Original Paper

The Outcome of Patients with Lupus Nephritis and the Impact of Cardiovascular Risk Factors

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ABSTRACT Background: Systemic lupus erythematosus (SLE) is the prototype of autoimmune connective tissue diseases. Renal disease is a frequent manifestation of SLE that influences the outcome of the patients. **The aim** of the current study was to determine and analyze the clinical features and subsequent outcome of 70 patients with LN, followed in our department over the past 5 years, focusing on the impact of cardiovascular risk factors in the renal outcome and mortality. **Patients and methods:** Our prospective study included 70 patients with SLE and LN and 70 patients with SLE without signs of renal involvement, all patients fulfilled the revised ACR (American College of Rheumatology) criteria for the classification of SLE. Demographical data, risk factors and comorbidities were recorded. **Results:** Patients with lupus nephritis had a mean age of 37 years (range 15-65, SD 1.8). During the study, we had a rate of drop off of 15 patients with lupus nephritis (21%) and 19 patients without nephritis (26%). Patients with LN had a higher prevalence of positive anti-dsDNA antibodies (85.4% vs 49%, p<0.001, RR=2.2) and a lower percent of rheumatoid factor (FR) positive (5.45% vs 15.68%, p=0.03, RR=0.34) compared with the controls, a higher prevalence of corticosteroid treatment (65.45% vs 7.83%, p<0.001, RR=2.1) and immunosuppressive treatment (AZA 27.27% vs 3.92%, p=0.01, RR=1.71, CFM 34.54% vs 0%, p<0.001, RR=2.16), a higher frequency of hypertension (47.27% vs 9.8%, p<0.001, RR=2.4), hyperlipidaemia (49.09% vs 1.96%, p<0.001, RR=1.81) and anti-PL antibodies (49.09% vs 20%, p=0.001, RR=2.70),and a higher mortality (16% vs 2%, p=0.02, RR=1.76). 20 patients (36.36%) from the survival group (55 patients), evoluated to renal failure, 9.09% of these with end -stage renal failure, results that are similar with the ones in other studies. **Conclusions:** The study reveals the fact that cardiovascular risk factors such as hypertension, hyperlipidaemia and antiphospholipid syndrome are associated with a higer rate of mortality and an evolution to end-stage renal disease.

KEY WORDS *systemic lupus erythematosus, renal involvement, cardiovascular risk factors, anti phospholipid antibodies.*

Introduction

Systemic lupus eythematosus (SLE) is an autoimmune pathology which due to the diversity of its clinical and immunological manifestations represents the prototype of autoimmune connective tissue diseases. It can virtually involve any self structure of the body and exhibits a large spectrum of clinical manifestations including cutaneous and joint disease, renal disease, haematological involvement and central nervous system disease (1, 2, 3).

Renal disease represents a frequent manifestation of SLE as well as an important outcome predictor in these patients. Although pathologically, the majority of patients with SLE may present a degree of renal involvement (glomerulopathy), a clinically relevant kidney disease occurs in about 50% of patients, mostly the consequence of the deposition or in situ formation of immune complexes containing anti-DNA in the kidney. As expected, the mortality has higher rates in patients with lupus nephritis (LN) than in those without renal disease, and some of these (10-60%) can develop end-stage renal failure that requires the substitution of the renal function.

The appropriate use of corticosteroids and newer immunosuppressive agents with a judicious application of the current guidelines in patients with LN had a pivotal role in increasing the survival rate of these patients for up to 80% at 10 years, but unfortunately, the exposure to these drugs predispose to several late complications (4, 5, 6, 7),

Study objective

The aim of the current study was to determine and analyze the clinical features and subsequent outcome of 70 patients with LN, followed in our department over the past 5 years, focusing on the impact of cardiovascular risk factors in the renal outcome and mortality.

Methods

Our prospective study included 70 patients with SLE and LN and 70 patients with SLE without signs of renal involvement, from 2004

until 2009. At study enrolment, all patients signed the informed consent and fulfilled the revised ACR (American College of Rheumatology) criteria for the classification of SLE.

Also, demographical data, risk factors and co-morbidities were recorded, regarding sex, age, smoking habit, menopausal status, the presence of metabolic disease - diabetes, hyperlipidaemia and hypertension.

Hypertension was defined according to ESC guidelines as blood pressure >140/90mmHg in two consecutive determinations. Patients with defined hypertension were under pressure lowering medication such as angiotensin-converting-enzyme inhibitors or angiotensin receptor antagonists, calcium-channel blockers, adding, if necessary, diuretics or beta-blockers.

