40-year follow-up of a Lupus Nephritis cohort

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ABSTRACT

Objectives: To characterize a lupus nephritis (LN) followed for cohort 40 years, assessing its main characteristics and analysing any changes in evolution to end-stage renal disease (ESRD) and mortality rates in the first 5-years after LN diagnosis.

Methods: This was an observational retrospective study of LN patients , followed from 1975 at University College Hospital. Patients were divided into four groups, depending of the decade on diagnosis - 75-85 (D1), 86-95 (D2), 96-05 (D3) and 06-15 (D4). Comparison between the four groups was performed with respect to demographic, clinical, serologic and histologic characteristics and outcome.

Results: 219 patients with LN, predominantly women (91%), were identified. There was a change in ethnic distribution, with a decreasing incidence in Caucasians (58.6% in D1 to 31.3% in D4, p=0.018) and increase in Afro-Caribbeans (17.2% in D1 to 39.6% in D4, p=0.04) and South-Asians (17.2% in D1 to 29.2% in D4, p=0.240). Serological and histological patterns changed, with a reduction in class IV nephritis (51.7% in D1 to 27.1% in D4, $p<0.05$) and increase in class II nephritis (10% in D2 to 18.8% in D4, $p<0.05$), positive ENAab (17.2% in D1 to 83.3% in D4, $p<0.05$) and specific ENAab subtypes. The 5-year mortality rates after LN diagnosis decreased from D1 (24.1%) to D2 (4%), stabilizing thereafter. The 5-year ESRD development after LN diagnosis remained stable.

Conclusion: Despite the changes in treatment of LN in the last 20 years we have reached a plateau in 5-year mortality and progression to ESRD rates, suggesting that new therapeutic and management approaches, and strategies to enhance compliance, are needed to improve outcomes. Ethnic distribution has changed in the last 40-years , as well as the serological and histological patterns.

KEYWORDS: Lupus nephritis, long-term follow-up, 40-year period, mortality, end-stage renal disease, ethnicity, autoantibodies.

KEY MESSAGES

- The outcome in LN has not changed significatively in the past 30-years.
- There is a change in ethnic distribution over the decades: a decreasing incidence of Caucasians and increasing of Afro-Caribbean and Asian patients.
- Serological and histological patterns changed throughout the years: reduction of class IV nephritis; increase of class II nephritis, positive ENAab and specific ENAab subtypes.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune rheumatic disease, involving the kidney in up to 50% of cases [1]. Lupus nephritis (LN) is the most frequent first manifestation of SLE in solid organs, and is evident in about one-quarter of patients at presentation [2]. Treating LN is challenging. It is important to identify clinical and serological factors that might predict and improve long-term outcome.

The literature shows that the geographic region of origin and ethnicity significantly influences the probability of developing LN [1]. In particular, non-European populations, notably Afro-Caribbean, Asian and Hispanic patients have the most active disease with the worst prognosis [3-5].

Among the classic LN biomarkers such as elevated serum creatinine, haematuria and proteinuria, the last is the strongest predictor of long-term renal outcome [3]. Renal disease usually develops within three to five years of lupus diagnosis. [5] There are conflicting data as to whether it is more common in men [3, 6, 7]. The sooner patients begin treatment, the less the likelihood of progressing to end-stage renal disease (ERSD) [3].

In spite of aggressive immunosuppression with regimes recommended by both the American College of Rheumatology (ACR) [8] and the European League against Rheumatism (EULAR) [9, 10] involving combinations of prednisolone, cyclophosphamide, azathioprine, mycophenolate mofetil or rituximab, approximately 10-30% of LN patients develop ESRD [11]. This failure almost certainly relates in part to poor compliance [12, 13]. Diffuse proliferative glomerulonephritis [class IV] carries the worst prognosis [14]. ESRD increases both morbidity and mortality in SLE [15].

This report extends a previous study [16], which assessed the outcome of patients with LN in a cohort of lupus patients followed up at Middlesex Hospital/University College Hospital (UCLH) between 1975 and 2005. We have followed up our patients for another decade (2006-2015), adding 48 patients to the cohort with a minimum of 5 years follow-up. Thus we have reviewed a cohort of LN patients over a 40-year period (n=219). Our objective was to characterize our LN cohort and assess its evolution over the years, analysing two major outcomes: evolution to ESRD and mortality rates. We were particularly interested to determine whether more recently diagnosed patients were less likely to develop ESRD.

PATIENTS AND METHODS

A cohort of approximately 650 patients were diagnosed with SLE between January 1975 and December 2015, at UCLH. These patients all met the revised criteria of the ACR [17]. 227 patients were considered to have LN. We included in this study patients with a minimum of 5 years of follow up and those who died within the timeframe (n=219). All patients had a kidney biopsy with 2003 International Society of Nephrology/Renal

Pathology Society (ISN/RPS) classification criteria for LN [18] or clinically overt evidence of renal involvement (increased proteinuria, raised creatinine, high blood pressure) in whom biopsy was not obtained (n=12), mostly due to patient refusal.

