Systemic lupus erythematosus : from diagnosis to prognosis

Rijnink, E.C.

Chapter 5

Clinical and Histopathologic Characteristics Associated with Renal Outcomes in Lupus Nephritis

Emilie C. Rijnink, Y.K. Onno Teng, Suzanne Wilhelmus, Mathilde Almekinders, Ron Wolterbeek, Karlien Cransberg, Jan A. Bruijn, Ingeborg M. Bajema

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ABSTRACT

Background

The prognostic significance of histopathologic (sub)classes in the current classification of lupus nephritis (LN) is controversial. We analysed clinical and histopathologic predictors of renal outcomes in LN outside the framework of the classification.

Methods

Variables (50 histopathologic and 10 clinical) were tested in mixed, linear, and Cox regression models for their association with renal flare, end-stage renal disease (ESRD), and estimated glomerular filtration rate (eGFR) during follow-up (1, 5, and 10 years) in 105 LN patients biopsied from 1987–2011.

Results

During median follow-up of 9.9 years (25^{th}–75^{th} percentile, 5.9−13.8), 47 patients experienced a renal flare and 21 progressed to ESRD. Renal flare was predicted by fibrinoid necrosis (hazard ratio [HR] 1.04 per % [95% confidence interval [CI], 1.00 to 1.07]) and non-Caucasian race (HR 2.23 [95% CI, 1.23 to 4.04]). ESRD was predicted by fibrinoid necrosis (HR 1.08 per % [95% CI, 1.02 to 1.13]), fibrous crescents (HR 1.09 per % [95% CI, 1.02 to 1.17]), interstitial fibrosis/tubular atrophy (IF/TA) ≥25% (HR 3.89 [95% CI, 1.25 to 12.14]), eGFR at baseline (HR 0.98 per mL/min/1.73 m^2 [95% CI, 0.97 to 1.00]), and non-Caucasian race (HR 7.16 [95% CI, 2.34 to 21.91]). A higher mean eGFR during follow-up was associated with normal glomeruli (+0.2 mL/min/1.73 m^2/% [95% CI, 0.1 to 0.4]). Like ESRD, a lower eGFR during follow-up was associated with fibrous crescents, IF/TA ≥25%, and non-Caucasian race, as well as with cellular/fibrocellular crescents (−0.4 mL/min/1.73 m^2/% [95% CI, −0.6 to −0.2]) and age (−0.8 mL/min/1.73 m^2/year [95% CI, −1.2 to −0.4]).

Conclusions

The LN classification should include an index of evidence-based prognosticators. Awaiting validation of a formal index, we suggest that at least fibrinoid necrosis, fibrous crescents, and IF/TA warrant explicit independent scoring to assess the risk of progressive renal dysfunction in conjunction with clinical findings.

INTRODUCTION

The disease manifestations and outcomes in lupus nephritis (LN) are heterogeneous, but 10–30% of patients progress to end-stage renal disease (ESRD) within 15 years.[1, 2] The prognosis can usually be improved by immunosuppression; although, there are severe and sometimes lethal adverse effects.[3] There is a constant need for refined and novel indicators to help clinicians predict outcomes and determine when intensive immunosuppression should be initiated for individual LN patients.

Currently, clinical guidelines for LN[4-6] reserve intensive immunosuppression primarily for patients with class III or IV (±V) LN according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification.[7] Although the ISN/RPS classification is useful in terms of standardisation and reproducibility of diagnosis,[8-10] studies concerning the power of this classification in predicting disease outcome reported conflicting results.[9, 11-18] Conflicting results may be due to the grouping of a wide variety of prognostically, pathogenically, and chronically different glomerular lesions in broad classes, thereby assuming that the prognosis of individuals within classes is equal regardless of the type of lesions.

A revision of the ISN/RPS classification is called for.[19, 20] An evidence-based approach, similar to the Oxford classification of IgA nephropathy would be the resolution for a future classification.[21, 22] In the current study, we aimed to identify evidence-based clinical and histopathologic predictors of renal outcome in LN outside the framework of the ISN/RPS classification.

