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Clinical and histological findings at second but not at first kidney biopsy predict end-stage kidney disease in a large multicentric cohort of patients with active lupus nephritis

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ABSTRACT

Objective To investigate second kidney biopsy as predictor of end-stage kidney disease (ESKD) in active lupus nephritis (LN).

Methods Patients with biopsy-proven LN (International Society of Nephrology/Renal Pathology Society 2003) who had undergone a second kidney biopsy between January 1990 and December 2018 were included. Clinical and histological findings at first and at second biopsy were analysed with Cox proportional hazard models to predict ESKD, defined as start of kidney replacement therapy. Survival curves were calculated with Kaplan-Meier method.

Results Ninety-two patients with LN were included, 87% females, mean follow-up 17.9±10.1 years. Reasons for second kidney biopsy encompassed nephritic flares (n=28, 30.4%), proteinuric flares (n=46, 50%) or lack of renal response (n=18, 19.5%). Class switch from first biopsy occurred in 50.5% of cases, mainly from non-proliferative towards proliferative classes. Class IV remained stable in over 50% of cases. Twenty-five patients (27.2%) developed ESKD, mostly belonging to the nephritic flare group (17/28, 60.7%). Independent predictors of ESKD at second biopsy were activity index (AI; (HR 95% CI) 1.20 (1.03 to 1.41), p=0.022), chronicity index (CI; 1.41 (1.09 to 1.82), p=0.008) and 24h-proteinuria (1.22 (1.04 to 1.42), p=0.013). AI≥2 (log-rank p=0.031), CI >4 (log-rank p=0.001) or proteinuria ≥3.5 g/day (log-rank=0.009) identified thresholds for higher ESKD risk. In a subgroup analysis, glomerular activity and tubular chronicity mostly accounted for AI and CI association with ESKD. No histological or laboratory predictors emerged at first biopsy (95% CI): AI: 0.88 to 1.19; CI: 0.66 to 1.20; proteinuria 0.85 to 1.08.

Conclusions Findings at second but not at first kidney biopsy in patients with persistently active or relapsing LN inform about ESKD development in a long-term follow-up.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Second biopsy may add pieces of information in defining lupus nephritis (LN) course.

WHAT THIS STUDY ADDS

⇒ We provide evidence that second diagnostic kidney biopsy is more informative than the first biopsy in predicting end-stage kidney disease (ESKD).

⇒ We identify thresholds for activity index ≥2, chronicity index >4 and proteinuria ≥3.5 g/day at the time of second biopsy as factors that predict subsequent ESKD in patients with persistently active or relapsing LN.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ This study identifies handy parameters at second biopsy to be followed up which may significantly influence patient prognosis.

⇒ It supports the utility of second biopsy for a better long-term stratification of patients with active LN, regardless of class switch.

⇒ The identification of high AI in the second kidney biopsy as long-term prognostic factor might have therapeutic implications.

INTRODUCTION

The performance of a second kidney biopsy in patients with lupus nephritis (LN) has been a matter of debate for a long time.[1–6] In fact, while it is accepted that an early renal response is associated with a better renal outcome,[7–10] predictors of renal dysfunction and end-stage kidney disease (ESKD) are difficult to pinpoint in a short-to-mid timeframe, when an overt decline of renal function may not be apparent.[11] In this regard, the validity of clinical predictors is still debated,[12] whereas increasing evidence suggests that a second biopsy may provide more solid information concerning the risk of renal flares[4 6] and eventually kidney failure,[2 3 6] occurring in up to 30% of patients.[13 14] Moreover, a systematic, multicentric trial aiming at investigating the prognostic effect of repeating kidney biopsy

in LN is underway (REBIOLUP).[12] The blossoming of data regarding renal rebiopsy testifies the eagerness of clinical scientists to improve LN management; on the other hand, it underlines the uncertainty concerning the interpretation of renal findings as long-term predictors.

Indications for rebiopsy are not unanimously accepted outside recommendations for LN flares,[15 16] overlooking a relevant portion of patients with suboptimal response.