The following parameters were considered in the evaluation of the renal status: normal renal function defined as a plasma creatinine <1,1mg/dl, proteinuria appreciated as of nephrotic range when urinary protein excretion exceeded 3g/day and non-nephrotic when it was between 0.2-3g/day, altered renal sediment considered when >3 red blood cells or >5 white cells or when any casts (granular, tubular, red cell or mixed) were observed per field.

Renal biopsy specimens were interpreted according to the WHO (World Health Organization) classification of lupus nephritis: normal or minimal changes lupus nephritis (class I), mesangial prolipherative nephritis (class II), focal proliferative (class III), diffuse proliferative (class IV), membranous nephritis (class V) advanced sclerosing glomerulonephritis (class VI) (8). Patients without renal disease were considered those ones with a normal creatinine value, a proteinuria <0.2g/day and an inactive urine sediment. The outcome parameters evaluated were renal function/failure, end-stage renal failure and death.

The immunological profile included the most relevant autoantibodies for patients with SLE and included the determination of antinuclear antibodies (ANA) by indirect immunofluorescence, measurement of anti-dsDNA antibodies by Farr's techique, anti-Sm antibodies, anti-RNP antibodies, anti-Ro/SSA and anti-La/SSB antibodies by immunelectrophoresis and rheumatoid factor (FR) by latex fixation or Waaler-Rose tests. Complement factors (C3 and C4) were estimated by the nephelometry, IgG and IgM anticardiolipine antibodies were determined by ELISA technique and the lupus anticoagulant (LA) was determined by coagulation assays (prothrombin time, activated partial TP time, Russel's time).

The statistical analysis included the use of $\varkappa^2$ test and Fisher's exact test to analise qualitative differences, Student's test for the comparison of means and the non-parametric Mann-Whitney test. A value of $p<0.05$ was considered to indicate the statistical significance.

Results

General characteristics of all patients with lupus nephrirtis. We enrolled seventy patients with lupus nephritis, with a mean age of 37 years (range 15-65 years, SD1.8) and recorded signs of renal involvement as follows: an altered urine sediment in 28 (40%) patients, nephrotic syndrome in 24 patients (34%), renal failure in 8 (12%), and other features of renal disease in 10 patients (14%). Renal tissue was obtained in 63 (90%) patients and showed nephritis class I in 5 (8%) patients, class V in 8 (13%) patients, class II in 13 (20%), class III in 16 (25%) and class IV in 21 patients (34%) patients. At the begining of the study none of the patients had nephritis class VI.

27 patients, with severe disease, recevied immunosuppresive agents and corticosteroids: cyclophosphamide (CFM) 1.5mg/kgc/day in 17 patients (15 intravenously and 2 orally) and azathioprine (AZA) 2mg/kgc/day in 12 patients; 2 patients recevied both therapies. CFM was administrated monthly 6 months and every 3 months during the following 1.5 years. AZA was administrated for 2 years. 32patients recevied oral prednisone (>0.5mg/kgc/day) alone.

Comparative analysis of SLE patients with LN and without renal involvement. During the study we had a frequency of drop off of 15 (21%) patients from the LN group and 19 (26%) from the group without nephritis, so to the end we compared 55 patients with lupic nephritis and 51 patients without renal disease. The comparative analysis was done recording their clinical and immunological features (autoantibody profile), cardiovascular risk factors (smoking, hypertension, diabetes mellitus, hyperlipidaemia) and therapy (corticosteroids, immunosupressive therapy). Extensive data were presented in Table 1.

The analysis of the two groups showed in patients with lupus nephritis a significant statistical higher prevalence of anti-dsDNA antibodies (85.4% vs 49%, $p<0.001$, RR=2.2) thus being known their pathogenic role and the correlation with renal disease, as well as a lower prevalence of FR (5.45% vs 15.68%, $p=0.03$, RR=0.34).

Table 1: Demographic, immunological characteristics, risk factors and therapy in SLE patients with/without LN

Characteristics	SLE with LN n=55	SLE without LN n=51	Statistic p
Gender (females)	50 (90.9%)	47	-
Age	37.2± 1.8	36.9±1.8	-
Anti-dsDNA ab (>10U/l)	47 (85.4%)	25 (49%)	<0.001
RF	3 (5.45%)	8 (15.68%)	0.04
Anti-RNP ab	7 (12.72%)	9 (17.64%)	-
Anti-Ro/SS-A ab	17 (30.90%)	8 (15.68%)	-
Anti-La/SS-B ab	5 (10.90%)	4 (7.83%)	-
Anti-Sm ab	8(14.54%)	9 (17.64%)	-
Low C3	33 (60%)	19 (37.25%)	-
Low C4	33 (60%)	25 (49%)	-
Corticosteroids >0.5mg/kg/day	36(65.45%)	4 (7.83%)	<0.001
Azathioprine	15 (27.27%)	2 (3.92%)	0.01
Ciclophosphamide	19 (34.54%)	0 (0%)	<0.001
Hypertension	26 (47.27%)	5 (9.8%)	<0.001
Diabetes mellitus	3 (5.45%)	0 (0%)	-
Hyperlipidaemia	27 (49.09%)	1 (1.96%)	<0.001
Smoking	5(10.90%)	1 (1.96%)	-
Menopause	8(14.54%)	9 (17.64%)	-
Anti-PL ab	26 (49.09%)	11(20%)	0.01

