in the kidneys, the most common symptoms observed were arthritis (76%), malar rash (66%), hematological disorders (64%), photosensitivity (60%) and serositis (40%). Discoid rash was observed less frequently in six patients (12%), and oral ulcers in five of the patients (10%).

A comparison of the prevalence of these symptoms in the groups showed the existence of differences in the clinical picture of lupus. Arthritis occurred significantly less frequently in the N+ group of patients (92% in N- group and 76% in N+ group; p = 0.0291).

Another symptom that shows the difference between the two groups was serositis, which was observed more frequently in the N+ group (p = 0.0153).

The average number of criteria in the study observed in patients with renal involvement was 5.6 ± 1.5, which is significantly higher (p < 0.0001) than the average number of criteria recognized in patients with no kidney changes (4.2 ± 1.1).

In Table 5, a comparison of the analyzed indicators of disease activity for each group can be observed. The N+ patients had significantly higher average erythrocyte sedimentation

values (median: 23 mm/h in the N+ group and 18 mm/h in the N- group; p = 0.0193) and lower average concentration of γ globulin in the blood (arithmetic mean values were 14.4 ± 6.6 for the N+ group and 21.4 ±13.6 g/dl for the N- group; p = 0.0151) compared with the N- patients.

The examined groups did not differ greatly in average concentrations of CRPor the level of α1-globulin fraction, α-2 globulin and β globulin.

In the N+ group, chronic renal failure was observed in one patient and severe renal failure was observed in eight patients. In 38% of the patients in this group, the stage of disease remained at a moderate level and in 16% it remained at a slightly lower level.

In 28 patients of the N+ group (56%), relapses occurred, and seven patients from the N+ group had previously had dialysis.

Table 6 shows the values for morphology and total serum protein level, albumin level and lipid profile in patients with SLE according to renal involvement.

Analysis of blood morphology and biochemical parameters showed significant differences between the values of certain indicators, such as hemoglobin, erythrocytes, protein, albumin, cholesterol and LDL.

In addition to the significantly lower concentration of hemoglobin and erythrocytes in the blood of patients in group N+, this group also had significantly lower levels of total protein and albumin than those in the N- group.

Statistical analysis of the lipid profile observed in both groups of patients, the results of which are shown in Table 6, showed significant differences between both groups. Average concentrations of both total cholesterol and its LDL fraction were significantly higher in the group of patients with renal involvement, then in comparison to the group without renal involvement.

Characteristics of SLE patients with renal involvement

There was no correlation between patients' age, duration of disease, sex and histopathological results (Table 7).

The clinical picture of SLE patients with known renal involvement was dependent on the results of the histopathological examination.

Characteristics of the incidence of SLE diagnostic criteria in patients with different classes of renal histopathology results is presented in Table 8. Photosensitivity was frequently observed in class III and IV patients, but was rare in classes II and V. Similarly, arthritis and lower C4 was much rarer in class V than in other classes.

Table. 4. Antibody levels in the examined groups of patients.

Antibodies	Group N- (n = 50)	Group N+ (n = 50)	p-value
ANA (IU/ml)†	4.90 (2.70–9.50)	16.3 (10.4–22.3)	0.0321
Antibody dsDNA (IU/ml)†	14.6 (4.1–56.6)	25.5 (1.6–240.0)	0.0218
Anty-Sm (IU/ml)†	1.40 (0.60–2.50)	11.1 (1.3–14.0)	0.0458
Anticardiolipin IgG (IU/ml)†	8.80 (3.20–11.10)	46	NA
Antycardiolipin IgM (IU/ml)†	3.90 (2.00–6.40)	4.9 (1.4–9.5)	NS
Lupus anticoagulant†	1.41 (1.31–1.91)	1.53 (1.28–1.78)	NS
SS-A/Ro (IU/ml)†	3.35 (2.0–37.80)	7.8 (1.6–14.0)	NS
SS-B/La (IU/ml)†	2.80 (1.70–19.40)	3.9 (0.8–7.0)	NS
C3c complement level (ng/ml)‡	97.0 ± 21.4	79.7 ± 31.8	0.0062
C4 complement level (ng/ml)‡	16.6 ± 8.0	9.4 ± 5.6	<0.0001

†Values in brackets are lower and higher quartiles.
‡Values are mean ± standard deviation.
N+: With renal involvement; N-: Without renal involvement; NA: Not applicable; NS: Nonsignificant.