In this study, we investigated the clinical and histological findings at second diagnostic kidney biopsy in a large cohort of patients with persistently active or flaring LN on initial treatment and their prognostic value on the development of ESKD. We also compared the predictors of ESKD at the first and second kidney biopsy.

PATIENTS AND METHODS

We analysed a subgroup of patients selected from a multicentric cohort of 381 patients with LN[11] across four Italian Rheumatology and Nephrology referral centres who received standard of care for LN, consisting in prednisone in combination with synthetic immunosuppressants, including mycophenolate mofetil, azathioprine, calcineurin inhibitors or cyclophosphamide and addition of angiotensin-converting enzyme inhibitors.[15] We included in the present work adult (≥18 years old) patients with a biopsy-proven LN who underwent a second diagnostic kidney biopsy due to development of a renal flare or lack of complete response despite standard initial treatment,[11] limiting the time period for inclusion from January 1990 onwards, due to data availability and homogeneity in the treatment schedule.

Exclusion criteria were the presence of other renal diseases and the pre-existent need for kidney replacement therapy. Patients displaying incomplete clinical records were as well excluded. A retrospective analysis of prospectively collected clinical and histological data was performed.

The set outcome was the development of ESKD and the primary aim was to evaluate predictors of ESKD at second kidney biopsy. The secondary aim was the comparison between predictors at second versus first kidney biopsy.

Patient assessment

LN was classified according to the 2003 International Society of Nephrology/Renal Pathology Society (ISN/RPS) criteria;[17] chronicity index (CI) and activity index (AI) were assessed according to Austin *et al*.[18]

Demographics, clinical and laboratory features at first and second kidney biopsy and at last observation were recorded in a shared database. End of follow-up was defined as development of ESKD, or last observation or death in patients who did not develop ESKD.

Renal variables

Normal renal function: serum creatinine ≤1 mg/dL and estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m2 by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration).

ESKD: start of kidney replacement therapy.[19]

Proteinuria: measured by benzethonium chloride on the urine collected over 24 hours expressed as g/24 hours.

Arterial hypertension: mean of three consecutive measurements of systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg in sitting position.

The reasons for rebiopsy were classified as follows:

Nephritic flare: increase in serum creatinine of at least 30% over the last value associated with nephritic urinary sediment, with or without increased proteinuria.[20]

Proteinuric flare: increase in proteinuria without modification of serum creatinine of at least 2 g/24 hours if the previous proteinuria was <3.5 g/24 hours, or a doubling if previous proteinuria was ≥3.5 g/24 hours.[20]

Lack of complete response: proteinuria ≥0.7 g/day for more than 1 year from the start of induction treatment and normal or near-normal eGFR.[11 15]

Patient and public involvement

This research was carried out without patient involvement. Patients were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Statistical analysis

Descriptive statistics are reported as mean and SD for continuous variables, and as counts and percentages for dichotomous variables. The difference of continuous variables between groups was tested with non-parametric Mann-Whitney test for independent samples. χ^2 test was used to test associations of qualitative or dichotomised variables among groups of patients.

Inferential analysis was performed using the Cox proportional hazard model. Both univariable and multivariable analysis were performed. Kaplan-Meier estimate was used to draw survival curves and Mantel-Cox log-rank test was used to test their difference.

In the multivariate analysis, we tested variables with potential clinical significance.[21]

Patients lost to follow-up were censored at last observation. The SPSS statistical package 26 (Chicago, Illinois, USA) was used.

RESULTS

Study cohort

Ninety-two patients with LN were included in the study, 84 (91.3%) of Caucasian ethnicity, with a mean age 35.1±10.56 years at second kidney biopsy.

Reasons for repeating kidney biopsy were nephritic flare (Group 1, n=28, 30.4%), proteinuric flare (Group 2, n=46, 50%) or lack of complete response LN (Group 3, n=18, 19.5%). Demographic, histological, therapeutic intervention and clinical features of patients from different groups are reported in table 1.

