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Use of cyclophosphamide and other immunosuppressive drugs in the treatment of patients with lupus nephritis

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Systemic lupus erythematosus (SLE) is a chronic relapsing systemic autoimmune disease; one of the most serious complications is renal involvement, which is occurring in almost 50% of all patients at the beginning of the disease. The aim of the present study was to compare renal function, proteinuria, activity markers and treatment regimen of active and inactive SLE patients with renal involvement. We analyzed the correlation of serum blood urea nitrogen, creatinine level, glomerular filtration rate, urine total protein/serum creatinine (uTP/creat), CRP to classic activity markers of SLE (serum complement 3, -4 level, anti-dsDNA antibody). Moreover we analyzed the treatment modalities of patients with lupus nephritis (LN). Data of 418 SLE patients were analyzed, out of these patients 128 had biopsy proven lupus nephritis or had more than 3+ proteinuria by urine dipstick analysis (30% of all cases). Results: Data of 128 patients with lupus nephritis were analyzed (mean age 32.18 ± 11.48 year, time between the diagnosis of SLE and LN was 2.78 ± 4.59 year). 48% of patients had diffuse proliferative glomerulonephritis, 75% of them received cyclic cyclophosphamide treatment. UTp (total protein)/creatinine level was significantly higher in active LN group ($p = 0.03$), and correlated to erythrocyte sedimentation rate ($p = 0.002$, $R = 0.52$). Mean anti-dsDNA level of patients with active LN was significantly higher ($p < 0.001$). Conclusions: Patients with active lupus nephritis are at higher risk of developing renal failure, activity markers and urine protein are elevated in these patients as compared to inactive patients, early aggressive immunosuppressive treatment needs to be started to prevent end-stage renal failure.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic relapsing systemic autoimmune disease leading to organ damage. Morbidity and mortality is increased, but early diagnosis, therapy and follow-up led to a significant improvement in patients' outcome. 5-year and 10-year survival rates of lupus nephritis (LN) range 83–92% and 74–84% respectively. Renal involvement is one of the most common complications, and is present at the beginning in 25–50% of patients; moreover 60% of all patients develop immune complex-mediated lupus nephritis during the course of their disease (Lateef and Petri 2012). Renal disorder could be serious and life-threatening affecting quality of life, treatment, and outcome.

Anti-double stranded deoxyribonucleic acid (DNA) antibodies and serum complement levels are used to monitor disease activity, but there are several other candidate biomarkers which are more sensitive to predict flares (Illei and Lipsky 2004; Brúgós and Zeher 2010). Anti-C1q antibodies correlate with renal disease activity (Akhter et al 2011).

Usually the first sign of renal involvement is proteinuria; urine total protein, serum creatinine level, glomerular filtration rate (GFR), urine total protein/creatinine are used to follow renal activity. Nowadays a lot of other novel urinary biomarkers have been proposed to be more sensitive and specific to predict renal

flares, for example urinary monocyte chemoattractant Protein - 1 (uMCP - 1), neutrophil gelatinase-associated lipocalin (NGAL), tumor-necrosis factor-like weak inducer of apoptosis (TWEAK) (Mok CC 2010). Renal biopsy is usually performed in lack of contraindications. Histological classification of lupus nephritis is based on the 2003 International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system (Weening et al 2004).

Patients with lupus nephritis need an early aggressive therapy to prevent end-stage renal failure; the standard therapy is use of glucorticoids and cyclophosphamide. It is well known that cyclophosphamide could cause serious adverse effects as infections, malignancies and infertility. Mycophenolate mofetil could also be used for induction therapy having fewer side effects (Appel et al. 2009). Azathioprine is effective in maintenance therapy (Lateef and Petri 2012). B-cells have an important role in the pathogenesis of SLE, rituximab, a chimeric anti-CD20 antibody, efficiently depletes CD20-positive B cells and could be use in refractory lupus nephritis (Gumarsson and Jonsdottir 2013).

Hydroxychloroquine has been shown to improve response rates, decrease flares and improve survival, is a commonly used drug in treating SLE patients. Renal protective treatment is also very important (angiotensin-converting enzyme inhibitors, statins).