METHODS

We collected a cohort of patients, biopsied from 1987–2011, with biopsy-confirmed LN from the pathology archives at the Leiden University Medical Center (LUMC). Inclusion criteria were: patients with a first renal biopsy available for re-evaluation with ≥5 scorable glomeruli, fulfilling ≥4 of the revised American College of Rheumatology[23, 24] or Systemic Lupus International Collaborating Clinics[25] classification criteria for SLE, and with clinical follow-up at the LUMC, Bronovo hospital (The Hague, the Netherlands), or Erasmus Medical Center (Rotterdam, The Netherlands). In accordance with the ethics committee guidelines at the LUMC, all patient data were coded and kept anonymous. Biopsies were processed for light and immunofluorescence microscopy according to standard techniques in our centre, including haematoxylin and eosin, periodic acid-Schiff, and methenamine-silver staining. Immunofluorescence reports were originally prepared by four experienced nephropathologists who consistently scored immunofluorescence intensity on a 0–3+ scale.

Histopathology definitions and scoring

Definitions of histopathologic lesions are shown in **Appendix 5.1**. Slides were scored by an experienced nephropathologist (IMB). All glomerular variables were determined for each scorable glomerulus separately and tubulointerstitial parameters were scored categorically (**Appendix 5.1**). We treated glomerular parameters as continuous variables and expressed them as the percentage involved of all scorable glomeruli. We considered 50 histopathologic variables (**Figure 1**). To reduce the number of candidate variables, we excluded lesions with a low prevalence (occurring in ≤5 patients) and included only one of two strongly correlated variables ($r/\rho > 0.8$). The decision on which of the correlated variables to be included was based on relevance and ease of scoring.

Figure 1 Histopathologic variables that were assessed.

Glomerular variables	
Global sclerosis	% glomeruli with global sclerosis
Normal glomeruli	% glomeruli noted as normal
Normal glomeruli/minimal leukocyte influx	% glomeruli noted as normal/containing 1−3 leukocytes
Minimal leukocyte influx	% glomeruli with 1−3 leukocytes
Ischemic glomeruli	% glomeruli noted as ischemic
Mesangial 1†	Mean score for mesangial hypercellularity
Mesangial 2	% glomeruli with mesangial hypercellularity
Mesangial 3	% glomeruli with mesangial matrix expansion
Segmental sclerosis	% glomeruli with segmental sclerosis
Endocapillary 1	% glomeruli with segmental endocapillary hypercellularity
Endocapillary 2	% glomeruli with segmental/global endocapillary hypercellularity
Endocapillary 3†	% glomeruli with segmental/global endocapillary hypercellularity/minimal leukocyte influx
Endocapillary 4	% glomeruli with global endocapillary hypercellularity
Endocapillary inflammatory infiltrate†	% glomeruli with influx of ≥4 inflammatory cells
Endocapillary granulocytes	% glomeruli with ≥4 endocapillary granulocytes
Endocapillary lymphocytes	% glomeruli with ≥4 endocapillary lymphocytes
Endocapillary monocytes†	% glomeruli with ≥4 endocapillary monocytes
Endothelial swelling†	% glomeruli with endothelial cell swelling
Wire loops	% glomeruli with wire loops
Adhesions	% glomeruli with adhesions
Fibrinoid necrosis	% glomeruli showing fibrinoid necrosis
Extracapillary 1†	% glomeruli with cellular crescents
Extracapillary 2	% glomeruli with cellular/fibrocellular crescents
Extracapillary 3†	Mean cellular + fibrocellular crescent score
Extracapillary 4	% glomeruli showing fibrous crescents
Extracapillary 5†	Mean fibrous crescent score
Extracapillary 6	% glomeruli with fibrocellular + fibrous crescents
Karyorrhexis	% glomeruli with karyorrhexis

Figure 1 Continued.

Glomerular variables	
Microthrombi*	% glomeruli with microthrombi
Pseudothrombi*	% glomeruli with pseudothrombi
Double contours	% glomeruli with double contours
Spikes/vacuoles	% glomeruli with spikes/vacuoles
Tubulointerstitial variables	
Tubular atrophy‡	Tubular atrophy score
Acute tubular injury*	Acute tubular injury (+/-)
Tubular casts	Tubular casts (+/-)
Tubular luminal macrophages	Tubular luminal macrophages (+/-)
Tubular reabsorption droplets	Tubular reabsorption droplets (+/-)
Tubular regeneration*	Tubular regeneration (mitoses) (+/-)
Tubulitis*	Tubulitis (+/-)
Interstitial infiltration	Interstitial infiltration score
Interstitial lymphocytes†	Interstitial lymphocyte-dominant infiltrate (+/-)
Interstitial granulocytes	Interstitial granulocyte-dominant infiltrate (+/-)
Interstitial fibrosis‡	Interstitial fibrosis score (+/-)
Focal cortical atrophy*	Focal cortical atrophy (+/-)
Vascular variables	
Vasculitis*	Vasculitis (+/-)
Fibrinoid necrosis in large vessels*	Fibrinoid necrosis in large vessels (+/-)
Thrombosis*	Thrombosis (+/-)
Hyaline arteriolosclerosis*	Hyaline arteriolosclerosis (+/-)
Fibrous intimal hyperplasia*	Fibrous intimal hyperplasia (+/-)
Arterial intimal fibrosis	Arterial intimal fibrosis (+/-)