As in the previous study [16], the following patient data were collected: gender, ethnicity, age at SLE diagnosis and kidney biopsy, ISN/RPS class, levels of serum C3, antinuclear antibodies (ANA), anti-dsDNA, antibodies to extractable nuclear antigens (ENAab) and antiphospholipid antibodies (APL). ANA was measured by indirect immunofluorescence using Hep-2 as the substrate (positive ANA ≥ 1:80); ENAab, including Sm, RNP, Ro, La, were measured by enzyme-linked immunosorbent assays (ELISA); anti-dsDNA were measured by ELISA and indirect immunofluorescence with Crithidia luciliae as the substrate; lupus anticoagulant (LAC) measured by Dilute Russell Viper Venom Screen (DRVVT); anticardiolipin (IgM and IgG ACA) antibodies measured by ELISA; and C3 by laser nephelometry.

We also analysed ESRD patients with regard to the time from renal involvement to ESRD, the type and number of years in dialysis, and whether they had a renal transplant (RTx). The time to the development of ESRD and mortality rate at 5 years were also evaluated.

The treatment of LN has evolved over the years. Standard treatment in our centre was prednisolone, antimalarials and anti-hypertensives combined with one or more immunosuppressives, notably: azathioprine and cyclophosphamide throughout. From the late 1970's until 2003 most of our patients with proliferative nephritis were given steroids plus a high dose of cyclophosphamide (total 15 gm). From 2003 we have used the safer and equally effective low dose regime (3gm) proposed by the Euro-Lupus Nephritis Trial [20]. Mycophenolate mofetil has been used since the late 1990s [19]. Rituximab has been used in the patients failing conventional therapy since 2000 [22]. We began to offer Rituximab to LN patients in early stages (at the time of diagnosis) in 2008.

We divided the population into four groups, according to the decade of onset LN after kidney involvement became obvious: 1975-1985 (D1), 1986-1995 (D2), 1996-2005 (D3) and 2006-2015 (D4). When collecting the data for this study, we identified a small number of differences compared to the cohort presented in the previous work. Some LN patients had a renal biopsy performed in another hospital before they came under our care. Every effort was made to review the reports of these biopsies and, where possible, our pathologists requested permission to review the slides themselves.

Statistical analysis

The collected data were analysed using SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA). Categorical variables are presented as frequencies and percentages, and continuous variables as means and standard deviations (SD) or medians and interquartile ranges (IQR) for variables with skewed distributions. Normal distribution was checked using skewness and kurtosis and Kolmogorov-Smirnov or Shapiro-Wilk tests. Clinical and serological manifestations, plus mortality and progression to ESRD were compared between the four groups of patients.. Comparisons of continuous

variables were made using parametric Student's T test or ANOVA analysis of variance, or non-parametric Wilcoxon/Mann-Whitney tests. Chi-square test, and Fisher's exact test were used to compare categorical variables when appropriate. To compare the medians of two independent variables we used the Mann-Whitney test as a non-parametric test. All reported p values are two-tailed, with a probability level less than 0.05 indicating statistical significance. Odds Ratio coefficient and respective confidence interval of 95% were also analysed.

RESULTS

Demographic and Histologic characterization

From 1975 to 2015, 219 patients (33% of the cohort) were identified with LN and a minimum of 5-year follow-up out of a cohort of 650 patients with SLE. The Demographic, clinical and histological characteristics in the four decades are shown in Table 1.

Gender distribution, age at SLE and LN diagnosis were similar between the four decades. There was an approximately 90% prevalence of females within each decade. The overall median age at SLE diagnosis was 24 years (IQR=16), whereas the median age at LN diagnosis was 27 years (IQR=16).

Regarding the ethnic distribution across the decades, Caucasians were the most common ethnicity, both overall (44.7%) and within each decade. However, over the 40-year period the percentage of Caucasian patients fell from 58.6% to 31.3% (p=0.018). In contrast, the number of both Afro-Caribbeans (17%.2 to 39.6%, p=0.04) and South-Asians (17.2% to 29.2%, p=0.240) rose sharply, especially after the end of the 2nd decade onwards.

No significant differences between the four decades were observed regarding time from SLE to LN diagnosis. Most (81.8%) of the patients develop LN within the first 5 years after SLE diagnosis (median = 1, IQR = 4). 57.1% were diagnosed with LN within the first year, and almost 25% of the patients between the first and the fifth years. This pattern was maintained throughout the decades.

The most frequent ISN/RPS classes identified across the years were class IV (42.9%), class III (18.7%) and V (17.4%). When comparing the last decade with the previous ones, we observed a considerable reduction in the proportion of patients with class IV nephritis (D4 vs D1, p=0.035; D4 vs D2, p=0.039; D4 vs D3, p=0.006; D4 vs all other decades, p=0.005). In contrast, a statistically significant increase of class II (D4 vs D3, p=0.01; D4 vs all other decades, p=0.005) and a trend towards an increase of class III (D4 vs all other decades, p=0.08) was noted. These findings are especially prominent in the 4th decade. The prevalence of other ISN/RPS classes remained stable over the years. Class V was more frequent among Afro-Caribbean patients (OR 2.2, 95% CI: 1.1-4.6, p=0.026).

In our cohort, patients received various combinations of the available recommended therapies in each decade (Table 2). Overall, 37.4% had rituximab and cyclophosphamide.