There were no significant differences between the criteria of lupus (Table 8), creatinine levels, eGFR (Table 9), morphology parameters (Table 9) and lipid profiles (Table 9) observed in patients with different classes of histopathological findings.

Discussion

Development of the specific serologic diagnostic marker for lupus nephritis patients would allow the disease to be diagnosed earlier and help with establishing a correct prognosis. It is possible that such a marker could decrease the need for kidney biopsy.

In our study, we compared the serological profile (antibody type) in SLE patients with and without renal involvement according to their disease activity and histopathology in order to find the possible presence of specific antibodies correlated with renal involvement in SLE patients.

Table 5. Erythrocyte sedimentation rate, C-reactive protein, total protein and its fractions in systemic lupus erythematosus patients with and without renal involvement.

Parameters	Group N- (n = 50)	Group N+ (n = 50)	p-value
ESR (mm/h)†	18.0 (7.5–34.6)	23.0 (20.0–52.0)	0.0193
CRP (mg/l)†	1.15 (0.5–2.90)	1.80 (1.10–2.56)	0.4085
α-1 globulin (g/dl)†	4.10 (2.00–8.20)	4.10 (3.70–4.72)	0.6071
α-2 globulin (g/dl)†	13.4 (8.7– 6.5)	12.5 (11.1–13.9)	0.6072
β globulin (g/dl)†	11.4 (9.0–19.50)	13.0 (11.4–16.0)	0.6181
γ globulin (g/dl)‡	21.4 ± 13.6	14.4 ± 6.6	0.0151

†Values in brackets are lower and higher quartiles.
‡Values are mean ± standard deviation.
CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; N+: With renal involvement; N-: Without renal involvement.

Table 6. The results of blood morphology, total protein, albumin levels and lipid profile in patients with systemic lupus erythematosus according to renal involvement.

Parameters	Group N-	Group N+	p-value
Hemoglobin (g/l)†	12.8 ± 1.3	11.2 ± 2.3	<0.0001
Erythrocytes (T/l)†	4.28 ± 0.42	3.82 ± 0.71	<0.0001
Leukocytes (g/l)†	6.46 ± 2.57	5.74 ± 3.14	0.2109
Trombocytes (g/l)†	214.0 ± 69	213.0 ± 86	0.9796
Protein (g/dl)†	7.07 ± 0.77	6.07 ± 1.06	<0.0001
Albumin (g/dl)†	4.15 ± 0.55	3.27 ± 0.70	<0.0001
Cholesterol (mg/dl)†	186.1 ± 31.2	260.3 ± 74.6	<0.0001
HDL (mg/dl)†	55.7 ± 27.6	63.9 ± 19.0	0.4978
LDL (mg/dl)†	120.3 ± 7.6	165.5 ± 64.7	0.0095
Tryglycerides (mg/dl)‡	115.0 (78–133)	155.0 (97.0–253.0)	0.0515

†Values are mean ± standard deviation.
‡Values in brackets are lower and higher quartiles.
HDL: High-density lipoprotein; LDL: Low-density lipoprotein; N+: With renal involvement; N-: Without renal involvement.

In our study, the difference between the group with and without lupus nephritis was in the value of ANA antibodies, a higher value was found in patients with lupus nephritis. Eighteen patients without renal involvement (36%) were observed to have dsDNA antibodies. A total of 36% patients without renal involvement had dsDNA antibodies, while 46% patients with renal involvement had dsDNA antibodies with higher value.

SLE is the most common immunological disorder accompanied by a secondary antiphospholipid syndrome. So far it is not clear to what extent the activity of renal disease in SLE is dependent on the concomitant manifestation of antiphospholipid syndrome [10]. In our study, anticardiolipin IgM and IgG were only found in a few patients with and without renal involvement. LA was found in 16% of SLE patients without renal involvement and in 4% of patients with renal involvement.