For definitions of histopathologic lesions, see Appendix 5.1. Percentages represent the proportion of involved scorable glomeruli. Crescent score: a multiplication factor of 1 was used for segmental crescents, and 2 for circumferential crescents. * Excluded from analyses because of low prevalence (≤5 patients). † Excluded from analyses because the variable was strongly correlated with another variable ($r/\rho>0.8$). ‡ Interstitial fibrosis and tubular atrophy were combined to form the composite variable "IF/TA" (interstitial fibrosis/tubular atrophy; whichever was the higher value).

Clinical dataset

The following clinical parameters were recorded for each patient at the time of biopsy$_0$: age, sex, race, mean arterial pressure ([MAP] diastolic blood pressure + ⅓ pulse pressure), antihypertensive medication, previous immunosuppression, induction immunosuppression, time since SLE diagnosis, estimated glomerular filtration rate (eGFR), 24h proteinuria, erythrocyturia, and the presence of antinuclear, anti-double stranded DNA, and antiphospholipid antibodies. The Cockcroft-Gault[26] (normalised to a body surface area of 1.73 m^2 and Schwartz [27-29] formulas were used to calculate eGFR for adults and children, respectively. The eGFR and 24h proteinuria were registered at 1, 5, and 10 years (±0.5 year) and at

the last follow-up. Clinical data were studied throughout follow-up for the occurrence of renal flare and/or ESRD.

Brief study outcomes and statistical methods

A complete outline of this section is given in **Appendix 5.2**. Outcomes were studied in two settings: (i) the complete cohort of patients with all observed LN classes who received various therapies and (ii) a subset of patients with class III or IV (±V) LN who received induction immunosuppression including cyclophosphamide (CYC), mycophenolate mofetil (MMF), or azathioprine (AZA). Prespecified variables for multivariable analyses were: variables from the reduced histopathology dataset; interaction terms of these with race, age, and induction immunosuppression; and the clinical variables sex, race, time since SLE diagnosis, age_0, $proteinuria_0$, $erythrocyturia_0$, MAP_0, induction immunosuppression, and decade during which the patient was biopsied.

Renal flare and ESRD

Time to first LN flare was calculated for patients who achieved (partial) remission from the date of biopsy until the date of flare for patients who reached this endpoint; the remaining patients were censored at the last follow-up or at the time of ESRD. Time to ESRD was calculated analogously. Patients who reached ESRD before 10 years follow-up were regarded as having eGFR=0 mL/min/1.73 m^2 at the remaining time points. Multivariable Cox proportional-hazards models included the prespecified variables and were simplified by stepwise removal of the least significant variables.

eGFR during follow-up

The extent by which variables were associated with irreversible nephron loss was investigated by modelling eGFR during follow-up. The prespecified variables were tested for their potential to predict a change in the intercept of the adjusted average level of decline in multivariable random intercept/slope linear mixed-effects models, which were simplified by removing the least significant variables (Wald test) and comparing the goodness of fit of nested models (maximum likelihood ratio test).

Progressive eGFR decline

To investigate progressive eGFR decline that did not necessarily result in ESRD and/or renal flare, variables were analysed in association with progressive eGFR decline over 1, 5, and 10 years relative to its linear prediction based upon $eGFR_0$.

Normally distributed data were expressed as mean ± standard deviation (SD). Non-normally distributed data were expressed as median (25^{th}–75^{th} percentile). Correlations between clinical and histopathologic variables were assessed using Pearson and Spearman tests, as appropriate. Given the chance of false-positives by multiple correlations of histopathologic variables, Bonferroni correction was performed (**Appendix 5.3**). All other *P*-values were two-tailed and considered significant at $P<0.05$. All analyses were performed using SPSS 23.0 (IBM, Armonk, New York).