In total, 25 (27.2%) developed ESKD after a mean time of 9.04±9.48 years from the second biopsy. The prevalence of patients developing ESKD varied significantly,

Table 1 Demographic, histological and clinical features at second renal biopsy and at disease outcome in patients from different groups (expressed as mean (SD) unless differently specified)

Variable	Overall	Group 1 Nephritic flare	Group 2 Proteinuric flare	Group 3 lack of response	P value
Number of patients	92	28	46	18	–
Age at second biopsy	35.1 (10.6)	34.7 (11.1)	36.6 (10.36)	31.7 (10.1)	0.32
Females, n (%)	80 (86.9)	26 (92.9)	39 (84.8)	15 (83.3)	0.54
Arterial hypertension, n (%)	47 (51)	24 (85.7)	17 (36.9)	6 (33.3)	0.008
Proteinuria, g/24 hours	4.1 (3.5)	6.0 (5.1)	3.75 (2.41)	2.1 (1.0)	0.003
SCr mg/dL	1.4 (1.3)	2.4 (1.7)	0.8 (0.17)	0.9 (0.3)	<0.001
GFR ml/min/1.73 m^2	71.9 (35)	33.3 (14.1)	94.8 (27.1)	82.8 (30.3)	<0.001
C3 mg/dL	67.3 (29.3)	61.8 (28.5)	67.6 (28.8)	75.6 (30.5)	0.28
C4 mg/dL	17.6 (21.3)	14.7 (20.8)	20.4 (24.9)	15.5 (.48)	0.37
Antibodies					
Anti-dsDNA in 87 pts, n (%)	79 (90.8)	26	39	14	0.84
aPL in 84 pts, n (%)	56 (66.7)	14	29	13	0.47
Anti-ENA in 69 pts, n (%)	35 (50.7)	8	21	6	0.93
Prednisone dosage, mg/day	8.8 (6.8)	9.73 (8.07)	8.68 (6.8)	7.5 (4.1)	0.82
Antimalarial use*, n (%)	16 (21.6)	8	4	4	0.69
Immunosuppression†, n (%)	44 (50.5)	5	20	8	0.053
MMF	18	2	12	4	0.13
AZA	11	2	5	4	0.30
CNI	3	0	3	0	0.84
CYC	1	1	0	0	0.81
Class ISN/RPS2003‡, n					
I	1	0	0	1	0.15
II	3	0	2	1	0.12
III	9	0	6	3	0.28
IV	42	21	15	6	<0.001
V	14	0	10	4	0.09
Mixed	20	6	11	3	0.91
AI	4.7 (3.8)	5.9 (3.7)	4.5 (3.8)	3.8 (3.7)	0.4
CI	4.0 (2.4)	6.1 (2.6)	2.9 (1.5)	3.9 (2.2)	<0.001
Time between first and second biopsy (years)	6.7 (4.9)	8.2 (5.1)	6.9 (4.6)	3.5 (4.1)	<0.001
Time from repeated biopsy to end of follow-up (years)	10.3 (9.8)	8.9 (9.4)	10.4 (9.1)	12.1 (12.4)	0.61
ESKD, n (%)	25 (27.2)	18 (60.7)	6 (13)	2 (11)	<0.001

Comparison between continuous variables: one way ANOVA with Bonferroni correction; comparison between proportions: χ^2 test.
*Data available for 74 patients.
†Data and referred percentages are calculated 87 patients.
‡Data for 89 patients.
eGFR glomerular filtration rate; aPL antiphospholipid antibodies; AI, activity index; ANOVA, analysis of variance; anti-dsDNA, antibodies antidouble stranded DNA; anti-ENA, antiextractable nuclear antigens antibodies; AZA, azathioprine; CI, chronicity index; CNI, calcineurin inhibitors; CYC, cyclophosphamide; ESKD, end-stage kidney disease; ISN/RPS, International Society of Nephrology/Renal Pathology Society; MMF, mycophenolate mofetil; pts, patients; SCr, serum creatinine.

being predominant within Group 1, that is, nephritic flare group (60.7% (17/28) Group 1 vs 13% (6/46) Group 2 vs 11% (2/18) Group 3, p<0.001)).