Majdan showed that high titers of anticardiolipin IgM and anti-β2-glycoprotein I IgM and the presence of LA combined to impair GFR in patients with SLE. The relationship between increased titers of specific antiphospholipid antibodies and GFR reduction was most pronounced in patients with SLE with secondary antiphospholipid syndrome [11].

Currently the gold standard for assessment of the nature and degree of severity of lesions in the kidney is based on the result of kidney biopsy. However, clinical studies performed using the classification of the International Sopciety of Nephrology (ISN)/Renal Pathology Society (RPS) broadly differ as to the predictive value of this classification and its clinical use as an indicator for the selection of treatments [11].

Unfortunately, in our study analyzing the clinical picture of patients in the groups with different histopathological results proved to have no regularity, maybe owing to a small number of patients with different histopathological classes.

DsDNA antibodies were usually found in classes II–IV using the ISN/RPS classification (40, 37.5 and 59.1%, respectively), and anti-Sm antibodies were frequently observed in class V (33.3%). LA was also found in class V (33.3%). Antibodies SS-A/Ro and SS-B/La were observed in class III and IV, but were only found in isolated cases.

Christopher-Stine *et al.* evaluated the results of renal biopsies from 21 patients with proteinuria less than 1 g daily and microscopic hematuria, ten subjects had class III, and two patients had class IV changes according to the ISN/RPS [12]. This showed no correlation between the serological profile and the type of antibodies present in each group.

In the sera of patients with SLE, a wide spectrum of antibodies were found, which were useful in the diagnosis, monitoring of progress and treatment of the disease. The world is still performing research into newer, more specific and sensitive immunoassays, designed to increase the clinical utility of antibodies. To date, the recognized immunological markers of SLE were antibodies directed against native DNA (dsDNA) and against Sm antigen [10].

It was found that antinucleosome antibodies may be a valuable marker in the diagnosis of SLE immunoassay, and may correlate with the

Table 7. Characteristics of the study group of systemic lupus erythematosus patients with renal involvement, depending on the result of the histopathological examination of the kidneys.

Parameters	Class II (n = 5)	Class III (n = 17)	Class IV (n =24)	Class V (n = 7)	p-value
Age (years)†	30.4 ± 8.0	35.5 ± 9.9	32.8 ± 8.9	41.5 ± 17.9	0.2415
Disease duration (years)‡	3.0 (2–3)	5.0 (0–17)	6.0 (0–9)	0.5 (0–5)	0.6046
Female, n (%)	4 (80)	15 (93.7)	20 (87)	5 (83.3)	0.6663

†Values are mean ± standard deviation.
‡Values in brackets are lower and higher quartiles.

Table 8. Characteristics of the incidence of systemic lupus diagnostic criteria in patients with different classes of renal histopathology results.

Parameters	Class II (n = 5); n (%)	Class III (n = 16); n (%)	Class IV (n = 23); n (%)	Class V (n = 6); n (%)	p-value
Malar rash	2 (40)	8 (50.0)	19 (82.6)	4 (66.7)	0.0820
Discoid rash	0	0	5 (21.7)	1 (16.7)	0.4166
Photosensitivity	1 (20)	9 (56.3)	18 (78.3)	2 (33.3)	0.0329
Oral ulcers	0	2 (12.5)	3 (13)	0	1.000
Arthritis	3 (60)	14 (87.5)	19 (82.6)	2 (33.3)	0.0410
Serositis	3 (60)	5 (31.25)	11 (47.8)	1 (16.7)	0.3708
Renal disorders	5 (100)	16 (100)	23 (100)	6 (100)	
Neurologic disorders	0	1 (6.25)	8 (34.8)	0	0.0670
Hematologic disorders	2 (40)	12 (75.0)	16 (69.6)	2 (33.3)	0.2094
dsDNA+	2 (40)	6 (37.5)	13 (59.1)	2 (33.3)	0.5611
Anty-Sm	0	0	0	2 (33.3)	1.000
aCL IgG	0	0	3 (13.0)	0	0.8553
aCL IgM	0	0	1 (4.3)	0	1.000
LA	0	0	0	2 (33.3)	1.000
SS-A /Ro	0	1 (6.25)	2 (8.7)	0	0.9822
SS-B/La	0	1 (6.25)	1 (4.3)	0	1.000
ANA	5 (100)	16 (100)	23 (100)	6 (100)	1.000
C3c (IU/ml)	113.0	80.8 ± 18.6†	76.0 ± 39.8†	85.0 ± 16.8†	0.7150
C4 (IU/ml)	17.8	9.6 ± 4.8†	9.6 ± 6.1†	6.0 ± 3.7†	0.2885
Low C3 complement level	4 (80)	14 (87.5)	23 (100)	4 (66.7)	0.0421
Low C4 complement level	4 (80)	16 (100)	23 (100)	4 (66.7)	0.0179

