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Changing patterns in clinical-histological presentation and renal outcome over the last five decades in a cohort of 499 patients with lupus nephritis.

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Objectives. To evaluate changes in demographic, clinical and histological presentation, and prognosis of lupus nephritis (LN) over time.

Patients and Methods. We studied a multicentre cohort of 499 patients diagnosed with LN from 1970 to 2016. The 46-year follow-up was subdivided into three periods (P): P1 1970-1985; P2 1986-2001; P3 2002-2016, and patients accordingly grouped based on the year of LN diagnosis. Predictors of patient and renal survival were investigated by univariate and multivariate proportional hazards Cox regression analyses. Survival curves were compared using the log-rank test.

Results. A progressive increase in patient age at the time of LN diagnosis ($p<0.0001$) and a longer time between SLE onset and LN occurrence ($p<0.0001$) was observed from 1970 to 2016. During the same period, the frequency of renal insufficiency at the time of LN presentation progressively decreased ($p<0.0001$) and that of isolated urinary abnormalities increased ($p<0.0001$). No changes in histological class and activity index were observed, while chronicity index significantly decreased from 1970 to 2016 ($p=0.023$). Survival without end-stage renal disease (ESRD) was 87% in P1, 94% in P2 and 99% in P3 at 10 years, 80% in P1 and 90% in P2 at 20 years ($p=0.0019$). At multivariate analysis, male gender, arterial hypertension, absence of maintenance immunosuppressive therapy, increased serum creatinine, and high activity and chronicity index were independent predictors of ESRD.

Conclusions. Clinical presentation of LN has become less severe in the last years, leading to a better long-term renal survival.

Keywords: Lupus nephritis, Systemic Lupus Erythematosus, Research outcomes, Treatment

INTRODUCTION

Lupus nephritis (LN) is a frequent and severe manifestation of systemic lupus erythematosus (SLE) and is characterized by a relapsing and remitting clinical course.[1-4] Renal involvement occurs at the time of SLE diagnosis or during the course of the disease in up to two thirds of patients.[5-6] Clinical presentation varies from asymptomatic urinary abnormalities to chronic irreversible renal insufficiency.[7] Although renal involvement is still considered a strong predictor of death and end-stage renal disease (ESRD),[8,9] both patient and renal survival have significantly improved in the last few decades [10-13] and the rate of renal flares has considerably decreased over time as well.[3] The improvement in LN prognosis has been attributed to many factors including the better understanding of SLE pathogenesis, new treatment options and strategies, and improved management of hypertension, infections and other co-morbidities.[14]

To the best of our knowledge, no studies have evaluated whether changes in demographic, clinical and histological features at the time of LN presentation have occurred over the last decades and whether these changes have had an influence on the disease management and outcome.

The objective of our study was to examine, the changes in demographic, clinical and histological features at the time of LN onset in a large cohort of patients during a 46-year follow-up. We looked at changes in LN prognosis during the course of the follow-up and searched for the prognostic factors associated with patient and renal outcomes.

PATIENTS AND METHODS

Four hundred and ninety-nine patients were included in this retrospective study of prospectively collected data. Inclusion criteria were: ACR criteria-based diagnosis of SLE [15] and biopsy proven LN performed between January 1970 and December 2016. Patients were followed in four Italian referral centres: Renal Divisions of Ospedale Maggiore Milano, San Carlo Hospital Milano, and University of Parma, and Rheumatology Unit of Padova University. Since the eighties, according to the good clinical practice, patients undergoing renal biopsy in Italy signed informed consent that includes the consent for using clinical data for scientific purposes, while in previous years no

consent was required for this type of studies. The study was approved by the local Ethics Committees. The 46-year follow-up was subdivided into three periods (P), 15 years each: P1 from January 1970 to December 1985, P2 from January 1986 to December 2001, and P3 from January 2002 to December 2016, and patients accordingly grouped based on the year of LN diagnosis. Detailed data on the source population and study design are reported in Supplementary Text S1 and Supplementary Table S1.

All patients received a renal biopsy which was classified according to the International Society of Nephrology/Renal Pathology Society (IRS/RPS) classification criteria [16]. Since 2003 all renal biopsies performed before 2002 were reclassified according to the same IRS/RPS classification criteria by the clinicians and pathologists based on written reports of light microscopy and immunofluorescence or the re-evaluation of slides, where necessary. Activity and chronicity indices were calculated according to the score proposed by Austin et al.[17] Estimated glomerular filtration rate (eGFR) was calculated according to the Cockcroft and Gault formula based on gender, serum creatinine, age and body weight of the patients. Normal renal function was defined as serum creatinine ≤1mg/dl and eGFR >60ml/min that correspond to the definition of CKD 1 and 2. Proteinuria was measured by benzethonium chloride on the urine collected over 24 hours expressed as g/24 hours. Arterial hypertension was defined as the mean of three consecutive measurements of systolic blood pressure >140 mm/Hg and/ or diastolic blood pressure >90 mm/Hg in sitting position.

Definitions

Clinical syndromes at presentation were defined as follows:

- *Isolated urinary abnormalities*: normal renal function, proteinuria < 3.5 g/24 hours and >0.5g/24 hours, and/or microscopic haematuria (urinary red blood cells >5/high power field (HPF) after having excluded non-renal causes;

- *Nephrotic syndrome*: normal renal function, proteinuria ≥3.5 g/24h, and serum albumin <3.5g/dl;

- *Acute nephritic syndrome*: acute renal dysfunction (serum creatinine >1 mg/dl and eGFR <60ml/min), macroscopic or severe microscopic haematuria (urinary red blood cells >20/HPF), and/or erythrocytes casts, arterial hypertension and variables degrees of proteinuria;

- *Rapidly progressive renal insufficiency*: rapid deterioration of renal function leading to CKD stage 3 to 5 within a few weeks, with oliguria, arterial hypertension and severe haematuria. Renal states at last observation were defined as follows: *Complete renal remission*, serum creatinine <1mg/dl with eGFR≥60 ml/min, proteinuria <0.5g/day, and inactive urinary sediment; *Partial renal remission*, serum creatinine <1mg/dl with eGFR ≥60 ml/min, and proteinuria <3.5g/day and ≥0.5g/day; *CKD*, serum creatinine >1.0mg/dl with eGFR <60ml/min and inactive urinary sediment, confirmed by at least three determinations; *ESRD*, the need of renal replacement therapy; *Poor renal outcome*, CKD or ESRD.