RESULTS

We retrieved 293 reports of LN patients from the pathology archives at the LUMC. Of these, 134 patients were not followed at any of the specified centres, and 54 did not have a retrievable renal biopsy or their biopsy was of insufficient quality. Thus, 105 patients were included. Baseline clinical and laboratory findings are summarised in **Table 1** and histopathologic findings in **Table 2.** A complete overview of histopathologic findings and assessment of scorable glomeruli are found in **Appendix 5.4**. The histopathology dataset (**Figure 1**) was reduced from 32 to 27 glomerular and 18 to 9 tubulointerstitial variables by excluding lesions occurring in ≤5 patients, excluding one of two strongly correlated variables, and combining interstitial fibrosis and tubular atrophy (IF/TA, see **Appendix 5.3**). Correlations between histopathologic variables and MAP_0, $eGFR_0$, and $proteinuria_0$ are shown in **Appendix 5.5**.

Treatment and outcome

The median follow-up was 9.9 years (25^{th}–75^{th} percentile, 5.9–13.8). Induction immunosuppression was given to 102 patients (**Table 1**). Patients biopsied before 2000 received significantly more frequently AZA and less often CYC or MMF than patients biopsied after 2000 (all $P<0.001$). Five patients required dialysis at the time of renal biopsy and did not regain renal function during follow-up. Of 100 patients without ESRD at the time of biopsy, 99 achieved (partial) remission; of these, 47 experienced a renal flare during follow-up. Fifteen of the patients who experienced a renal flare eventually developed ESRD. In addition to the five patients with ESRD at the time of biopsy, 16 patients progressed to ESRD and did not regain renal function (**Figure 2**). Five patients died during follow-up due to renal failure (n=1), infection (n=2), cardiovascular disease (n=1), and trauma (n=1). A comparison between patients biopsied before and after 2000 revealed no difference in renal survival ($P=0.34$) and renal flare rate ($P=0.66$).

Table 1 Clinical characteristics of 105 patients with LN at the time of renal biopsy.

Characteristic	All patients (n=105)
Decade of renal biopsy, n (%)	
1980–1989	7 (7)
1990–1999	36 (34)
2000–2009	56 (53)
2010–2011	6 (6)
Age, years	
Mean ± SD	29.8 ± 13.2
<18 years, n (%)	22 (21)
Females, n (%)	83 (79)
Race, n (%)	
Caucasian	68 (65)
Asian	24 (23)
Afro-Caribbean	13 (12)
Previous diagnosis of SLE, n (%)	77 (73)
Years since SLE diagnosis, median (25^{th}–75^{th} percentile)	4.2 (1.1–7.9)
Diagnosis of SLE at the time of biopsy, n (%)	28 (27)
BMI, kg/m^2	
Mean ± SD	22.4 ± 4.4
Diastolic BP ≥90 mm Hg, n (%) [total=101]	31 (31)
MAP (mm Hg)	
Mean ± SD	97 ± 17
Taking antihypertensive medication, n (%) [total=103]	34 (33)
Treated with RAS blockade, n (%) [total=103]	19 (18)
Induction immunosuppression, n (%)	
No immunosuppression	3 (3)*
CS alone	4 (4)†
CS + CYC NIH	26 (25)
CS + CYC EuroLupus	27 (26)
CS + MMF	14 (13)
CS + AZA	31 (30)
$eGFR_0$, mL/min/1.73 m^2	
Mean ± SD	76.5 ± 36.2
CKD stage, n (%)	
1	36 (34)
2	34 (32)
3	24 (23)
4	9 (9)
5	2 (2)
$Proteinuria_0$, g/24h	
Median (25^{th}–75^{th} percentile)	2.48 (1.25–5.00)
Erythrocyturia, n	
−/+/++/+++	5/22/22/56
Previous immunosuppression, n (%)	19 (18)
ANA, n (%)	104 (99)
Anti-dsDNA, n (%) [total=101]	77 (76)
Antiphospholipid antibodies, n (%) [total=57]	29 (51)