†*Values are mean ± standard deviation.*

development of lupus nephritis [13]. Szewczyk *et al.* have studied the sera of 74 patients diagnosed with ANA using the immunofluorescence method (sera of 19 patients in this group were from patients with SLE) [13]. In 37% of patients with SLE, antibodies against nucleosomes were detected, which occurred in conjunction with ANA, and in one case these antibodies were the only ones identified. Further clinical analysis showed that the antinucleosome antibodies (AnuA) were present in patients with SLE with renal changes. Amoura *et al.* found that the nucleosome antibodies of IgG, in particular the class IgG3 may be a new marker of SLE, especially lupus nephritis [14]. Yin *et al.* demonstrated that there is a correlation between the presence of antibodies AnuA and changes in kidney. The study was conducted on 233 patients identified with SLE [15].

In addition, Haddouk *et al.*, in work involving 200 patients with established SLE, showed that antinucleosome antibodies may be a useful marker for active lupus nephritis [16]. Antinucleosome antibodies may be even better than the dsDNA antibodies. In our work, patients with these antibodies were not studied.

Bertolaccini *et al.* found that there is a correlation between the presence of antiribosomal (anti-P) antibodies and the active form of lupus manifested by changes in the kidneys [17]. Tests were carried out on 81 patients with SLE in whom renal biopsy were performed. At the same time, immunoassays were performed on these patients. The antiribosomal antibodies were detected in 18 (22%) patients. Positive anti-P antibodies in most patients correlated with class V renal involvement by ISN/RPS classification. The antiribosomal antibodies have the ability to penetrate into the cell, induce proinflammatory cytokine production and initiate apoptosis [18]. In our work, these antibodies were not tested.

Kim *et al.* showed that there is a close correlation between the level of antichromatin and anti-dsDNA antibodies, complement components, the level of white blood cells and SLEDAI scale [18]. A study of 100 patients with a diagnosis of SLE suggests that antichromatin antibodies may

Table 9. Analysis of blood morphology, erythrocyte sedimentation rate, C-reactive protein, total protein and its fractions, and lipid profile in systemic lupus erythematosus patients with renal involvement according to histopathology classes.