Statistical analysis

Mean±standard deviation (SD) or median and interquartile range (IQR) were used for descriptive statistics, according to variable distribution. Temporal trends of clinical parameters were tested through Pearson or Spearman correlation analysis, according to parametric or non-parametric variable distribution. Survival curves were drawn using the Kaplan-Meier estimate and compared using the log-rank test. Univariate and multivariate proportional hazards Cox regression analyses were used to investigate the prognostic value of continuous and binary (dichotomised) variables. Patients lost to follow-up were 2/106 (1.9%) in P1, 6/158 (3.8%) in P2, and 13/235 (5.5%) in P3. These low numbers of patients and the lack of a significant clinical deterioration at their last available follow-up suggest that censoring due to lost to follow-up was likely to be minimal and non-informative. The statistical package S-Plus was used to analyse sample data.[18]

RESULTS

Demographic characteristics

Four hundred and ninety-nine patients (427 women, 85.6%) were included in the study; they were followed for a median period of 10.6 years (IQR 4-18). All but 51 (10.2%) patients were Caucasian.

Demographic, clinical and histological features of the cohort at the time of LN diagnosis are reported in Table 1. The cohort was subdivided into three groups according to the year of LN diagnosis: group 1 included 106 patients (21%) diagnosed with LN in P1; group 2 encompassed 158 patients (32%) diagnosed with LN in P2; group 3 comprised 235 patients (47%) diagnosed with LN in P3.

Table 1. Clinical features at the time of lupus nephritis diagnosis in all patients and according to the three different periods

	Overall 499 patients	Period 1 106 patients	Period 2 158 patients	Period 3 235 patients	P value
Gender, Female, N. (%)	427 (85.6)	99 (93.4)	139 (88)	189 (80.4)	0.004
Age at SLE diagnosis, years	28.11±12.0	27±10.3	26.3±11.2	29.8±13	0.01
Age at LN diagnosis, years	31.4±12.5	28.4±10.4	29±11.5	34.4±13.3	0.001
Disease duration before LN diagnosis, years	3.3±5.3	1.3±1.3	2.6±4.5	4.6±6.3	<0.0001
Follow-up duration, years	12.7±9.8	20.5±13	15.8±7.8	6.8±4.3	
Weight, Kg	61.7±12.2	57.4±10.4	62±11.2	63.3±13.1	ns
Hypertension, N. (%)	240 (48.2%)	56 (52.8%)	77 (48.7%)	107 (45.9%)	ns
Serum creatinine, mg/dl	1.2±1.1	1.8±1.8	1.2±0.8	1.0±0.7	<0.0001
Creatinine clearance, ml/min	86.3±41	72.2±45.1	83.7±36.6	94.1±40.2	0.0001
Proteinuria, g/24h	4.1±3.7	3.6±2.7	4.5±4.0	4.1±3.9	ns
Urinary erythrocytes /HPF	27.7±45.7	18.6±18.6	24.2±24.3	34.1±61.9	0.01
Serum albumin, g/dl	3.0±0.7	2.7±0.7	3.0±0.7	3±0.7	0.005
Haematocrit, %	33.5±6.2	33.3±7.3	33.8±5.5	33.4±6	ns
White blood cells /10^3/mL	6252±3223	6258±2842	6180±2888	6299±3603	ns
Platelets /10^3/mL	240302±96198	230422±103282	252193±97365	236641±91640	ns
C3, mg/dl	62.1±25.4	65.1±22.6	58.7±25.4	63.1±26.3	ns
C4, mg/dl	13.7±14.3	20.7±20.2	14.7±15.8	10.2±8	0.001
Anti-dsDNA, positive N. (%) [NA 25]	414 (87.3)	82 (93.6)	128 (85.3)	204 (90.3)	ns
Urinary abnormalities Nephrotic syndrome Nephritic syndrome Rapidly progressive renal insufficiency	203 (40.7) 174 (34.9) 92 (18.4) 30 (9.0)	28 (26.4) 32 (30.2) 31 (29.2) 15 (14.2)	60 (38) 59 (37.3) 32 (20.3) 7 (3.9)	115 (48.9) 83 (35.4) 29 (12.4) 8 (3.4)	<0.0001 n.s. 0.0001 <0.0001
Histological classes, N. (%) II III* IV* V VI	 22(4.4) 115 (23.1) 267 (53.7) 93 (18.7) 2 (0.4)	 5 (4.8) 23 (21.9) 56 (53.3) 21 (20) 1(0.9)	 4 (2.5) 28 (17.8) 91 (58) 34 (21.7) 1 (0.6)	 13 (5.5) 64 (27.2) 120 (51.1) 38 (16.2) 0 (0)	 ns ns ns ns ns
Activity index	6.4±4.9	6.2±4.9	6.6±4.9	5.9±4.5	Ns
Chronicity index	2.0±2.2	2.6±2.5	2.0±2.2	1.6±2	0.0023

Period 1: 1970-1985; Period 2: 1986-2001; Period 3: 2002-2016

LN, lupus nephritis; SLE, systemic lupus erythematosus; N., number; HPF, High power field; ; C3/C4, complement components; aPL, antiphospholipid antibodies; CKD, chronic renal insufficiency; ESRD, end stage renal disease; NA, not available; ns, non significant

*Class III+V: Overall, 4 patients; P1, 3 patients; P2, 1 patient, P3, no cases. Class IV+V: Overall, 31 patients; P1, 2 patients, P2, 8 patients; P3, 21 patients.