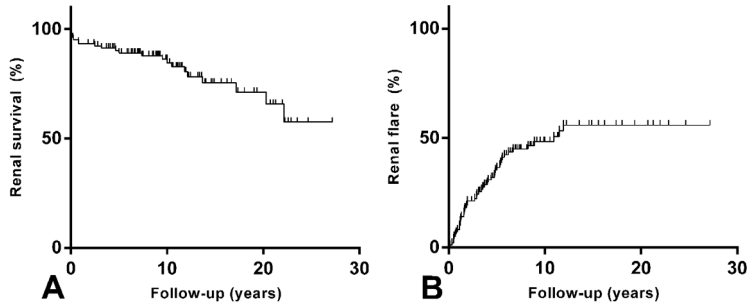
The number of patients with valid registrations is indicated if data were missing for some patients. -/+/++/+++, erythrocyturia score ([-] 0-18 erythrocytes/μL; [+] 19–25 erythrocytes/μL; [++] 26–40 erythrocytes/μL; [+++] >40 erythrocytes/μL). ANA, anti-nuclear antibody; anti-dsDNA, anti-double stranded DNA antibody; AZA, azathioprine; BP, blood pressure; CS, corticosteroids; CYC, cyclophosphamide; $eGFR_0$, eGFR at the time of biopsy; MAP, mean arterial pressure; MMF, mycophenolate mofetil; RAS, renin-angiotensin system. *Patients (n=3) with class III, IV, and III+V LN. †Patients with class III/IV (n=2) and class V (n=2) LN.

Table 2 Distribution of selected histopathologic lesions in 105 patients with LN.

Scorable glomeruli, n	
Median (25^{th}–75^{th} percentile)	13 (10–19)
ISN/RPS class, n (%)	
I	1 (1)
II	3 (3)
III	24 (23)
IV	60 (57)
III/IV + V	12 (11)
V	5 (5)
Normal glomeruli, n (%)	
absent	47 (45)
1–24% of glomeruli	37 (35)
25–49% of glomeruli	16 (15)
≥50% of glomeruli	5 (5)
Global sclerosis, n (%)	
absent	71 (68)
1–24% of glomeruli	21 (20)
25–49% of glomeruli	10 (10)
≥50% of glomeruli	3 (3)
Mesangial hypercellularity, n (%)	
absent	22 (21)
1–24% of glomeruli	38 (36)
25–49% of glomeruli	34 (32)
≥50% of glomeruli	11 (10)
Endocapillary hypercellularity, n (%)	
absent	12 (11)
1–24% of glomeruli	26 (25)
25–49% of glomeruli	17 (16)
≥50% of glomeruli	50 (48)
Crescents, n (%)	
absent	38 (36)
1–24% of glomeruli	30 (29)
25–49% of glomeruli	24 (23)
≥50% of glomeruli	13 (12)
Interstitial infiltration, n (%)	
absent	56 (53)
1–24% of glomeruli	37 (35)
25–49% of glomeruli	4 (4)
≥50% of glomeruli	8 (8)
IF/TA, n (%)	
absent	67 (64)
1–24% of glomeruli	26 (25)
25–49% of glomeruli	8 (8)
≥50% of glomeruli	4 (4)

IF/TA, interstitial fibrosis or tubular atrophy.

Figure 2 Probabilities of renal survival (A, n=105) and renal flare (B, n=99) among patients in the cohort.



Time to end-stage renal disease (ESRD) and time to first renal flare are depicted according to the Kaplan-Meier method. A: during follow-up, 21 patients progressed to ESRD. The probability of renal survival was 93% at 1 year, 90% at 5 years, 85% at 10 years, and 71% at 20 years of follow-up. B: only patients were considered who achieved (partial) remission after renal biopsy and were not censored because they already reached ESRD. During follow-up, 47 patients experienced a renal flare. The probability of renal flare was 8% at 1 year, 35% at 5 years, and 48% at 10 years.

Predictors of renal outcome

Predictors of renal outcome were similar for the complete cohort and the subset (**Tables 3**, **4**, and **Appendix 5.6**). Below, parameter estimates refer to the complete cohort.

Renal flare and ESRD

Non-Caucasian race (HR 2.23 [95% confidence interval (95% CI), 1.23 to 4.04]) and fibrinoid necrosis (HR 1.04 for each percent of glomeruli [95% CI, 1.00 to 1.07]) independently predicted renal flare (**Table 3**). The following variables independently predicted ESRD (**Table 3**): non-Caucasian race (HR 7.16 [95% CI, 2.34 to 21.91]), $eGFR_0$ (HR 0.98 for each mL/min/1.73 m^2 [95% CI, 0.97 to 1.00]), fibrous crescents (HR 1.09 for each percent of glomeruli [95% CI, 1.02 to 1.17]), fibrinoid necrosis (HR 1.08 for each percent of glomeruli [95% CI, 1.02 to 1.13]), and the presence of IF/TA ≥25% (HR 3.89 [95% CI, 1.25 to 12.14]).