More than half of LN patients were treated with mycophenolate, azathioprine or antimalarials (55.7%, 61.6% and 64.8%, respectively), and the majority concomitantly treated with steroids (90%). Blood pressure medication was prescribed in 59.4% of cases with angiotensin converting enzyme inhibitors or aldosterone receptor antagonist. Only four (1.8%) out of 219 LN patients have been treated with rituximab at the time of diagnosis.

Serological analysis

The overall antibody profile is shown in Table 3; the distribution and comparisons between specific antibodies and ethnicity or main ISN/RPS classes are shown in Supplementary Table 1 and in Table 4 - respectively. There is a statistically significant increasing prevalence over the decades of a positive ENAab profile (D4 vs D1, p=0.0001), as well as each of the specific ENAab subtypes: anti-Ro, anti-RNP, anti-Sm antibodies (D4 vs D1, P<0.001), and anti-La antibodies (p=0.047). However, there were no significant differences between the four decades regarding the anti-dsDNA, low C3 level, ANA or APL-antibodies profiles. Most patients had positive ANA (>95%), elevated anti-dsDNA-antibodies (78.1%) and low C3 levels (81.7%). APL-antibodies were found in 29.7% of cases, ranging from 31% in D1 to 22.9% in D4 (p=0.408).

A strong positive association was found between the Afro-Caribbean population and the presence of ENAab (p=0.0001), as well as with all specific ENAab subtypes (p <0.007). This antibody profile was significantly less common in Caucasian patients (p <0.007).

The presence of ENAab was found to be statistically significantly associated with a longer time (over 10-years) from SLE to LN diagnosis (p=0.04).

When comparing histological with serological aspects some interesting patterns emerged (Figure 1A and Table 4). There was a statistically significant association between ENAab, anti-RNP or anti-La antibodies and development of ISN/RPS class II (OR 3.7, 95% CI: 1.0-13.0, p=0.034; OR 3.5, 95% CI: 1.3-9.8, p=0.01 and OR 3.2, 95% CI: 1.1-9.2, p=0.037, respectively). There was a strong negative association between ENAab and anti-RNP antibodies and development of ISN/RPS class IV (OR 0.54, 95% CI: 0.3-0.9, p=0.028; OR 0.50, 95% CI: 0.3-0.9, p=0.015, respectively). There was a positive association between the presence of low C3 levels or anti-Sm antibodies and the development of ISN/RPS class V (OR 4.6, 95% CI: 1.1-20.4, p=0.025; OR 2.3, 95% CI: 1.1-4.8, p=0.021, respectively).

Outcomes - Mortality and ESRD

We analysed two outcome measures, mortality and progression to ESRD (Table 1). Due to the relatively small number of patients analysed, a combination outcome of mortality or progression to ESRD was also considered.

The 5-year mortality rates from diagnosis of LN were 24.1% in D1, 4% in D2, 4.3 % in D3 and 6.3% in D4. There were no significant differences between the decades with respect

to the mean age at death (44.05 years, SD 17.07). However, an association was found when comparing the D4 with previous decades with respect to the time from LN diagnosis to death, which was interestingly shorter in the fourth decade (1.7 years in D4 vs 9.5 years, 12.3 years and 8.7 years in the D1, D2 and D3, respectively, P<0.05). In our cohort, although not statistically significant, there was a trend towards greater mortality rates in the Afro-Caribbean population (OR 1.9, 95% CI: 0.96-3.9, p=0.062) (Table 5). Those patients who developed LN one to five years after being diagnosed with SLE were more likely to die than those whose nephritis became evident in the first year or five years after SLE diagnosis (OR 2.25, 95% CI: 1.05-4.61, p=0.025). In contrast, patients with positive anti-Sm antibodies were less likely to die (OR 0.4, 95% CI: 0.2-1.0, p=0.05) (Figure 1B). There were no statistically significant associations found between histological patterns or other auto-antibodies and mortality alone. The main causes of death were infections and cardiovascular diseases (31% each), cancer (16.7%) and renal failure (4.8%).

Progression to ESRD occurred in 38 patients (17.4%). Amongst these, 11 patients (28.9%) developed ESRD within the first 5 years following the LN diagnosis. 36.8% of patients developed ESRD more than 10 years after LN diagnosis. There were no significant differences comparing the decades regarding the proportion of patients developing ESRD within the first 5 years after the diagnosis of LN. The mean age at ESRD diagnosis was 37.4 years (SD 12.8). Considering ethnicity, the Afro-Caribbean population predominated (44.7% out of 38 patients with ESRD), followed by Caucasian (31.6%) and South-Asian populations (18.4%). Afro-Caribbean ethnicity was associated with ESRD (OR 2.4, 95% CI: 1.2-4.9, p=0.017) (Table 5). The majority of patients (n=26, 68.4%) presented with ISN/RPS class IV, followed by ISN/RPSclass III (n=7, 18.4%). A strong association was found between ISN/RPS class II or IV and the development of ESRD (OR 0.81, 95% CI: 0.75-0.86; and OR 3.1, 95% CI: 1.5-6.5, p=0.002, respectively). Those patients with positive anti-RNP antibodies were less likely to develop ESRD (OR 0.43, 95% CI: 0.19-0.97, p=0.038) (Figure 1B). No other statistically significative associations were found between histologic, serologic or demographic data and ESRD alone.