Six out of 25 patients (24%) who had developed ESKD died along the study, vs 5/67 (7.4%) patients who had not developed ESKD (p=0.06).

Demographic, histological and clinical features of patients according to development of ESKD are reported in table 2.

Predictors of ESKD at second kidney biopsy and comparison with first biopsy

At univariate analysis, several clinical and histological factors present at second biopsy were associated with ESKD (table 3). Cox multivariable analysis highlighted CI (HR (95%CI): 1.41 (1.09 to 1.82), p=0.008), AI 1.20 (1.03 to 1.41), p=0.022), and proteinuria at second biopsy (1.22 (1.04 to 1.42), p=0.013) to be independent predictors of ESKD (table 3). Values of CI >4, AI≥2 and

Table 2 Demographic, histological and clinical features at second renal biopsy according to renal outcome (reported as mean (SD) if not otherwise specified)

Variable	ESKD	Non ESKD	P value
Number of patients	25	67	–
Age at second biopsy	35.9 (11.5)	34.7 (10.3)	0.6
Females, n (%)	21 (84)	59 (88)	0.7
Arterial hypertension, n (%)	22 (88)	1 (1.5)	<0.001
Proteinuria, g/24 hours	5.4 (5.02)	2.8 (2.6)	0.02
SCr mg/dL	2.2 (1.9)	1.05 (0.52)	0.01
GFR ml/min/1.73 m^2	51.3 (35.6)	79.8 (31.6)	0.001
C3 mg/dL	67.6 (30.9)	67.2 (28.6)	0.9
C4 mg/dL	18.2 (22.6)	17.4 (20.1)	0.9
Antibodies			
Anti-dsDNA in 87 pts (%)	22/25 (88)	57/62(92)	0.6
aPL in 84 pts (%)	13/22 (59)	43/62 (69.3)	0.4
Anti-ENA in 69 pts (%)	5/12 (41.6)	30/57 (52.6)	0.5
Prednisone dosage, mg/day	8.9 (8.5)	8.5 (6.1)	0.4
Antimalarials*, n (%)	1/21 (4.8)	15/53 (28.3)	0.030
Immunosuppression†, n (%)	5/20 (25)	28/52 (53.8)	0.036
MMF	0	18	0.01
AZA	5	11	0.7
CNI	1	8	0.25
CYC	1	0	0.45
Class ISN/RPS2003‡			
II	1	10	0.15
III	2	8	0.6
IV	18	26	0.005
V	1	15	0.038
Mixed	6	17	0.7
AI	5.7 (3.6)	4.33 (3.8)	0.2
CI	4.8 (2.9)	3.7 (2.09)	0.09

Comparison between continuous variables: one way ANOVA with Bonferroni correction; comparison between proportions: χ^2 test.
*Data available for 74 patients.
†Data and referred percentages are calculated on 72 patients.
‡Data for 89 patients; one patient with class I is not listed.
aPL antiphospholipid antibodies; eGFR glomerular filtration rate; AI, activity index; ANOVA, analysis of variance; anti-dsDNA, antibodies antidouble stranded DNA; anti-ENA, antiextractable nuclear antigens antibodies; AZA, azathioprine CNI calcineurin inhibitors; CI, chronicity index; CYC, cyclophosphamide; ESKD, end-stage kidney disease; ISN/RPS, International Society of Nephrology/Renal Pathology Society; MMF, mycophenolate mofetil; pts, patients; SCr, serum creatinine.

proteinuria ≥3.5 g/day identified patients at higher risk for ESKD at Mantel-Cox analysis (figure 1 and online supplemental table S1).

Importantly, histological indexes and proteinuria at first kidney biopsy did not predict subsequent ESKD (AI: 95% CI 0.88 to 1.1, p=0.8; CI: 95% CI 0.08 to 1.4, p=0.6; proteinuria 95% CI 0.9 to 1.1, p=0.8), whereas arterial hypertension did (HR 7.20 (2.11 to 24.52), p=0.002). Independent predictors at first and second biopsy are summarised in online supplemental table S1).