Parameters	Class II (n = 5)	Class III (n = 16)	Class IV (n = 23)	Class V (n = 6)	p-value
ESR (mmHg)†	33.3 (33.3–33.3)	23.0 (21.0–39.0)	26 (12.5–54.5)	21.5 (11.0–50.0)	0.8998
CRP (mg/l)†	1.50 (1.5–1.5)	1.90 (1.30–2.10)	1.80 (1.10–3.00)	1.65 (0.65–4.0)	0.9936
α1 globulin (g/dl)†	4.50 (4.0–4.70)	3.80 (3.70–4.30)	4.30 (3.90–4.90)	3.85 (2.9–4.9)	0.3636
α2 globulin (g/dl)†	13.4 (12.0–13.8)	11.8 (10.2–13.0)	12.1 (11.0–13.9)	13.8 (12.8–16.6)	0.1951
β globulin (g/dl)†	15.7 (13.0–16.3)	12.5 (10.0–15.0)	13.5 (10.4–16.0)	12.6 (12.5–17.0)	0.5035
γ globulin (g/dl)†	15.8 (10–17)	13.8 (11.0–16.9)	12.65 (8.0–20.1)	9.8 (7.3–15.9)	0.8126
Hemoglobin (g/l)‡	10.9 ± 3.9	11.2 ± 1.7	10.6 ± 2.2	13.4 ± 1.7	0.1519
Erythrocytes (T/l)‡	3.89 ± 1.22	3.88 ± 0.55	3.63 ± 0.69	4.33 ± 0.44	0.2711
Leukocytes (G/l)‡	6.85 ± 2.95	5.88 ± 3.40	4.82 ± 2.84	7.92 ± 2.91	0.1457
Thrombocytes (G/l)‡	237.0 ± 76.0	225.0 ± 93.0	192.0 ± 89.0	243.0 ± 46.0	0.4647
Protein (g/dl)‡	5.77 ± 1.14	6.27 ± 1.09	6.06 ± 0.62	5.87 ± 1.11	0.7425
Albumin (g/dl)‡	3.02 ± 0.39	3.50 ± 0.80	3.18 ± 0.62	3.23 ± 0.95	0.4401
Cholesterol (mg/dl)‡	250.0 ± 42.1	225.1 ± 49.5	282.6 ± 88.4	247.5 ± 26.3	0.3119
HDL (mg/dl)‡	53 ± 19.6	68.9 ± 17.3	61.3 ± 21.3	66.3 ± 16.9	0.7713
LDL (mg/dl)‡	153 ± 49.3	126.1 ± 41.6	183.9 ± 75.9	129 ± 65.5	0.2075
Triglycerides (mg/dl)†	220.0 (220–220)	128.0 (100–155)	230.0 (97–311)	154.0 (106–194)	0.3640
Creatinine (mg/dl)†	1.23 (0.90–1.40)	1.09 (0.75–2.00)	1.30 (0.80–2.10)	1.10 (0.70–1.30)	0.9219
eGFR (ml/min/1.73m^2)†	52.5 (47.9–100.1)	62.0 (31.1–87.4)	52.2 (28.2–95.3)	55.6 (48.7–92.3)	0.9338

†Values in brackets are lower and higher quartiles.
‡Values are mean ± standard deviation.
CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

be a useful parameter in the future to diagnosis patients with suspected SLE. In our work, these antibodies were not tested.

Conclusion

The serological profile of SLE patients did not fully reflect the type and degree of the severity of renal changes in these patients. The histopathological examination still remains the gold standard.

Higher values of ANA and anti-dsDNA antibodies were found more often in patients with lupus nephritis. In SLE patients with lupus nephritis, serositis occurred more often and arthritis was rarely seen in patients without lupus nephritis.

Photosensitivity and arthritis were more commonly observed in class III and IV lupus nephritis then other classes.

Patients with lupus nephritis had higher disease activity characterized by SLEDAI and higher chronic damage characterized by SLICC damage scale.

Future perspective

Perhaps new examinations including a larger number of lupus nephritis patients will be conducted in order to find a correlation between dsDNA, Sm antibodies and the results of histopathological changes in the kidney. It is also very important to find this correlation between new antibodies, such as antinucleosome antibodies, antichromatin antibodies and anti P-antibodies, with the results of histopathological changes in the kidney in SLE patients.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Lupus nephritis is characterized by a higher value of ANA, dsDNA, antinucleosome antibodies, antiribosomal antibodies and antichromatin antibodies, as well as higher disease activity characterized by the Systemic Lupus Erythematosus Disease Activity Index and higher chronic damage characterized by the Systemic Lupus International Collaborating Clinics damage scale.
- Despite the higher values of the abovementioned antibodies they are not, to date, sufficient to diagnose the type and severity of lupus nephritis.
- Class III and IV lupus nephritis is connected with photosensitivity and the presence of arthritis.
- Despite advances in serological investigations, histopathological examination remains the gold standard in diagnosing lupus nephritis patients.

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