When looking at the composite endpoint comprising mortality or development of ESRD, some other interesting associations emerged. Both, the Afro-Caribbean population and ISN/RPS class IV, positively associated with the combined endpoint (OR 2.1, 95% CI: 1.1-3.9, p=0.018 and OR 2.2, 95% CI: 1.2-4, p=0.01, respectively). However, ISN/RPS class V was found to be protective when compared to the composite endpoint (OR 0.4, 95% CI: 0.16-1.0, p=0.046). ENAab notably anti-RNP antibodies were also found to be negatively associated with the composite endpoint (OR 0.53, 95% CI: 0.29-0.95, p=0.033; and OR 0.5, 95% CI: 0.26-0.95, p=0.034, respectively). Time from SLE to LN diagnosis between 1 and 5 years was associated with mortality or ESRD (OR 1.92, 95% CI: 1.0-3.7, p= 0.047).

Regarding renal replacement therapy, 14 patients received haemodialysis and 11 patients had peritoneal dialysis. Few had combined dialysis therapy (n=3). Sixteen patients underwent renal transplantation, which was more frequent among those diagnosed in D3 (n=6).

DISCUSSION

Our data, based on the long-term follow-up of 219 patients, indicate that the outcome for LN has not changed significatively in the past 30-years, in spite of several changes in therapeutic approach. These include the introduction of Mycophenolate mofetil in the mid-90s [19], the reduction in the standard dose of Cyclophosphamide from a total of 15g to a 3g regiment [20], the more thorough control of high blood pressure [21], and, more recently, the use of Rituximab [22]. Despite these measures, we appear to have reached a plateau in 5-year mortality and progression to ESRD, suggesting that new therapeutic and management approaches, as well as strategies to enhance compliance, are needed to improve outcomes in LN further . Other key messages were that in our cohort we noticed a change in ethnic distribution over the decades, characterized by a decreasing incidence of Caucasians and increase in Afro-Caribbean and South-Asian patients. Linked to this change, the serological and histological patterns have changed, with a reduction of ISN/RPS class IV nephritis, balanced by an increase in ISN/RPS class II, as well as variations in anti-ENAab profiles. These findings will be discussed in more detail below.

This report extends a previous one [16], with the objective of looking at trends in the demographics, biomarkers and outcome of LN patients. There are few studies with such long-term follow-up of a large cohort with biopsy proven LN [23-27], and we are aware of only one other study with a 40-year follow-up [28].

Women were more often affected than men (9:1), as reported elsewhere [29], without significant differences between the decades. The median age at LN diagnosis was 27 years (IQR=16), lower than reported by some others [28]. Younger age at the time of SLE diagnosis, (our cohort was 24 years (IQR=16), was found to be significantly associated with LN in a previous report [5]. As in other studies [5], renal disease frequently developed within five years of the lupus diagnosis – 57.1% within the first year and almost 25% between the first and the fifth years. This pattern was maintained throughout the decades.

Interestingly, we found a change in ethnic distribution over time, with a reduction in the Caucasian population, but increased involvement of South-Asians and Afro-Caribbeans. These findings, especially prominent after the second decade, were reported in our previous study [16] and reinforced by the present one. They might reflect patterns of population movements in central-London, where our hospital is located. As noted by others [3-5, 30, 31], in our cohort Afro-Caribbean ethnicity was overall associated with a worse prognosis (Table 5). Patients of African ancestry died or developed ESRD more frequently compared to other ethnic groups. Other studies reported that these patients responded less well to treatment and had more aggressive disease progression [30, 31]. Surprisingly, we also found that AC patients developed ISN/RPS class V nephritis significatively more frequently. This group is often characterized by nephrotic proteinuria, may occur in combination with classes III or IV LN and is unlikely to progress

quickly to ESRD [38]. That our Afro-Caribbean patients had a worse outcome despite the association with ISN/RPS class V nephritis, suggests that other factors (cardiovascular or thromboembolic increased risks) have contributed to enhance the risk in this specific population.

Over the past 40 years, we observed a considerable reduction of patients with ISN/RPS class IV (diffuse proliferative) glomerulonephritis but an increase in class II (mesangial) nephritis. These findings were especially prominent in the fourth decade, which raises interesting questions. Did the increasing awareness that excessive delay between the onset of LN and the kidney biopsy is associated with higher risk of progression to ESRD [32] lower the threshold for performing renal biopsy? Early biopsy is recommended by EULAR/ERA-EDTA [39] even when the levels of proteinuria are as low as 500mg in 24h-urine test. Thus, was LN identified at earlier stages of disease progression? Does early treatment with Rituximab (an anti-CD20 monoclonal antibody) slow progression towards more severe histologic LN stages? We cannot be sure of the answers to these questions.