In order to check for overfitness and to test the influence of other clinically meaningful independent variables at second kidney biopsy, we tested different Cox regression models (online supplemental table S2) which confirmed the above stated predictors for ESKD in the whole patient cohort.

Itemised data for AI and CI scores at second biopsy were available for a subgroup of patients (n=29, including 11 patients developing ESKD). Within this subgroup, glomerular activity and especially presence of subendothelial deposits was associated with increased ESKD risk while tubular chronic lesions showed a trend towards increased ESKD risk, at univariable Cox regression analysis (table 4).

Histological class switch from first to second kidney biopsy

At first kidney biopsy, 3 (3.2%) patients displayed class I or II, 15 (16.3%) class III, 49 (53.3%) class IV, 14 (15.2%) class V, 11 mixed (5 III+V and 6 IV+V). At second kidney biopsy, histological record was available for 89/92 patients and showed a total amount of class switch of 50.5% (45/89, (online supplemental table S3).

Table 3 Predictors of ESKD at second kidney biopsy

	Univariable analysis			Multivariable analysis		
Variable	**HR**	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Age at second biopsy	1.01	0.94 to 1.10	0.33	–	–	–
Male gender	2.21	0.74 to 6.64	0.16	–	–	–
Time elapsed between first and second biopsy	1.05	0.91 to 1.13	0.22	–	–	–
Use of IS at time of second biopsy	0.82	0.28 to 2.33	0.72	–	–	–
Serum creatinine mg/dL	1.61	1.34 to 1.91	<0.001	1.20	0.79 to 1.85	0.39
eGFR mL/min/1.73 m^2	0.97	0.95 to 0.98	0.001	–	–	–
Proteinuria g/day	1.23	1.11 to 1.36	<0.001	**1.22**	**1.04 to 1.42**	**0.013**
Arterial hypertension	11.71	1.6 to 87.1	0.016	4.9	0.6 to 40.4	0.14
Proliferative Class ISN/RPS 2003	7.41	0.99 to 54.9	0.050	–	–	–
AI	1.14	0.99 to 1.32	0.059	**1.20**	**1.03 to 1.41**	**0.022**
CI	1.32	1.02 to 1.51	0.030	**1.41**	**1.09 to 1.82**	**0.008**

Variables included in the model: Serum Creatinine, hypertension, proteinuria, AI, CI.
Significant variables at multivariable analysis are highlighted in bold
AI, activity index; CI, chronicity index; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; IS, immunosuppressants; ISN/RPS, International Society of Nephrology/Renal Pathology Society.

Class IV remained stable in 29/49 (59%) cases, while patients with initial class I and II progressed to a proliferative class. No independent predictors of class switch were found.

Modification of CI and AI between first and repeated biopsy

The overall mean CI significantly increased (mean±SD, 1.43±1.61 vs 4.11±2.42, p<0.001) while the AI decreased (7.04±3.89 vs 4.83±3.43, p=0.002) from the first to the second biopsy. The greatest CI increase occurred in Group 1, being as well apparent in Group 3 (lack of response) (online supplemental figure S1A), which may at least partially account for the increased proteinuria in this group. Proportions of patients displaying AI≥2 across groups are shown in online supplemental figure S1B (Group 1, 87.5%; Group 2 65.5%; Group 3 61.8%; p<0.001).

DISCUSSION

This study, performed on a large real-life cohort of patients with LN with persistently active or flaring LN on initial treatment, has shown that the histological findings at second diagnostic biopsy were the best predictors of ESKD. Importantly,