eGFR during follow-up

The adjusted mean eGFR at the time of renal biopsy was 116.5 mL/min/1.73 m^2, with an average change of −0.7 mL/min/1.73 m^2 per year (**Table 4**). Cellular/fibrocellular crescents independently predicted an overall lower eGFR (i.e. lower adjusted mean eGFR at baseline and during follow-up) of −0.4 mL/min/1.73 m^2/%glomeruli (95% CI, −0.6 to −0.2), as did fibrous crescents (−1.4 mL/min/1.73 m^2/%glomeruli [95% CI −2.4 to −0.5]) and the presence of IF/TA ≥25% (−40.5 mL/min/1.73 m^2 [95% CI, −56.2 to −24.8]). Conversely, each percent of normal glomeruli, including glomeruli with 1−3 leukocytes, independently predicted an overall higher eGFR (+0.27 mL/min/1.73 m^2/%glomeruli [95% CI, 0.1 to 0.4]). Clinically, non-Caucasian race and age_0 independently predicted an overall lower eGFR (−11.4 mL/min/1.73 m^2 [95% CI, −21.9 to −0.8] and −0.7 mL/min/1.73 m^2/year [95% CI, −1.2 to −0.4], respectively).

Table 3 Multivariable prediction models for renal flare and end-stage renal disease (ESRD).

Model	ISN/RPS Class I–V (n=105)		ISN/RPS Class III/IV (±V)* (n=91)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Renal flare (n=99)				
Non-Caucasian	2.23 (1.23; 4.04)	0.008	2.08 (1.09; 3.98)	0.03
% Fibrinoid necrosis (glomerular)	1.04 (1.00; 1.07)	0.04	1.04 (1.00; 1.08)	0.04
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
ESRD (n=105)				
Non-Caucasian	7.16 (2.34; 21.91)	0.001	9.12 (2.85; 29.22)	<0.001
Age_0, y	1.02 (0.99; 1.06)	0.18	1.02 (0.99; 1.06)	0.23
$eGFR_0$, mL/min/1.73 m^2	0.98 (0.97; 1.00)	<0.05	0.99 (0.97; 1.00)	0.12
% Fibrinoid necrosis (glomerular)	1.08 (1.02; 1.13)	0.004	1.07 (1.02; 1.13)	0.01
% Fibrous crescents	1.09 (1.02; 1.17)	0.02	1.10 (1.02; 1.19)	0.01
IF/TA ≥25%	3.89 (1.25; 12.14)	0.02	4.53 (1.40; 14.73)	0.01

* Patients with class III/IV (±V) LN who received induction immunosuppressive treatment with cytotoxic drugs were analysed separately from the complete cohort. CI, confidence interval; $eGFR_0$, eGFR at the time of renal biopsy; HR, hazard ratio; IF/TA, interstitial fibrosis/tubular atrophy.

Table 4 Multivariable prediction model for the course of eGFR during follow-up†.

Model	ISN/RPS Class I–V (n=105)		ISN/RPS Class III/IV (±V)‡ (n=91)	
	β (95% CI)	*P*	β (95% CI)	*P*
(Intercept)	116.5 (100.1; 132.8)	<0.001	116.4 (98.9; 133.9)	<0.001
(Time, y)	−0.7 (−1.5; 0.0)	0.06	−0.6 (−1.4; 0.2)	0.13
Baseline predictors				
Non-Caucasian	−11.4 (−21.9; −0.8)	0.04	−13.5 (−25.2; −1.7)	0.03
Age_0, y	−0.8 (−1.2; −0.4)	<0.001	−0.8 (−1.2; −0.4)	<0.001
% Normal glomeruli/minimal leukocyte influx	0.2 (0.1; 0.4)	0.01	0.2 (0.0; 0.5)	0.03
% Cellular/fibrocellular crescents	−0.4 (−0.6; −0.2)	0.001	−0.4 (−0.6; −0.1)	0.003
% Fibrous crescents	−1.4 (−2.4; −0.5)	0.004	−1.6 (−2.6; −0.5)	0.004
IF/TA ≥25%	−40.5 (−56.2; −24.8)	<0.001	−41.4 (−58.3; −24.4)	<0.001

β indicates eGFR in mL/min/1.73 m^2. eGFR at time t is given by: eGFR(t) = intercept + β_{Time}*t + Z, where Z is the value given by the baseline predictors of the patient: $Z = \beta_{Age0}*Age_0 + \beta_{\%Normal\ glomeruli/minimal\ leukocyte\ influx}$*%Normal glomeruli/minimal leukocyte influx + $\beta_{\%cellular/fibrocellular\ crescents}$*%cellular/fibrocellular crescents + $\beta_{\%fibrous\ crescents}$*%fibrous crescents + $\beta_{non\text{-}Caucasian}$ (if Non-Caucasian) + $\beta_{IF/TA\ \geq 25\%}$ (if IF/TA ≥25%). † Mixed model analysis. ‡ Patients with class III/IV (±V) LN who received induction immunosuppression with cytotoxic drugs were analysed separately from the complete cohort. CI, confidence interval; IF/TA, interstitial fibrosis/tubular atrophy.