P values refers to T-test, Kruskal-Wallis test or chi-square test (with 2 degrees of freedom), according to the type and distribution of variables.

The number of male patients progressively increased over the three periods: 6.6% in P1, 12% in P2, and 19.6% in P3 (p=0.004). The lag-time between SLE and LN diagnosis (p<0.0001) progressively increased from 1970 to 2016 The mean age at the time of LN occurrence increased from 28.4±10.4 in P1, to 29±11.5 in P2, and to 34.4±13.3 in P3 (p<0.001)

Clinical and histological presentation

The mean values of serum creatinine progressively decreased overtime: 1.8±1.8 mg/dl in P1, 1.2±0.8 mg/dl in P2, 1.0±0.7 mg/dl in P3 (p<0.0001). Consistently, a significant decrease in the frequency of acute nephritic syndrome (p=0.0001) and rapidly progressive renal insufficiency (p=0.0001) was observed, together with a significant increase in the prevalence of isolated urinary abnormalities from the first to the third period (p<0.001) (Figure 1A). The rate of nephrotic syndrome presentation was similar in the three periods. Creatinine serum levels, eGFR, proteinuria and urinary red blood cells in patients with the different clinical syndromes at the time of LN diagnosis by the three periods are reported in Supplementary Table S2.

No differences in the percentage of histological classes in the three periods were observed (Table 1 and Figure 1B). Interestingly, an increase in mixed forms (class III+IV and IV+V) from P1 (4.7% of cases) to P2 (12.6%) and P3 (17.4%) (p=0.006) was noted. Activity index did not significantly change over the three periods either when all the classes were considered (Table 1) or when patients with class III (4.95±2.9 in P1, 5.6±3.1 in P2 and 5.9±4.5 in P3, p=ns) and class IV (9.4±4.9 in P1, 9.4±3.7 in P2 and 9.4±3.8 in P3, p=ns) were separately analysed. Conversely, chronicity index significantly decreased (p=0.0023) from P1 to P3 (Table 1).

Treatment

More than 2/3 of patients in each period were treated with methylprednisolone pulses as induction therapy. In P1, 29% of patients received corticosteroids alone for induction therapy in comparison to 17.9% in P2 and 5.4% in P3 (p<0.0001). Immunosuppressive drugs were added to corticosteroids for maintenance therapy in 30.5% of patients in P1, 65.5% in P2 and 89.1% in P3 (p<0.0001). The immunosuppressive drugs used in induction and maintenance therapy during the three periods are reported in Table 2. More than 50% of patients in each period received cyclophosphamide as induction therapy (Supplementary Table S3). A decrease in the use of azathioprine as induction therapy from P1 to P3 was counterbalanced by an increase in the use of mycophenolate mofetil (MMF). As far as maintenance therapy is concerned, the proportion of patients receiving azathioprine remained stable in the first two periods and decreased in the 3rd period (p<0.0001), while MMF use significantly increased in the last period compared with the previous ones (p<0.0001). Notably, the proportion of patients who were not treated with induction therapies progressively decreased over time (p<0.0001).

Table 2. Induction and maintenance therapy, and outcomes in all patients and according to the three different periods.

	Overall 499 patients	Period 1 106 patients	Period 2 158 patients	Period 3 235 patients	P value
Methylprednisolone pulses, N. (%)	351 (70.3)	63 (67.7)	120 (83.9)	168 (73.7)	0.01
Immunosuppressive drugs, induction					
None, N. (%)	66 (13.2)	28 (29)	26 (17.9)	12 (5.4)	<0.0001
Cyclophosphamide, N. (%)	258 (51.7)	49 (51)	95 (65.5)	114 (51.3)	0.016
Azathioprine, N. (%)	42 (8.4)	15 (15.6)	18 (12.4)	9 (4.0)	<0.0001
Mycophenolate, N. (%)	79 (15.8)	0	4 (2.7)	75 (33.8)	<0.0001
Others*, N. (%)	17 (3.4)	3 (3.1)	2 (1.4)	12 (5.4)	ns
Immunosuppressive drugs, maintenance					
None, N. (%)	140 (28)	66 (68.7)	50 (34)	24 (10.9)	<0.0001
Cyclophosphamide, N. (%)	7 (1.4)	1 (1)	5 (3.4)	1 (0.45)	ns
Azathioprine, N. (%)	152 (30.4)	27 (28)	58 (39)	67 (30.6)	ns
Mycophenolate, N. (%)	143 (28.6)	1 (1)	22 (15.1)	120 (54.8)	<0.0001
Others*, N. (%)	18 (3.6)	0	11 (7.5)	7 (3.2)	Ns
Outcomes #					
Partial renal remission, N. (%)	122 (25.5)	7 (6.9)	43 (28.1)	72 (32.1)	<0.0001
Complete renal remission, N. (%)	246 (51.4)	41 (49.6)	74 (48.4)	131 (58.5)	0.01
CKD, N. (%)	31 (6.4)	8 (7.9)	13 (8.5)	10 (4.5)	<0.0001
ESRD, N. (%)	42 (8.8)	25 (24.8)	14 (9.1)	3 (1.3)	<0.0001
Death, N. (%)	37 (7.7)	20 (19.8)	9 (5.9)	8 (3.6)	<0.0001

Period 1: 1970-1985; Period 2: 1986-2001; Period 3: 2002-2016

CKD, chronic kidney disease; ESRD, end stage renal disease.
* "Others" includes cyclosporin A, methotrexate, rituximab.
[#] Outcome was available in 478 patients (P1, 101 patients; P2, 153 patients; P3, 224 patients)
P values refers to chi-square test with 2 degrees of freedom.