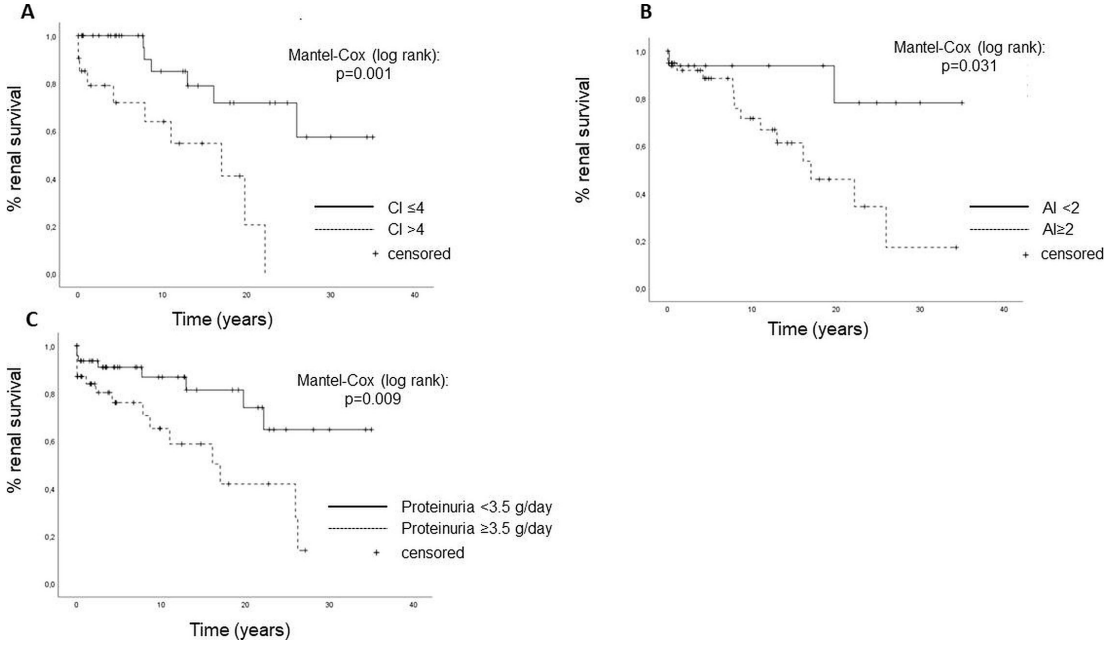

Figure 1 Risk graphs divided by histological parameters and proteinuria show critical values. (A) Patients with CI>4 or (B) AI≥2 at second biopsy are at significantly higher risk of ESKD during their clinical course. (C) Patients with proteinuria ≥3.5 g/day at second biopsy are at significantly higher risk of ESKD during their clinical course. Survival calculated with Mantel-Cox long-rank test. AI, activity index; CI, chronicity index; ESKD, end stage kidney disease.

Table 4 Subgroup analysis (Cox regression univariable models) testing itemised AI and CI scores as predictors of ESKD

Variable	HR	95% CI	P value
Glomerular activity	**1.384**	**1.029 to 1.861**	**0.032**
Subendothelial deposits	**4.824**	**1.129 to 16.301**	**0.033**
Necrosis/karyorrhexis	1.247	0.329 to 4.273	0.746
Endocapillary hypercellularity	2.196	0.472 to 10.207	0.316
Cellular crescents	2.890	0.250 to 9.825	0.089
Leucocyte infiltration	2.898	0.766 to 10.940	0.117
Glomerular chronicity	1.302	0.582 to 2.915	0.520
Tubular activity	0.649	0.270 to 1.561	0.334
Tubular chronicity	1.628	0.920 to 2.874	0.090
Tubular atrophy	1.220	0.354 to 4.203	0.750
Interstitial fibrosis	0.712	0.143 to 3.553	0.679

Results from proportional hazards regression (Cox regression) univariable models, with ESKD as dependent variable and NIH itemised AI or CI score (ref 18) as predictors under investigation. Signifcant variables are highlighted in bold.
AI, activity score; CI, chronicity score; ESKD, end stage kidney disease.

AI and CI at second biopsy outperformed the same parameters evaluated at the first biopsy in terms of prediction of ESKD.

The concept of findings at second biopsy being more predictive for renal survival has been acknowledged in the last decade,[6 12 13 16 22 23] with most data concerning prediction of renal relapses rather than terminal renal disease.[5 6] In fact, despite the experience linking renal damage to worsened renal outcome,[4 6] the true prognostic value of histological lesions in the long run is hard to assess both in clinical trials and real-life cohorts,[11 14] likely due to an extensive follow-up required to tell apart hard endpoints such as ESKD.