Progressive eGFR decline

Briefly, a decline of eGFR over 1 and 5 years was independently predicted by non-Caucasian race, age_0, fibrinoid necrosis, fibrous crescents, and IF/TA ≥25%. Contrastingly, wire loops and endocapillary lymphocytes were associated with eGFR recovery over 10 years follow-up (**Appendix 5.6**).

Influence of therapy, race, and age on the predictive values of histopathologic variables

In the complete cohort, cytotoxic immunosuppression and/or ACE inhibition were not associated with any of the renal outcomes (**Appendix 5.2**). Of the histopathologic variables, only segmental/global endocapillary hypercellularity (P=0.03) and cellular crescents (P=0.05) were associated with cytotoxic immunosuppression (CYC, MMF, or AZA). Therapy showed no interactions with histopathologic variables for the different outcomes, with the exception of global glomerulosclerosis with ESRD (HR 1.03 for each percent glomeruli [95% CI, 1.00 to 1.06]) only in patients treated with AZA within the class III/IV subset (**Appendix 5.7**). Spikes/vacuoles and tubular reabsorption droplets were associated with a lower eGFR during follow-up in patients with Afro-Caribbean race compared with other races (**Appendix 5.7**). None of the prespecified clinical variables were correlated both with spikes/vacuoles or tubular reabsorption droplets and race. Age_0 showed no interactions with histopathologic variables for the different outcomes (**Appendix 5.7**).

Immunofluorescence in relation to outcome

The intensities of the individual immunoglobulins (IgA, IgG, and IgM) and complement factors (C3 and C1q) were not associated with ESRD, renal flare, or eGFR during follow-up (data not shown); neither were different immunofluorescence patterns, including <1+ IgG and C1q or the full house pattern.

ISN/RPS classes in relation to outcome

ISN/RPS classes were not significantly associated with overall renal survival (P=0.72) and renal flare (P=0.29). LN classes were significantly associated with eGFR during follow-up (P<0.05), with the lowest eGFR in class IV-S LN. For detailed results, see **Appendix 5.8**.

DISCUSSION

Controversies surrounding the prognostic significance of histopathologic (sub)classes in the classification of LN indicate that the prognosticators should be restructured from scratch.[19, 20] Without preconceptions, we analysed clinical and histopathologic predictors of renal outcomes in 105 patients with class I–V LN, including a subset of 91 patients with class III or IV (±V) LN treated relatively uniformly with cytotoxic immunosuppression. Essentially, our analysis of the different outcomes in the complete cohort and its subset revealed two categories of clinical and histopathologic predictors: (i) variables predictive

of the level of eGFR during follow-up and (ii) variables predictive of renal flare, progressive eGFR decline, and ultimately ESRD. The variables that emerged as independent predictors in the complete cohort were consistent with the predictors in the class III or IV (±V) LN subset. Here, we discuss the clinicopathologic predictors of renal outcome as identified among patients with class I–V LN.

In our study, the percentage of normal glomeruli with <4 leukocytes, in the absence of other abnormalities, was the only independent histopathologic predictor associated with a higher eGFR during follow-up. The prognostic significance of glomeruli that are normal by light microscopy most likely indicates that the relatively unaffected part of the kidney is vital in determining renal function. This notion is well known in the setting of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis, where biopsies in which ≥50% of glomeruli are normal using identical definitions have been incorporated in a well-validated "focal class" in the classification.[30-35] Based on our results, glomeruli that are normal by light microscopy may similarly transcend the LN classes and warrant incorporation into a separate index.