The serological profile changed progressively (Table 3), with an increasing prevalence of a positive overall ENAab profile and of the specific ENAab subtypes – anti-Ro, anti-RNP, anti-Sm and anti-La. Some of these autoantibodies have been found to be deposited in kidney biopsies of patients with SLE [33] and are more common in Afro-Caribbeans [34, 35]. This is probably why the prevalence of these autoantibodies increased significantly over time. We also found a statistically significative positive association between Afro-Caribbean ethnicity and all specific ENAab subtypes. Although anti-dsDNA and anti-nucleosome antibodies are more overtly involved in the pathogenesis of LN [36, 37], our findings suggest a role for these ENAab antibodies in SLE with renal involvement.

When comparing histological with serological aspects some interesting patterns emerge. Anti-RNP and anti-La antibodies were found more frequently in ISN/RPS class II nephritis, suggesting a probable association with better outcome. However, only patients with this anti-RNP did progress less frequently to ESRD and had less ISN/RPS class IV nephritis, (known to be associated with poorer prognosis [37]). Patients with low C3 levels or anti-Sm antibodies were more likely to develop ISN/RPS class V nephritis and might be biomarkers for slowly progressive renal disease and good prognosis, especially the anti-Sm antibody – the only antibody linked to a reduced mortality.

There was a reduction in mortality rates from the first to the second decades, stabilizing for the next 3 decades, with no differences found between the mean age at death (44.05 years ± 17.07). This plateau in mortality rates for the last 30 years strongly suggests that we have now optimised the use of conventional steroids and immunosuppressives.

In the fourth decade, the time between the LN diagnosis to death was considerably lower compared to previous decades. This probably relates to the obviously short follow-up period in this decade or might be explained by the intrinsic aggressiveness of the disease *per se* in these patients, with less time to respond to therapies.

Patients whose nephritis developed between one to five years after SLE diagnosis had a worse prognosis. We suspect that those developing LN in the first year were treated more aggressively and thus had better prognosis. This finding is in concordance with other evidence that excessive delay between the onset of LN and the kidney biopsy is associated with higher risk of progression to ESRD and death [32], and also with EULAR/ERA-EDTA´s recommendation that biopsies should be performed within the first month after disease onset [39]. Those patients whose LN was diagnosed five years after SLE diagnosis probably had less aggressive disease, thus explaining why they did better than those diagnosed between one and five years.

Overall, 38 patients (17.4%) developed ESRD, which concurs with earlier reports that 10-30% of LN patients progress to kidney failure [11]. Eleven patients (28.9%) developed ESRD in the first 5 years after LN diagnosis. No differences were found between the decades with regard to ESRD development in the 5 years after LN diagnosis. The mean age at ESRD diagnosis was 37.4 years (± 12.8). The majority of patients with ESRD (n=26, 68.4%) presented with ISN/RPS class IV nephritis. In contrast, patients with ISN/RPS class II nephritis were less likely to develop terminal renal disease.

A more detailed analysis of the treatment approach in our centre was referred to in previous studies [21, 40, 41].

We are aware of the limitations of this study. Being a retrospective study over 40-years, some data are missing and the cause of death in some instances has been hard to verify. Being a single-centre makes it hard to generalise our results. However, it does allow for a consistency of approach, and we believe that our key observation that newer modes of therapy are needed to improve both mortality and morbidity is correct.

In summary, LN is a concerning complication of SLE. It contributes significatively to morbidity and mortality. LN requires prompt recognition and rapid initiation of appropriate therapy. Breakthroughs in translational and clinical research are needed to improve renal outcomes in the future.

AUTHOR CONTRIBUTIONS

All authors contributed to manuscript preparation and approved the final manuscript.

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ETHICAL APPROVAL INFORMATION

This is an observational retrospective study of medical records collected over a period of over 40 years. All data were collected based on available information obtained in clinical management. No patients underwent extra questionnaires or research procedures. Data were analysed in an anonymized fashion and the results presented in an aggregate form (with no patient identifiable information). No individualised or identifiable data are presented in this study. Therefore, ethical approval and informed consent were not required.

DISCLOSURES

The authors have declared no conflicts of interest.

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Table1: Characterization of the cohort - overall and within each decade.

		D1 (n=29)	D2 (n= 50)	D3 (n=92)	D4 (n=48)	Overall (n=219)
Females, n (%)		28 (96.5)	44 (88)	85 (92.4)	43 (89.6)	200 (91.3)
Ethnicity, n (%)	**As**	5 (17.2)	7 (14)	27 (29.3)	14 (29.2)	53 (24.2)
	AC	5 (17.2)	12 (24)	27 (29.3)	19 (39.6)	63 (28.8)
	C	17 (58.6)	29 (58)	37 (40.2)	15 (31.3)	98 (44.7)
	other	2 (6.9)	2 (4)	1 (1.1)	0	5 (2.3)
Age at SLE diagnosis, mean ± SD or median (IQR), (min; max)		28 (10) (14; 66)	23.3 ± 9.6 (1; 45)	22.5 (20) (7; 75)	25.7 ± 9.9 (9; 47)	24 (16)
Age at LN diagnosis, mean ± SD or median (IQR), (min; max)		29 (12) (14; 66)	26.3 ± 10 (3; 45)	25.5 (18) (7; 75)	29.2± 11.3 (14; 52)	27 (16)
Time from SLE to LN diagnosis, years	**<=1,** n (%)	19 (65.5)	25 (50)	54 (58.7)	27 (56.3)	125 (57.1)
	1 to <=5, n (%)	7 (24.1)	12 (24)	23 (25)	12 (25)	54 (24.7)
	5 to <=10, n (%)	2 (6.9)	10 (20)	10 (10.9)	4 (8.3)	26 (11.9)
	>10, n (%)	1 (3.4)	3 (6)	5 (5.4)	5 (10.4)	14 (6.4)