We have pinpointed high activity at second biopsy as an independent predictor of ESKD. Notably, an AI ≥2 at second biopsy conferred a significant higher risk of ESKD, thereby suggesting that even a moderate histological activity contributes to kidney failure and not only to susceptibility to subsequent flaring. Our observations are consistent with recently published data on increased AI being associated with higher probability of renal function deterioration defined as ≥120% increase of baseline serum creatinine;[6] here, we further show this association to hold true for ESKD intended as actual kidney replacement therapy, therefore stressing the need for a profound control of histological activity within an optimised therapeutic decision making. Interestingly, a subgroup analysis highlighted glomerular activity and particularly the presence of subendothelial deposits as major drivers of AI hazardousness.

In line with previous studies,[1–6] we have as well shown that high chronicity at second kidney biopsy heralds future ESKD, with values of CI >4 conferring the highest risk. Among itemised domains, tubular chronic lesions showed a trend towards increased ESKD risk in the subgroup analysis, thereby confirming tubular-interstitial lesions as major contributors to renal damage.

It is worth noting that neither active nor chronic lesions at the first biopsy in our cohort could predict ESKD, which differs from earlier cohorts yet adds up to more recent data.[6] Hence, it may be reasoned that the lack of prognostic value of histology at first biopsy may be due to a timely diagnosis of SLE and LN, entailing an earlier performance of the diagnostic biopsy in the last few decades,[12 24] and to a more aggressive treatment in severe forms,[24] which possibly dampens their long-term impact.

Besides, proteinuria at second kidney biopsy resulted as an independent predictor of ESKD and was not correlated to either serum creatinine or histological parameters. Particularly, nephrotic proteinuria ≥3.5 g/day conferred a higher risk in our cohort. In keeping with observations concerning AI and CI, proteinuria at diagnostic biopsy did not predict ESKD. This may be explained by the initial proteinuria reflecting ongoing inflammation, while acting as a persistent damaging stimulus over time,[25] independent of the underlying histology. While the association between low proteinuria and favourable outcome has been confirmed,[7] the long-term predictive value of increased proteinuria is still debated, as this parameter was not found to predict adverse long-term prognosis in other cohorts.[6] Differences in duration of follow-up and patient groups may account for different observations and should raise awareness on the interpretation of clinical findings.

The rate of class switch between first and second biopsy was around 50%, in keeping with other cohorts,[26–28] while proliferative classes remained mostly stable. Because an increased AI at second biopsy signified an increased risk in ESKD, this highlights the importance of performing a second kidney biopsy in patients with suboptimal/no response or flaring LN as a tool for long-term risk stratification, regardless of LN class be changed or not.

This study has both strengths and limitations. Among the latter, most are connected to its real-life nature, encompassing the retrospective analysis of prospectively available data and the heterogeneity of the underlying reasons for a repeated kidney biopsy. Additionally, a detailed characterisation of the glomerular and tubule-interstitial compartment in the whole cohort was lacking. On the other hand, this is among the largest studies on the topic of second kidney biopsy in LN in the real-world, providing handy tools for patient stratification which may help to consolidate second biopsy as an advised clinical act.

CONCLUSION

In summary, we have shown that both high activity and chronicity at second, but not at first, diagnostic kidney biopsy predict ESKD in patients with LN and lack of response or flaring after standard therapy. Besides, proteinuria is confirmed as an independent damaging factor for the kidney which may benefit from additional normalising approaches. Altogether, our data identify easy-to-interpret parameters at second kidney biopsy which may significantly impact on

patient prognosis and highlight the need of multicentric studies assessing the indispensability and cost-effectiveness of repeating kidney biopsy in LN, at least in specialised centres, to improve prognostic stratification and prevent long-term deterioration of renal function.

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Contributors Study conception and design: AD, GM, MG, LI. Data collection: all authors. Data analysis: MG, GM, MZ. Data interpretation: MG, GM, AD, LI. Critical reading for important intellectual content: all authors. AD is held responsible for the overall content as guarantor.

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Patient consent for publication Not applicable.

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