Renal outcome and predictors of renal survival

Outcome was available in 478 patients (95.8%) (Table 2). At last observation, complete renal remission was observed in 49.6% of patients in P1, 48.4% in P2 and 58.5% in P3 (p=0.01) (Table 2). CKD and ESRD occurred in 7.9% and 24.8% of patients in P1, in 8.5% and 9.1% in P2 and in 4.5% and in 1.3% in P3, respectively (p<0.0001 for all comparisons). Twenty patients in P1 died (19.8%), in comparison with 9 (5.9%) in P2 and 8 (3.6%) in P3 (p<0.0001). The CKD free survival at 10 and at 20 years was 75% and 66% in P1, 85.5% and 80.2% in P2 and 91.5% in P3, respectively (p=0.0069) (Figure 2A). The ESRD free survival at 10 and at 20 years were respectively 87% and 80% in P1, 94% and 90% in P2 and 99% in P3, respectively (p=0.0019) (Figure 2B). Predictors of CKD and ESRD at univariate analyses are reported in Table 3.

Table 3. Univariate Cox proportional Hazard Regression analysis among the clinical characteristics at presentation of lupus nephritis for end stage renal disease and chronic kidney disease

	Univariate analysis ESRD			**Univariate analysis CKD**		
	RR	**95% CI**	**P**	**RR**	**95% CI**	**P**
Year of LN diagnosis	0.941	0.914-0.967	**<0.0001**	0.964	0.945-1.058	**0.00017**
Male gender	1.84	0.810-4.188	0.14	1.53	0.824-2.836	0.18
Age at diagnosis of LN	0.998	0.969-1.027	0.9	1.01	0.987-1.026	0.5
Duration of SLE before diagnosis of LN	0.925	0.835-1.024	0.13	0.961	0.906-1.019	0.19
Histological classes: II+V vs III+IV	3.01	1.067-8.456	**0.037**	1.79	0.987-3.251	0.055
Activity index *	1.15	1.085-1.26	**<0.0001**	1.11	1.065-1.167	**<0.0001**
Chronicity index *	1.39	0.935-1.531	**<0.0001**	1.3	1.197-1.414	**<0.0001**
Urinary abnormalities + Nephrotic syndrome vs Nephritic syndrome + Rapidly progressive renal insufficiency	3.19	2.202-4.620	**<0.0001**	2.35	1.88-2.943	**<0.0001**
Log. Serum creatinine **	5.03	3.52-7.26	**<0.0001**	3.72	2.838-4.838	**<0.0001**
Creatinine clearance	0.967	0.864-1.082	**<0.0001**	0.974	0.967-0.981	**<0.0001**
Proteinuria g/24h	1.04	0.969-1.110	0.28	1.03	0.979-1.083	0.24
Urinary erythrocytes	0.996	0.984-1.008	0.56	1.002	0.997-1.006	0.46
Serum albumin	0.551	0.36-0.84	0.0058	0.716	0.53-0.96	0.026
Arterial hypertension	8.35	3.277-21.177	**<0.0001**	4.15	2.480-6.900	**<0.0001**
Haematocrit	0.91	0.875-0.946	**<0.0001**	0.926	0.899-0.953	**<0.0001**

White blood cells count	1	1.000-1.000	**<0.0001**	1	1.000-1.000	**0.008**
Platelets count	1	1.000-1.000	0.33	1	1.000-1.000	0.07
C3	0.993	0.979-1.005	0.26	0.997	0.988-1.005	0.5
C4	0.998	0.977-0.995	0.8	0.997	0.982-1.011	0.68
Methyprednisolone pulses/oral prednisolone	1.01	0.45-2.26	0.97	0.913	0.530-1.571	0.74
Immunosuppressive induction therapy	2.23	1.079-4.623	**0.03**	0.724	0.420-1.244	0.24
Immunosuppressive maintenance therapy	0.693	0.34-1.41	0.31	0.857	0.53	1.38

* for any unit increase in activity or in chronicity index; ** for any unit increase in log. serum creatinine
ESRD, end stage renal disease; CKD, chronic kidney disease; LN, lupus nephritis; SLE; systemic lupus erythematosus; C3/C4, complement components.

At multivariate analysis, carried out in the entire cohort, several factors at the time of the diagnosis of LN were independently associated with poor renal outcomes (CKD or ESRD) including baseline serum creatinine, high activity and chronicity index, arterial hypertension and the absence of maintenance immunosuppressive therapy (Table 4). In addition, male gender, older age and high serum creatinine were predictors of death (Table 4).

Table 4**.** Predictors of chronic kidney disease, end stage renal disease and death at multivariate Cox proportional Hazard Regression analysis.

	Coefficient	**RR**	**95% CI**	**P value**
Dependent variable: chronic kidney disease				
Logarithm of serum creatinine	0.8708	2.39*	1.57-3.65	<0.0001
Activity index	0.0611	1.06**	1-1.13	0.038
Chronicity index	0.1188	1.13**	1.01-1.26	0.034
Hypertension	1.4243	4.16	2.15-8.03	<0.0001
No immunosuppressive drugs for maintenance	0.7341	2.08	1.14-3.82	0.018
Dependent variable: end stage renal disease				
Logarithm of serum creatinine	1.0001	2.72*	1.5-4.92	0.00095
Male gender	1.2057	3.34	1.25-8.93	0.016
Activity index	0.0936	1.1**	1.02-1.19	0.02
Chronicity index	0.2545	1.29**	1.11-1.49	0.00069
Hypertension	1.7835	5.95	1.99-17.75	0.0014
No immunosuppressive drugs for maintenance	1.1106	3.04	1.37- 6.74	0.0063
Dependent variable: death				
Logarithm of serum creatinine	0.6355	1.8*	1.1-3.25	<0.0001
Male gender	1.0584	2.88	1.17-7.1	<0.0001
Older age	0.0711	1.07***	1.04-1.11	<0.0001

RR, relative risk; CI, confidence interval.
Clinical characteristics at presentation of lupus nephritis were analyzed as independent variables.
* for any unit increase in log. serum creatinine; ** for any unit increase in activity or in chronicity index; *** for any increase in 1 year of age.