Table 1: Demographic data of patients

	Age at onset of SLE (year ± SD)	Age at onset of LN (year ± SD)	Time between the dg of SLE and LN (year ± SD)
Female (n = 113)	29.21 ± 11.27	32.18 ± 11.48	2.78 ± 4.59
Male (n = 15)	29.17 ± 12.26	30.38 ± 10.95	2.14 ± 3.71

Table 2: Clinical data of patients with active or inactive diseases

Patients Signs	Active LN (n = 31)	Inactive LN (n = 97)
Age (year ± SD)	26.16 ± 8.9	29.49 ± 12.09
SLEDAI	6.85 ± 1.78	2.66 ± 3.25
Butterfly rash (n =)	13	33
Non-erosive arthritis (n =)	26	74
Cerebrovascular event (n =)	2	11
Pleuritis (n =)	6	36
Increased aDNA level (n =)	20	43
Proteinuria (> 0.5 g/die) (n =)	12	20
Thrombocytopenia/leukopenia (n =)	17	46
Therapy: -methylprednisolone (n =)	31 (22.85 ± 10.4 mg)	97 (11.3 ± 4.67 mg)
-cyclophosphamide	26 (83,3%)	32 (32,9%)

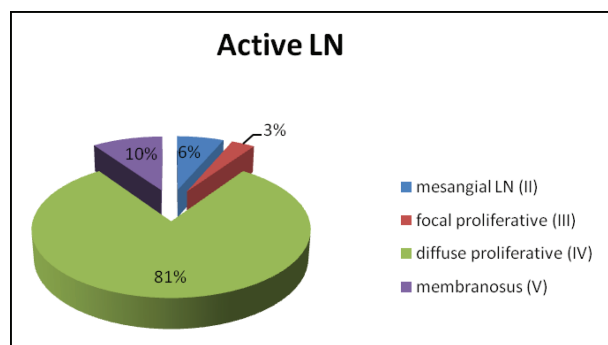


Fig. 1: Histological classification of patients with active LN.

One of the latest therapies in SLE and lupus nephritis is a new biologic, belimumab targeting B cell-stimulating factor (BLyS), also known as BAFF (B cell-activating factor from the tumor necrosis factor family). It was shown that BLyS circulating level is elevated in patients with SLE (Chiche et al. 2012; Harvey and Gordon 2013).

The goal of the present retrospective study was to find out the renal involvement of a large cohort of SLE patients, to find out the correlation between the activity of LN and classical laboratory parameters (serum complement levels, anti-DNA level), and to analyze treatment modalities of patients with lupus nephritis.

2. Investigations and results

Data of 418 SLE patients were analyzed (1974–2012), 30% of these patients, totally 128 patients had proteinuria or biopsy proven lupus nephritis. Demographic data of patients are shown in Table 1. 88.3% of patients were female. 76% of patients had renal biopsy. One patient had glomerulosclerosis based on renal histology, renal transplantation was needed in this patients.

48% of patients (n=61) had diffuse proliferative glomerulonephritis (Class IV) which is the most aggressive form of lupus nephritis. Most of the patients with diffuse proliferative glomerulonephritis were active. Histological distribution of patients is presented on Fig. 1 and Fig. 2.

5% of the patients had mesangioproliferative glomerulonephritis (GN) (Class II), 16% of them had focal proliferative GN

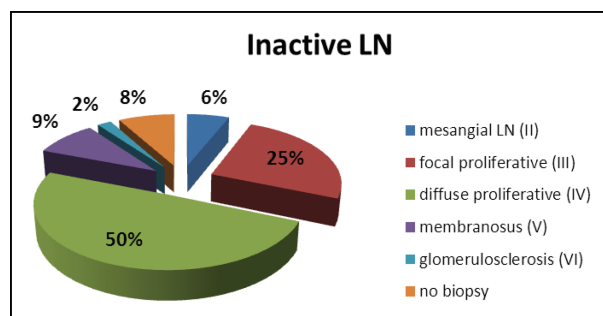


Fig. 2: Histological classification of patients with inactive LN.

(Class III), 6 % had membranous (Class V), and biopsy was not performed in 31 patients because of contraindications.

24% of patients had active lupus nephritis; clinical data of patients with active and inactive disease are listed in Table 2. In Class II patients the most common extrarenal symptom was erosive arthritis, while in patients with proliferative GN butterfly rash and serositis were usual.

Active patients had significantly higher sedimentation rate, dsDNA antibody levels and urine total protein/serum creatinine ratios (Table 3).