	Overall median (IQR)	0 (3)	1,5 (6)	1 (3)	1 (5)	1 (4)
ISN / RPS classes, N (%)	**I**	0	1 (2)	2 (2.2)	1 (2.1)	4 (1.8)
	II	0	5 (10)	4 (4.4)	9 (18.8)	18 (8.2)
	III	5 (17.2)	7 (14)	17 (18.5)	12 (25)	41 (18.7)
	IV	15 (51.7)	23 (46)	43 (46.7)	13 (27.1)	94 (42.9)
	V	3 (10.3)	10 (20)	16 (17.4)	9 (18.8)	38 (17.4)
	VI	0	0	1 (1,1)	0	1 (0.5)
	Mixed changes	0	2 (4)	7 (7.6)	2 (4.2)	11 (5)
	NA or NS	6 (20.7)	2 (4)	2 (2,2)	2 (4.2)	12 (5.4)
Mortality						
Nº of deaths 5 years after LN diagnosis, n(%)		7 (24.1)	2 (4)	4 (4.3)	3 (6.3)	16 (7.3)
Time from LN to death, mean ± SD or median (IQR), (min; max)		9.5 ± 9.9 (<1; 29)	12.3 ± 5.9 (4; 21)	8.1 ± 4.4 (<1; 17)	1.7 ±1.5 (<1; 3)	8.0 (11) (<1; 29)
Age at death, mean ± SD or median (IQR), (min; max)		41.9 ±14.6 (24; 81)	41.6± 13.3 (19; 56)	49.9± 20.7 (19; 87)	33 ± 12.2 (19; 41)	44 ± 16.9 (19; 87)
ESRD						
ESRD within 5 years of LN, n (%)		1 (3.4)	2 (4)	6 (6.5)	2 (4.2)	11 (5)
Age at ESRD, mean ± SD, (min; max)		44.9 ± 9.5 (34; 59)	31.3 ±11.1 (19; 55)	37.9 ±14.3 (19; 62)	35 ± 7.1 (30; 40)	37.6 ±12.8 (19; 62)
Time from LN diagnosis to ESRD, years	**<=1,** n	1	1	1	1	4
	1 to <=5, n	0	1	5	1	7
	5 to <=10, n	2	3	7	0	11
	>10, n	6	4	5	0	14
	Overall median (IQR)	17.5 (15)	10 (13)	6 (7)	2.3 (4)	9 (9)
No. of years in dialysis	**<=1,** n	4	6	4	1	15
	1 to <=5, n	1	2	11	1	15
	5 to <=10, n	2	0	2	0	4
	>10, n	2	1	1	0	4
Type of dyalisis	**CAPD,** n	2	1	7	1	11
	HD, n	1	4	8	1	14
	CAPD and HD, n	1	1	1	0	3
	NA, n	5	3	2	0	10
No. of RTx	**Related donor,** n	0	1	1	0	2
	Cadaver donor, n	1	1	2	0	4
	NA, n	4	3	3	0	10
Post-RTx immunosupressant	**CSA,** n	1	3	1	-	5
	Tacrolimus, n	1	-	4	-	5

D: decade; D1: 1975-1985; D2: 1996-1995; D3: 1996-2005; D4: 2006-2015; As: South-Asian; AC: Afro-Caribbean; C: Caucasian; CAPD: continuous ambulatory peritoneal dialysis; CSA: cyclosporine; ESRD: End-stage Renal Disease; HD: haemodialysis; IQR: inter-quartile range; ISN/RPS: International Society of Nephrology/Renal Pathology Society; LN: Lupus Nephritis; NA: not-available; NS: non-specific; SLE: Systemic Lupus Erythematosus; SD: standard-deviation;

Table 2: Treatment approach throughout the decades.

	Steroids	Antimalarials	AZA	MTX	CYC	MMF	RTX	IECA/ ARA
D1 (n=29), %	72.4	37.9	55.2	0	3.4	20.7	3.4	24.1
D2 (n= 50), %	88	52	68	18	44	44	36	58
D3 (n=92), %	93.5	65.2	60.9	8.7	50	58.7	41.3	70.7
D4 (n=48), %	95.8	93.8	60.4	12.5	27.1	83.3	52.1	60.4
Overall (n=219), %	90	64.8	61.6	10.5	37.4	55.7	37.4	59.4

The presented data refer to the realized treatment that patients diagnosed within each decade have done throughout the years (from the time of LN diagnosis until 2015).

Table 3: Serological profile distribution throughout the decades.