DISCUSSION

Our study outlines the most significant changes observed during the last 5 decades in demographic, clinical, and histological features of LN at presentation. These results were drawn from a large multicentric cohort of patients followed in four Italian referral centres from 1970 to 2016. In order to identify changes in LN presentation, the whole observational time was subdivided into three periods, 15 years each.

Historically, from 1970 to 1985 (P1) corticosteroid monotherapy was progressively replaced by combination treatment of corticosteroids with either azathioprine or cyclophosphamide probably due to the results of a pooled analysis that showed the superiority of combined immunosuppressive regimens over corticosteroids alone.[19] Intravenous methylprednisolone pulses were also largely used in this period, following the publication of several papers showing their efficacy in SLE.[20,21] From 1986 to 2001 (P2), high-dose intravenous cyclophosphamide was commonly used as induction and maintenance therapy following the positive results of long-term controlled trials carried out at the National Institutes of Health (NIH).[22] In the same period, the use of a combined oral immunosuppressive regimen as maintenance therapy became progressively more popular.[23] Interestingly, the proportion of our patients who received steroids alone as induction therapy decreased from 29% in P1 to 18% in P2 and further declined to 5% in P3. Finally, from 2002 to 2016 (P3), the evidence that MMF has a similar efficacy compared with cyclophosphamide in the induction phase and is more effective than azathioprine in the maintenance phase led to an increase in the use of MMF for induction as well as for maintenance therapy.[24-26]

The age of our patients at LN diagnosis progressively increased from 1970 to 2016 and LN developed progressively later after the onset of SLE. These changes may result from an earlier diagnosis of SLE, which leads to a closer surveillance of LN over time and, in turn, allows the identification of mild disease phenotypes, as well as from the earlier and more appropriate therapeutic intervention which includes the extensive use of antimalarial drugs,[27,28] MMF,[29,30] and biological drugs,[31-32] capable of hindering the development of LN.

The most interesting and innovative observation of our study is the progressively milder clinical presentation of LN from P1 to P3. Presentation with isolated urinary abnormalities significantly increased from 25% in P1 to about 50% in P3. This finding was accompanied by the progressive decrease in the frequency of renal insufficiency at presentation, whilst the percentage of nephrotic syndrome did not significantly change over time. The decreased severity in clinical presentation from 1970 to 2016 is in keeping with the progressive decline in serum creatinine at the time of LN diagnosis which is one of the most important predictors of renal adverse outcome in short- and long-term follow-up.[33-35]

Nevertheless, the distribution of the renal histological classes was similar in the three periods regardless of clinical presentation. Class IV accounted for more than 50% of cases in all periods, followed by class III in 25%, class V in around 20%, and class II in a minority of patients. There was a significant increase from P1 to P3 in mixed classes (class III+V and class IV+IV) that are considered to be associated with the worst prognosis in some [36-37] but not all studies.[38-39] Activity index remained unchanged from P1 to P3 either when we considered all histological classes or class III and IV separately. These data are consistent with the discrepancy between clinical and histological severity of LN at presentation reported in previous studies.[7] Proliferative forms of LN were observed even in the absence of urinary abnormalities [40-41] suggesting that a certain amount of time is required for histological lesions to give rise to clinical manifestations. On the other hand, the early diagnosis of renal involvement in recent years can account for the lower severity of clinical presentation which is in accordance with the significant progressive decrease in the chronicity index from P1 to P3. Moreover, in the last decades, the indication to renal biopsy has become wider due to the decrease in post-biopsy complications which has led to perform renal biopsy in a number of patients with less severe urinary abnormalities. The increasing number of class III and class IV LN diagnosed with isolated urinary abnormalities, yet with high activity index (unchanged over the three periods), has important implications in clinical practice. Indeed, this result emphasizes once again the importance of renal biopsy in defining the prognosis and tailoring therapeutic approaches

to LN. Notably, high activity and chronicity indexes were independent predictors of ESRD and CKD at multivariate analysis. Due to the decreasing trend of LN presentation with severe renal dysfunction, these histopathological variables remain a valuable tool aiding the physician in defining prognosis and taking treatment decisions in all patients.[42]

Arterial hypertension was another important predictor of both ESRD and CKD.[35,43-45] Around 50% of our patients had arterial hypertension at the time of LN diagnosis and this proportion was similar in the three periods. Thus, the effective control of blood pressure is of paramount importance in the management of LN. In keeping with previous reports,[46-49**]** male gender was associated with worse renal outcome in our cohort; however, according to a recent critical review of the literature there is limited evidence supporting the worse prognosis in male than in female patients.[50]

We observed that the proportion of male patients progressively increased over time but we have no explanation for the increase in number of males diagnosed in last decades and we think that this preliminary result needs to be confirmed in large multicentre studies. Another interesting result of our study is the significant and progressive improvement of renal survival from P1 to P3 which confirms previous data [10-13] and is probably the result of a wider indication to renal biopsy and improved treatment of LN over the last decades.[49] We are aware of a number of limitations of this study. It is a retrospective study of prospectively collected data and no information is provided on the number of patients who achieved remission after induction therapy, the duration of remission, the number of flares and the need of repeated renal biopsy. The majority of our patients were Caucasian hence the results may not be applied to other ethnic groups.

In conclusion, the clinical presentation at the time of kidney biopsy for suspected LN has apparently become less severe in the last years and is now characterized by an increase in isolated urinary abnormalities and a decrease in renal insufficiency. However, a concomitant decrease in histological active lesions was not observed. This emphasizes once again the importance of performing renal biopsy in the management of LN. The progressive improvement in renal survival in our cohort is the result of a comprehensive approach, which includes a prompt diagnosis of renal involvement, a

wider indication to renal biopsy, treatment based on renal biopsy, and increased clinical experience in the management of LN.