Table 2: Clinical and histological features of the patients with LN who develop renal failure, compared with those with normal renal function

Characteristics	Normal renal function n=35	Renal failure n=20	Statistic p
Gender (female)	32 (91.42%)	20(100%)	-
Age	36.7±2.1	37.5±1.9	-
Initial renal failure	3 (8.57%)	3 (25%)	-
Nephrotic syndrome	12 (34.28%)	11 (55%)	-
Altered urine sediment	37 (56.75%)	4 (20%)	0.010
WHO class I	0 (0%)	1 (5%)	-
WHO class II	14 (40%)	2 (10%)	0.016
WHO class III	8 (22.85%)	5 (25%)	-
WHO class IV	8 (22.85%)	10 (50%)	0.017
WHO class V	5 (14.28%)	2 (10.%)	-
WHO class VI	0 (0%)	0 (0%)	-
Corticosteroids >0.5mg/kg/day	20 (57.14%)	14 (70%)	-
Cyclophosphamide	10 (28.57%)	8 (40%)	-
Azathioprine	4 (11.42%)	9 (45%)	<0.001
Diabetes mellitus	1 (2.85%)	2 (10%)	-
Hypertension	14 (40%)	13 (65%)	0.001
Hyperlipidaemia	11 (31.42%)	15 (75%)	<0.001
Menopause	5 (14.28%)	3 (15%)	-
Smoking	1 (2.85%)	3 (15%)	-
Antiphospholipid antibodies	17 (48.57%)	9 (45%)	-

Regarding treatment administration in the studied patients, those with renal disease showed a higher prevalence of treatment with corticosteroids (65.45% vs 7.83%, p<0.001, RR=2.1) and immunosupressive treatment (azathioprine 27.27% vs 3.92%, p=0.01, RR=1.71 and cyclophosphamide 34.54% vs 0%, p<0.001, RR=2.16).

As for risk factors identified in patients with LN a higher prevalence of hypertension (47.27% vs 9.8%, p<0.001, RR=2.4), hyperlipidaemia (49.09% vs 1.96%, p<0.001, RR=1.81) and anti-PL antibodies (49.09% vs 20%, p=0.001, RR=2.70), while no significant differences were noticed between the two groups considering the menopausal status, the somking habit and the association of diabetes mellitus; in this group was a higher percentage of infections (47% vs 18%, p=0.001, RR=1.74) and a higher mortality (16% vs 2%, p=0.02, RR=1.76).

Renal outcome. At the last visit, 35 patients with LN (63.63%) had normal values of plasma creatinine, 20 (36.36%) had renal failure, of whom 5 (9.09%) had end-stage renal failure.

Patients with normal renal function had a higher prevalence of altered urinary sediment (56.75% vs 20 %, p=0.002, RR=1.58) and of nephritis class II (40% vs 10%, p=0.02, RR=1.59).

Patients that developed renal failure had a higher prevalence of nephritis class IV (50% vs 22.85%, p=0.02, RR=2.44). Patients treated with AZA had a higher risk to develop renal failure (45% vs 11.42%, p<0.001, RR=2.58). We also analysed if the presence of certain cardiovascular risk factors may predict progression to renal failure; the patients with hypertension (65% vs 40%, p<0.01, RR=2.58) and with hyperlipidemia (75% vs 31.42%, p<0.001, RR=4.52) had a higher prevalence of renal failure.

Finally, patients that developed renal failure had more infections 72% vs 35%, p=0.009, RR=2.90) and had a higher mortality (33% vs 8%, p=0.02, RR=2.56).

Mortality. One of the characterising parameters with a high relevance is mortality. 9 (16%) of the patients with LN died during the study, compared with 1 (2%) patient without LN (p=0.01, RR=2.51).The registered causes of death in the nine patients with were mostly vascular events (cardiovascular or cerebrovascular) in 6 patients (and interestringly, four of them had positive antiphospholipid antibodies), sepsis in 2 patients and ovarian cancer in one patient. The singular event in the group without renal disease was also due to a cardiovascular event.