	ENAab	Ro	La	RNP	Sm	ANA	dsDNA	Low C3	APL
D1 (n=29), %	17.2	17.2	3.4	3.4	0	96.6	69	75.9	31
D2 (n= 50), %	48	38	16	30	12	96	80	80	36
D3 (n=92), %	66.3	46.7	15.2	43.5	28.3	95.7	79.3	83.7	29.3
D4 (n=48), %	83.3	56.3	20.8	58.3	52.1	97.9	79.2	83.3	22.9
Overall (n=219), %	59.4	42.9	15.1	38.4	26	96.3	78.1	81.7	29.7
P-value (chi-square test)	0.0001	0.001	0.047	0.0001	0.0001		0.444	0.605	0.408

D1-D4 (see legend Table 1); APL: antyphospholipid antibodies; ENAab: anti-extractable nuclear antigen antibodies; LN: Lupus Nephritis; ANA: antinuclear antibodies;

P values refere to Chi-square test between D4 and D1. Relative values refere to the percentage of the antibody within each decade.

Table 4: Association between main ISN/RPS classes and Serological profile.

	II (n= 18)	III (n=41)	IV (n=94)	V (n=38)	Mixed (n=11)
ENAab, n (%)	15 (83.3)	23 (56.1)	47 (50)	25 (65.8)	9 (81.8)
	p = 0.034	p = 0.901	p = 0.028	p = 0.342	p = 0.128
Ro, n (%)	10 (55.6)	15 (36.69	36 (38.3)	15 (39.5)	9 (81.8)
	p= 0.274	p = 0.718	p = 0.524	p = 0.750	p = 0.011
La, n (%)	6 (33.3)	7 (17.1)	13 (13.8)	3 (7.9)	3 (27.3)
	p = 0.025	p = 0.538	p = 0.647	p = 0.342	p = 0.253
RNP, n (%)	12 (66.7)	17 (41.4)	26 (27.7)	17 (44.7)	7 (63.6)
	p = 0.011	p = 0.495	p = 0.015	p = 0.292	p = 0.112
Sm, n (%)	7 (38.9)	7 (17.1)	20 (21.3)	15 (39.5)	5 (45.5)
	p = 0.204	p = 0.326	p = 0.187	p = 0.021	p = 0.162
ANA, n (%)	17 (94.4)	39 (95.1)	90 (95.7)	37 (97.4)	11 (100)
	p = 0.559	p = 0.689	p = 0.551	p = 0.796	p =1.0
dsDNA, n (%)	12 (66.7)	33 (80.5)	73 (77.7)	32 (84.2)	10 (90.9)
	p = 0.188	p = 0.336	p = 0.789	p = 0.327	p = 0.464
Low C3, n (%)	15 (83.3)	31 (75.6)	74 (78.7)	36 (94.7)	9 (81.8)
	p = 0.922	p = 0.565	p = 0.108	p = 0.025	p = 1.0
APL, n (%)	6 (33.3)	14 (34.1)	24 (25.5)	15 (39.5)	2 (18.2)
	p = 0.660	p = 0.150	p = 0.884	p = 0.880	p = 0.306

Absolute and relative values refer to presence (positivity) of antibodies within each ethnic group. P values refer to Chi-square tests between each histologic group and the presence of an antibody.

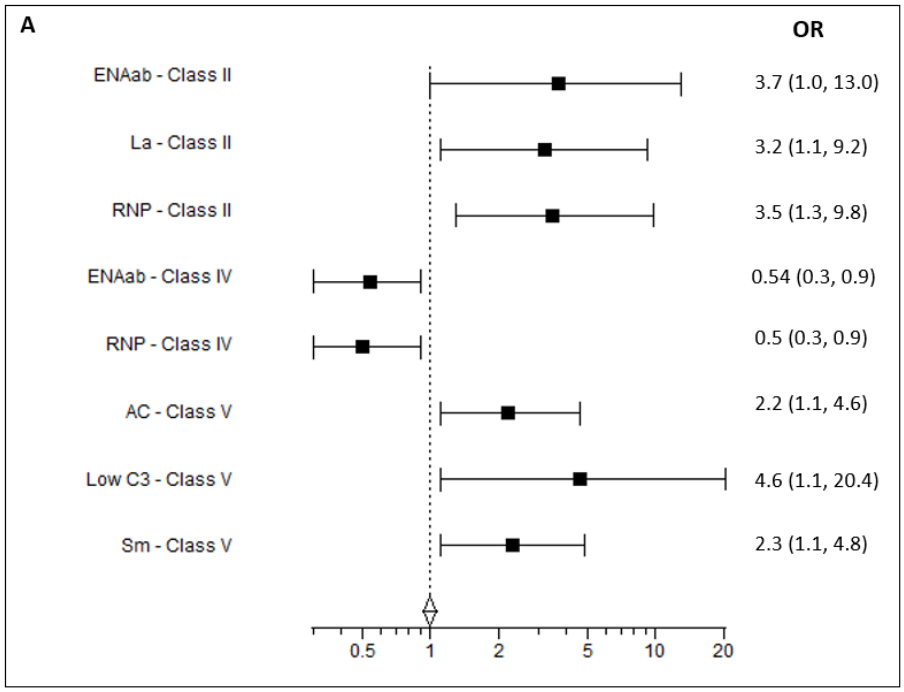
Table 5: Association between Ethnicity and Outcome.