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Competing interests

All Authors declare they have no competing interests to report.

Contributorship

Dr Moroni, Prof Doria, and Prof Sinico contributed to the conception and design of the work, interpreted the data, drafted and revised the manuscript for important intellectual content; Dr Quaglini and Dr Sacchi contributed to the statistical analysis; Dr Vercelloni, Dr Gatto, Dr Gianfreda, Dr Costantini, Dr Raffiotta, Dr Zen and Dr Urban followed up patients and contributed to the acquisition of data; Dr Messa, Dr Pieruzzi, and Dr Vaglio critical revised the final work.
All the authors approved the final version of the manuscript and gave their agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data sharing

There are no additional unpublished data from this study to share.

References

1) Ponticelli C, Moroni G. Flares in lupus nephritis: incidence, impact on renal survival and management. *Lupus* 1998;7:635-8.
2) Doria A, Iaccarino L, Ghirardello A, et al. Long-term prognosis and causes of death in systemic lupus erythematosus. *Am J Med* 2006;119:700-6.
3) Yap DYH, Tang C, Ma MKM, et al. Longterm data on disease flares in patients with proliferative lupus nephritis in recent years. *J Rheumatol* 2017; 44:1375-83.

4) Zen M, Bassi N, Nalotto L, et al. Disease activity patterns in a monocentric cohort of SLE patients: a seven-year follow-up study. *Clin Exp Rheumatol* 2012;30:856-63.
5) Cameron JS. Lupus nephritis. *J Am Soc Nephrol* 1999;10:413–24.
6) Hanly JG, Su L, Urowitz MB, et al. Longitudinal Analysis of Outcomes of Lupus Nephritis in an International Inception Cohort Using a Multistate Model approach. *Arthritis Rheumatol* 2016;68:1932-44.
7) Moroni G, Depetri F, Ponticelli C. Lupus nephritis: When and how often to biopsy and what does it mean? *J Autoimmun* 2016;74:27-40.
8) Cervera R, Abarca-Costalago M, Abramovicz D, et al. Systemic lupus erythematosus in Europe at the change of the millennium: lessons from the "Euro-Lupus Project". *Autoimmun Rev* 2006;5:180-6.
9) Yap DY, Tang CS, Ma MK, et al. Survival analysis and causes of mortality in patients with lupus nephritis. *Nephrol Dial Transplant* 2012;27:3248-54.
10) Moroni G, Quaglini S, Gallelli B,et al. Progressive improvement of patient and renal survival and reduction of morbidity over time in patients with lupus nephritis (LN) followed for 20 years. *Lupus* 2013;22:810-8.
11) Urowitz MB1, Gladman DD, Tom BD, et al. Changing patterns in mortality and disease outcomes for patients with systemic lupus erythematosus. *J Rheumatol* 2008;35:2152-8.
12) Fiehn C, Hajjar Y, Mueller K, et al. Improved clinical outcome of lupus nephritis during the past decade: importance of early diagnosis and treatment. *Ann Rheum Dis* 2003;62:435-9.
13) Tektonidou MG, Dasgupta A, Ward MM. Risk of End-Stage Renal Disease in Patients With Lupus Nephritis, 1971-2015: A Systematic Review and Bayesian Meta-Analysis. *Arthritis Rheumatol* 2016;68:1432-41.
14) Gatto M, Iaccarino L, Ghirardello A, et al. Clinical and pathologic considerations of the qualitative and quantitative aspects of lupus nephritogenic autoantibodies: A comprehensive review. *J Autoimmun* 2016;69:1-11.
15) Hochberg M. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
16) Weening JJ, D'Agati VD, Schwartz MM, International Society of Nephrology Working Group on the Classification of Lupus Nephritis; Renal pathology Society Working Group on the Classification of Lupus Nephritis. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004;65:521-30.
17) Austin HA 3rd, Boumpas DT, Vaughan EM, et al. Predicting renal outcomes in severe lupus nephritis: contributions of clinical and histologic data. *Kidney Int* 1994;45:544-50.

18) Venables WN, Ripley BD. Modern Applied Statistics with S-PLUS. 3rd Edition Springer, New York, Heidelberg, 1999.
19) Felson DT, Anderson J. Evidence for the superiority of immunosuppressive drugs and prednisone over prednisone alone in lupus nephritis: a pooled analysis. *N Engl J Med* 1984;311:1528-33.
20) Cathcart ES, Idelson BA, Scheinberg MA, et al. Beneficial effects of methylprednisolone "pulse" therapy in diffuse proliferative lupus nephritis. *Lancet* 1976;1:163–6.
21) Ponticelli C, Tarantino A, Pioltelli P, et al. High-dose methylprednisolone pulses in active lupus nephritis. *Lancet* 1977;1;1063.
22) Austin HA III, Klippel JH, Balow JE, et al. Therapy of lupus nephritis: controlled trial of prednisone and cytotoxic drugs. *N Engl J Med* 1986;314:614– 9.
23) Mok CC, Ying KY, Tang S, et al. Predictors and outcome of renal flares after successful cyclophosphamide treatment for diffuse proliferative lupus glomerulonephritis. *Arthritis Rheum* 2004;50:2559-68.
24) Henderson L, Masson P, Craig JC, et al. Treatment for lupus nephritis. Cochrane Database *Syst Rev* 2102;12:CD002922.
25) Hahn BH, McMahon MA, Wilkinson A, et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)*. 2012;64:797–808.
26) Bertsias GK, Tektonidou M, Amoura Z, et al. Joint European League against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012;71:1771–82.
27) Ponticelli C, Moroni G. Hydroxychloroquine in systemic lupus erythematosus (SLE). *Expert Opin Drug Saf* 2017;16:411-9.
28) Galindo-Izquierdo M, Rodriguez-Almaraz E, Pego-Reigosa JM, et al RELESSER Group, from Spanish Society of Rheumatology Systemic Autoimmune Diseases Study Group (EASSER). Characterization of Patients With Lupus Nephritis Included in a Large Cohort From the Spanish Society of Rheumatology Registry of Patients With Systemic Lupus Erythematosus (RELESSER). *Medicine (Baltimore)* 2016;95:e2891.
29) Mok CC. Mycophenolate mofetil for non-renal manifestations of systemic lupus erythematosus: a systematic review. *Scand J Rheumatol* 2007;36:329–37.