All patients received methylprednisolon, the cumulative dose for active patients was 22.8 ± 10.4 mg, for inactive patients 11.3 ± 4.6 mg. Azathioprin therapy was used for maintenance therapy in all groups: 95 ± 15.8 mg in active and 80.5 ± 24.3 mg for inactive patients. Cyclophosphamide was used monthly, 0.5–1 mg/m² dose (NIH protocol) in 75% of patients with diffuse proliferative glomerulonephritis and 70% of patients

Table 3: Laboratory data of patients

	Active LN	Inactive LN	p =
ESR (mm/h)	38 ± 19.6	25.35 ± 20.1	0.041
CRP (ng/ml)	5.61 ± 19.1	9.72 ± 11.7	NS
C3	0.66 ± 0.32	1.1 ± 0.3	NS
aDNA	74.3 ± 59.3	42.66 ± 52.9	0.001
UTP/kreat	225.58 ± 190	86.9 ± 83.2	0.03

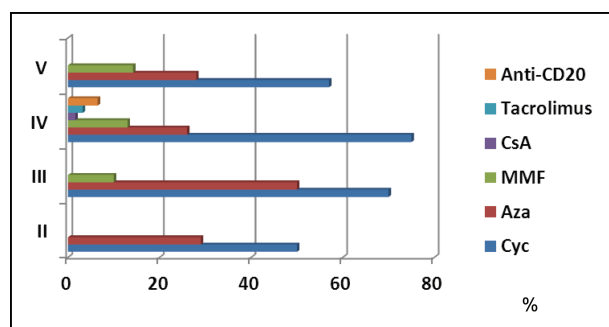


Fig. 3: Drugs used in patients with LN (antiCD20-rituximab, CsA-cyclosporinA, MMF-mycophenolate mofetil, Aza-azathioprin, Cyc-cyclophosphamide).

with focal proliferative GN. An average of 630.5 ± 154.8 mg cyclophosphamide was used in active patients (6.1 ± 2.5 cycles), while 531.4 ± 207 mg Cyc in inactive patients (5.8 ± 2.7 cycles). Other immunosuppressive drugs as mycophenolate mofetil, rituximab, tacrolimus, cyclosporinA were used in active group in some patients (Fig. 3).

3. Discussion

Systemic lupus erythematosus is a chronic, relapsing autoimmune disease involving several organs, the most serious complications are renal- and central nervous system involvement. New therapeutical strategies, regular follow-up of these patients improved the survival rate in the past decades, the 5-year survival rate increased from 50% to 80% (Kiss et al. 2011). Although survival of SLE improved, occurrence of end stage kidney is increasing (Fiehn et al. 2003).

Incidence of lupus nephritis among our patients was 30%, which is less than in patients treated and published by other authors (Cortez-Hernandez et al. 2004), due to the regular check-ups of these patients in order to treat activity signs properly and adequately.

In our patients proliferative lupus nephritis was the most common histological type, 48% of all patients had class IV glomerulonephritis, which needs the most aggressive immunosuppressive treatment. Our data are similar to other studies Bono et al. (1999), Martins et al. (2002) and Brugos et al. (2006), they had similar study populations. All patients received methylprednisolon, most of patients with proliferative lupus nephritis were treated either with cyclophosphamide or mycophenolate mofetil. In patients with refractory symptoms newer therapies as B-cell depleting (rituximab) therapies were used.

In patients with lupus nephritis the most common symptoms were antinuclear factor positivity and erosive arthritis. Erythrocyte sedimentation (ESR) rate of patients correlated to activity of lupus nephritis, moreover we found a positive correlation between ESR and UTp/creatinine levels ($p=0.002$, $R=0.52$). Mean UTp/creatinine- and anti-DNA (activity marker) level of patients with active LN was significantly higher.

The most aggressive immunosuppressive treatments, moreover use of newer biological therapies is needed in rapid proliferative forms of lupus nephritis (III and IV). The difference between laboratory parameters, clinical signs of patients with active or

inactive lupus nephritis could help us to decide the optimal therapeutic regimen to treat patients with lupus nephritis.

4. Experimental

Patients included in this study fulfilled four or more criteria of the American College of Rheumatology for the classification of SLE (Hochberg 1997), diagnosed, treated and followed-up by the Department of Internal Medicine, Division of Clinical Immunology, University of Debrecen, Hungary. Patient's clinical data, laboratory tests, 24 hour collected urine and UTp/creatinine ratio, classical activity markers and treatment regimen were analyzed.

All statistical analyses were carried out using SPSS program, Version 13.0, and values were expressed as the mean \pm SD. The significance of differences between laboratory tests of patients with active and inactive disease was assessed using the Mann-Whitney test. Correlation analysis was made using Pearson's parametric and Spearman's nonparametric tests. P values less than 0.05 were considered statistically significant.

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