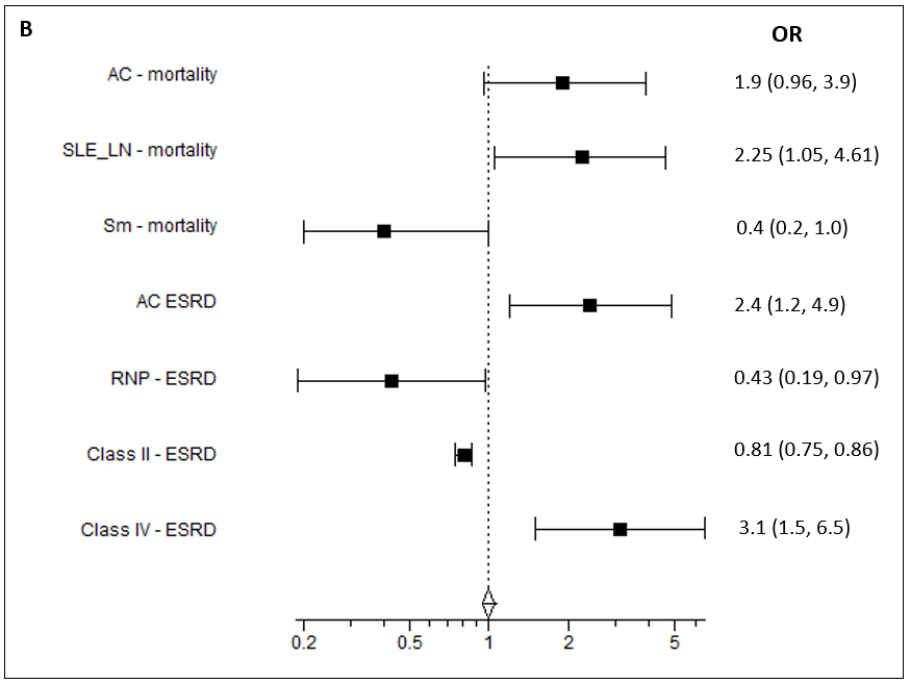
	As (n=53)	AC (n=63)	C (n=97)	Other (n=4)
ESRD, n (%)	7 (18.4%) p= 0.360	17 (44.7%) p= 0.017	12 (31.6%) p= 0.093	2 (5.3%)
Mortality, n (%)	8 (19%) p= 0.386	17 (40.5%) p= 0.062	15 (35.7%) p= 0.180	2 (4.8%)
M+ESRD, n (%)	15 (18.8%) p= 0.161	34 (42.5%) p= 0.018	17 (21.3%) p=0.180	4 (5%)

As, AC and C (see legend Table 1); ESRD: End-stage Renal Disease; M: Mortality;

Absolute and relative values refer to presence of endpoint within each ethnic group. P values refer to Chi-square tests between each ethnicity and the endpoints.

Figure 1: Forest Plot with the statistically significative associations.





A: variables associated with different histologic classes**; B:** variables associated with main outcomes (mortality and ESRD); AC: Afro-Caribbean ethnicity; ENAab: Anti-extractable nuclear antigen antibodies; SLE_LN: time between systemic lupus erythematosus diagnosis and development of lupus nephritis between 1 to 5 years; Class II, Class IV and Class V: histologic International Society of Nephrology/Renal Pathology Society classification criteria for LN

Supplementary Table 1: Association between Ethnicity and Serological profile.

	As (n= 53)	AC (n=63)	C (n=97)	Other (n=4)
ENAab, n (%)	29 (54.7) p = 0.402	49 (77.8) p = 0.0001	48 (49) p = 007	3 (75) p =0.650
Ro, n (%)	22 (41.5) p= 0.786	38 (60.3) p = 0.001	32 (32.7) p = 0.008	1 (25) p = 1.0
La, n (%)	6 (11.3) p = 0.373	16 (25.4) p = 0.007	10 (10.2) p = 0.097	0 p = 0.564
RNP, n (%)	23 (43.4) p = 0.403	36 (57.1) p = 0.0001	22 (22.4) p = 0.0001	2 (50) p = 0.376
Sm, n (%)	14 (26.4) p = 0.959	28 (44.4) p = 0.0001	12 (12.2) p = 0.0001	2 (50) p = 0.113
ANA, n (%)	50 (94.3) p = 0.365	63 (100) p = 0.197	93 (94.9) p = 0.703	4 (100) p = 1.0
dsDNA, n (%)	42 (79.2) p = 0.870	50 (79.4) p = 0.832	75 (76.5) p = 0.743	3 (75) p = 1.0
Low C3, n (%)	42 (79.2) p = 0.532	51 (81) p = 0.776	81 (82.7) p = 0.610	4 (100) p = 0.588
APL, n (%)	13 (24.5 p = 0.309	17 (27) p = 0.587	33 (33.7) p = 0.235	2 (50) p = 0.638

As, AC and C (see legend Table 1),

Absolute and relative values refer to presence (positivity) of antibodies within each ethnic group. P values refer to Chi-square tests between each ethnic group and the presence of an antibody.