30) Bijl M, Horst G, Bootsma H, et al. Mycophenolate mofetil prevents a clinical relapse in patients with systemic lupus erythematosus at risk. *Ann Rheum Dis* 2003;62:534–9.
31) Furie R, Petri M, Zamani O, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum* 2011;63:3918–30.
32) Iaccarino L, Bettio S, Reggia R, et al. Effects of Belimumab on Flare Rate and Expected Damage Progression in Patients With Active Systemic Lupus Erythematosus. *Arthritis Care Res (Hoboken)* 2017;69:115-123.
33) Austin HA 3rd, Boumpas DT, Vaughan EM, Balow JE. Predicting renal outcomes in severe lupus nephritis: contributions of clinical and histologic data. *Kidney Int* 1994;45:544-550.
34) Moroni G, Quaglini S, Gallelli B, et al. The long-term outcome of 93 patients with proliferative lupus nephritis. *Nephrol Dial Transplant* 2007;22:2531-9.
35) Contreras G, Pardo V, Cely C, et al. Factors associated with poor outcomes in patients with lupus nephritis. *Lupus* 2005;14:890-895.
36) Sloan RP, Schwartz MM, Korbet SM, et al. Long-term outcome in systemic lupus erythematosus membranous glomerulonephritis. Lupus Nephritis Collaborative Study Group. *J Am Soc Nephrol* 1996;7:299-305.
37) Adler SG, Johnson K, Louie JS, et al. Lupus membranous glomerulonephritis: different prognostic subgroups obscured by imprecise histologic classifications. *Mod Pathol* 1990;3:186-91.
38) Moroni G, Quaglini S, Gravellone L, et al. Membranous nephropathy in systemic lupus erythematosus: long-term outcome and prognostic factors of 103 patients. *Semin Arthritis Rheum* 2012;41:642-51.
39) Wong SN, Chan WK, Hui J, et al. Membranous lupus nephritis in Chinese children–a case series and review of the literature. *Pediatr Nephrol* 2009;24:1989-96.
40) Zabaleta-Lanz ME, Muñoz LE, et al. Further description of early clinically silent lupus nephritis. *Lupus* 2006;15:845-51.
41) Wakasugi D, Gono T, Kawaguchi Y, et al. Frequency of class III and IV nephritis in systemic lupus erythematosus without clinical renal involvement: an analysis of predictive measures, *J Rheumatol* 2012;39:79-85.
42) Rijnink EC, Teng YKO, Wilhelmus S, et al. Clinical and Histopathologic Characteristics Associated with Renal Outcomes in Lupus Nephritis. *Clin J Am Soc Nephrol* 2017;12:734-43.
43) Ginzler EM, Felson DT, Anthony JM, et al. Hypertension increases the risk of renal deterioration in systemic lupus erythematosus. *J Rheumatol* 1993;20:1694-1700.

44) Korbet SM, Lewis EJ, Schwartz MM, et al. Factors predictive of outcome in severe lupus nephritis. Lupus Nephritis Collaborative Study Group. *Am J Kidney Dis* 2000;35: 904–14.
45) Momtaz M, Fayed A, Wadie M, et al. Retrospective analysis of nephritis response and renal outcome in a cohort of 928 Egyptian lupus nephritis patients: a university hospital experience. *Lupus* 2017;26:1564-70.
46) Resende AL, Titan SM, Barros RT, et al. Worse renal outcome of lupus nephritis in male patients: a case-control study. *Lupus* 2011;20:561–7.
47) Schwartzman-Morris J, Putterman C. Gender differences in the pathogenesis and outcome of lupus and of lupus nephritis. *Clin Dev Immunol* 2012; 2012: 604892. doi: 10.1155/2012/604892 .
48) Ding Y, He J, Guo JP, et al. Gender differences are associated with the clinical features of systemic lupus erythematosus. *Chin Med J* 2012;125:2477-81.
49) Ponticelli C, Glassock RJ, Moroni G. Induction and maintenance therapy in proliferative lupus nephritis. *J Nephrol* 2010;23:9-16.
50) Murphy G, Isenberg D. Effect of gender on clinical presentation in systemic lupus erythematosus. *Rheumatology* (Oxford). 2013 Dec;52(12):2108-15

Figures:

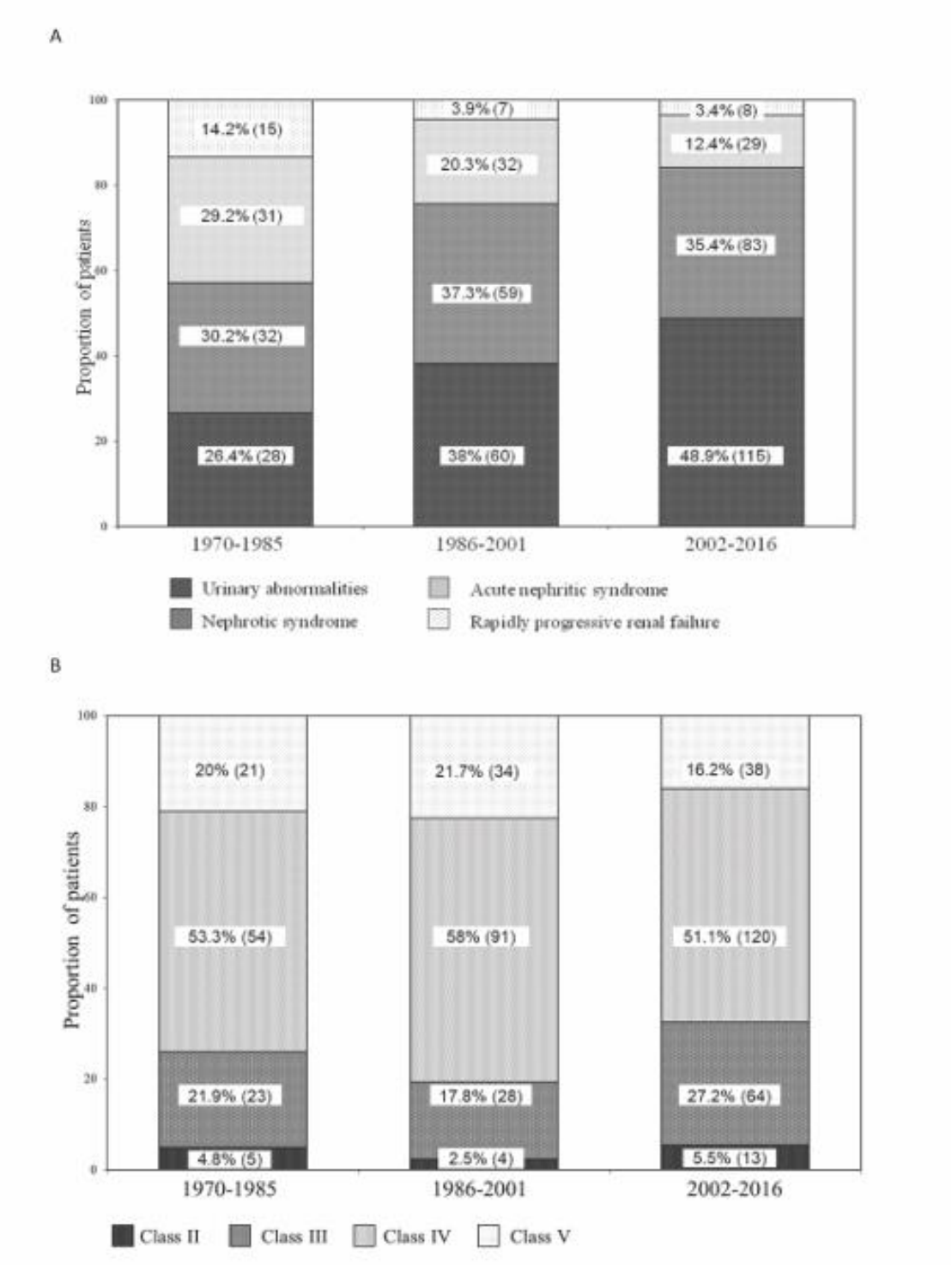

Figure 1

A: Clinical syndrome at presentation of lupus nephritis in three different periods.

B: Histological classes at renal biopsy in three different periods.

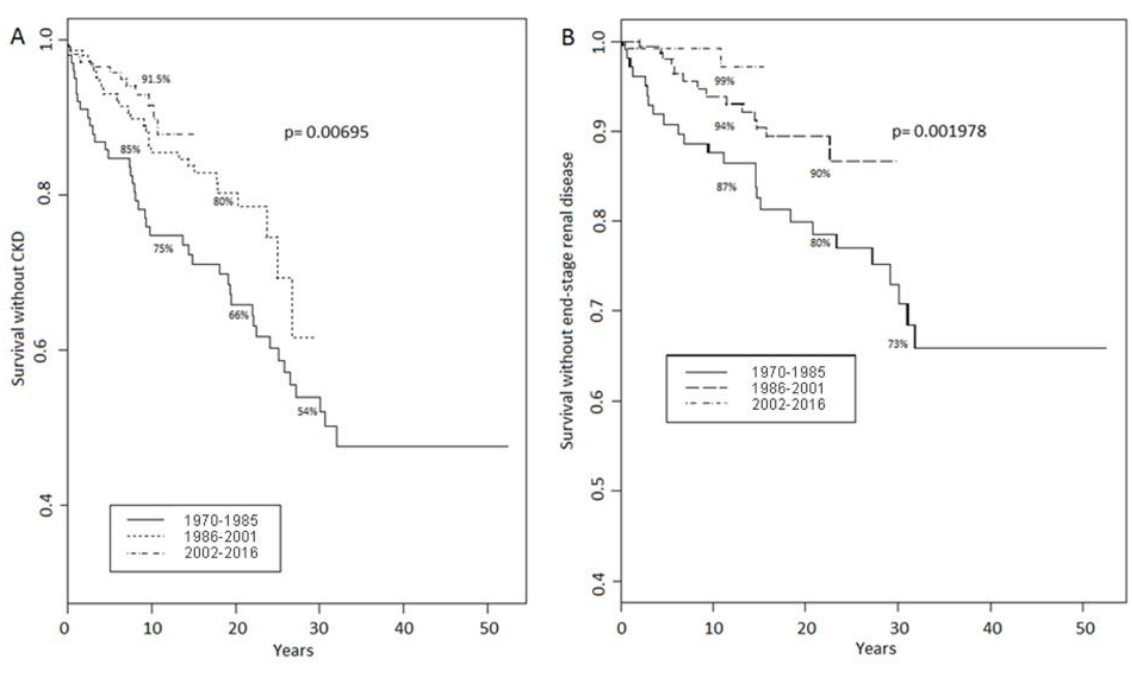

Figure 2

A: Survival without chronic kidney disease in three different periods.

B: Survival without end stage renal disease in three different periods.