In LN patients, mortality correlated with renal function at the last visit; 3(8.57%) of the 35 patients with normal renal function and 6 (30%) of the 20 who developed renal failure died (p=0.003, RR=4.11). We analysed the presence of certain features at follow-up that may predict mortality; the patients treated with AZA had a higher mortality (56% vs 37%,p=0.02, RR=4.48). We also analysed the presence of cardiovascular risk factors that can predict mortality. The presence of hyperlipidemia (66.66% vs 37%, p=0.03, RR=4.52) was correlated with a higher mortality.

Disscution

In the current study, the outcome of patients with lupus nephritis has been assessed, taking into consideration several variables, such as the variety of clinical manifestations, the histological pattern difficult to analyze, and the heterogeneity due to due to different risk factors or therapies that are used.

In this study, we analyzed the outcome of 70 patients with NL followed prospectively for a period of five years. 20 (36.36%) of the survival patients (55) progressed to renal failure, and 9.09% of these progressed to end-stage renal disease Table 3); these results are similar to the ones in other studies (9, 10).

Table 3: Clinical and histological features, cardiovascular risk factors and therapy at LN patients who died compared with living patients

Characteristics	Survival n=46	Death n=9	p
Gender (female)	44(95.65%)	7 (78%)	-
Age	36.7±1.8	34.9±2.2	-
SLE duration (months)	69.5±11.01	74.51±39.5	+
Initial renal failure	9 (19.56%)	0(0%)	-
Nephrotic syndorme	18 (39%)	3(33.33%)	-
Altered urine sediment	19 (41.30%)	5 (55.55%)	-
WHO class I	2 (4.34%)	0 (0%)	-
WHO class II	12 (26.08%)	3(33.33%)	-
WHO class III	11 (23.91%)	2 (22%)	-
OMS clasa IV	16 (34.78%)	2 (22%)	-
WHO class V	5 (11%)	2 (22%)	-
WHO class VI	0 (0%)	0 (0%)	-
Corticosteroids>0.5mg/kg/day	25 (56%)	5 (55.55%)	-
Cyclophosphamide	15 (33%)	1 (11%)	-
Azathioprine	8 (17.39%)	5 (56%)	=0.02
Diabetes mellitus	3 (6.52%)	0 (0%)	-
Hypertension	20 (43.47%)	3 (33,33%)	=0.02
Hyperlipidaemia	16 (36.08%)	6 (66.66%)	<0.001
Menopause	6 (13%)	3 (33.33%)	-
Smoking	3 (6.52%)	1 (11%)	-
Antiphospholipid antibodies	21 (45.65%)	6 (66.66%)	-

Using immunosuppressive therapy with azathioprine and cyclophosphamide as well as considerate dosage of corticosteroids allows preservation of a normal renal function for at least 5 years (Table 2) in more than half of the patients (67.27%).

The impact of several demographic factors (sex, age), was also assessed, as they were not found to be important for the outcome of renal function. The histological class represents maybe the most important factor in predicting the evolution to renal failure, as almost **1/3** of the patients with histological classes III, IV, V ultimately develop renal failure, compared with classes I and II, confirming the existing data from literature that confirm the useful predictive value of histological examination. We also observed a low prevalence of FR at the patients included in the study (Table 1, 2).

Our data shows that potentially risk factors such as hypertension and hyperlipidaemia as well as anti-PL antibodies or iatrogenic factors (corticosteroid theraphy), are associated with renal outcome and mortality in patient with renal disease with a higher prevalence of the above mentioned risk factors, compared with SLE patients without renal involvement.

Also, hypertension and hyperlipidaemia had a higher prevalence in LN patients who developed renal failure, 65% respectively 75%, as well as in the ones that died with renal involvement, 33% respectively 66%), confirming that the cardiovascular disease is one of the main causes of morbidity and mortality in SLE patients with LN. Hypertension and hyperlipidaemia have already been identified as important risk factors associated with atherosclerosis and coronary artery events in several previous SLE studies (11).

It is already also proven by several studies that corticosteroids and immunosuppressive therapy increase the risk of cardiovascular disease in patients with SLE, which is now the most important cause of mortality (12, 13, 14, 15) together with infection.

Conclusions

Our study focuses on shows that in patients with lupus nephritis hypertension, hyperlipidaemia and antiphospholipid syndrome are important risk factors associated with a higher mortality rate and with the development of renal failure even if they maintain a quite stable renal function over 5 years. In this direction, a tight control of the cardiovascular risk factors may be useful, with appropiate dietetic measures, smoking cessation, antihypertensive and antihyperlipidaemic treatment, using therapeutic drugs that protect the renal function, using lowest doses of corticosteroids, identifying antiphospholipidic syndrome with a proper treatment (16 - 20).

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