Only a limited number of active glomerular lesions had adverse prognostic value. Of the endocapillary lesions, wire loops and endocapillary lymphocytes were associated with eGFR recovery rather than decline over 10 years follow-up (**Appendix 5.6**), suggesting that the treatment of active endocapillary lesions was successful and damage was largely reversible for most patients. This result reflects the suggestion of the Oxford study on IgA nephropathy that endocapillary hypercellularity is a lesion more responsive to immunosuppression given the lack of its association with renal function decline among patients who received immunosuppression.[22] In contrast with active endocapillary lesions, the association of cellular/fibrocellular crescents with a lower eGFR during follow-up suggests active extracapillary lesions may result in irreversible damage. Yet, like active endocapillary lesions, active extracapillary lesions were not associated with progressive eGFR decline. The prognostic significance of extracapillary lesions also implies a treatment effect, but more in terms of halting the progression. In the current classification of LN, active endocapillary and extracapillary lesions contribute equally to the assignment of class III or IV LN. Our findings indicate that more weight should be given to extracapillary lesions.

In contrast with other active glomerular lesions, fibrinoid necrosis was not associated with $eGFR_0$, but rather with renal flare, progressive eGFR decline, and ESRD. Interestingly, fibrinoid necrosis was correlated with segmental, but not global endocapillary hypercellularity. This finding is consistent with the notion that global and segmental lesions represent distinct entities in class IV LN,[13, 14, 36] and that segmental lesions, with emphasis on fibrinoid necrosis, may have a different immunopathogenesis than global lesions.[13]

Apart from fibrinoid necrosis, chronic glomerular lesions were generally better predictors of eGFR and progressive eGFR decline than active lesions. Fibrous crescents were inde-

pendently associated with lower eGFR during follow-up, progressive eGFR decline, and ESRD. Correspondingly, it has been demonstrated that the composite chronicity index devised by Austin *et al*.[37] as well as its components individually, are excellent predictors of ESRD; whereas, the activity index and its components are weaker predictors.[37-42]

Currently, primarily the histopathologic class based on glomerular pathology determines the recommended clinical management of LN.[4-6] Our results confirm that, in addition to glomerular variables, tubulointerstitial variables including IF/TA, interstitial infiltrates, tubular casts, and arterial intimal fibrosis;[38, 39, 41-44] as well as clinical variables including non-Caucasian race, age, MAP_0, and $eGFR_0$, are also valuable predictors of renal outcome in LN.[39, 40, 45, 46]

Our study has some limitations. First, the predictors we identified apply to patients with the spectrum of clinical and histopathologic features observed in our cohort, in which the biopsies were scored by an experienced nephropathologist using our definitions. Indeed, a number of lesions, including microthrombi and vasculitis, were excluded from our analyses due to a low prevalence. These lesions may well have prognostic significance and should be evaluated in other cohorts. Second, we did not analyse electron microscopy (EM); which, if at hand, is the usual complement to light and immunofluorescence microscopy to comprehensively study LN pathology. Whereas in our study immunofluorescence microscopy did not confer prognostic significance, EM studies may reveal additional prognosticators. Third, our study is retrospective, whereas an ideal prognostic study would be prospective and standardise diagnostic and therapeutic procedures for all patients. However, our design allowed us to identify a cohort that could be followed to determine long-term outcomes.

Inherent to any histopathologic study investigating active LN, our results must be interpreted in the light of immunosuppression. Most patients (68%) in the class III or IV (±V) subset were treated relatively uniformly with regimens including CYC or MMF according to guidelines. Importantly, 29 (31%) patients were treated with AZA. Within the subset, no interactions between pathology variables and treatment were found, with one exception: global glomerulosclerosis was significantly associated with ESRD for patients treated with AZA, whereas in patients who received CYC or MMF, it was not. AZA has been associated with increased risk of renal flare and inferior efficacy compared with CYC in terms of delaying the progression of chronic lesions.[47, 48] The adverse prognostic significance of global glomerulosclerosis among patients treated with AZA and of chronic lesions in general provides circumstantial evidence that AZA should not be recommended as induction therapy in case of chronic lesions.

In conclusion, our results show that while ISN/RPS classes were poorly associated with the clinically relevant outcomes, prognostication in LN may benefit from the specific assessment of clinical variables and lesions currently obscured in the classification. Normal glomeruli, cellular/fibrocellular crescents, fibrous crescents, and IF/TA were potent predictors of eGFR during follow-up. Importantly, particularly active endocapillary lesions were

likely responsive to immunosuppression since they were not associated with renal function deterioration. Contrastingly, fibrinoid necrosis, fibrous crescents, and IF/TA predicted progressive renal dysfunction even in the uniformly treated subset, implying that these lesions are unlikely to benefit from immunosuppression and thereby correspond to the clinically most relevant category of predictors. An evidence-based era of classifying LN is on the horizon, with an international effort to refine the histopathologic classification already on its way. Our results suggest that LN classes should be expanded with an evidence-based index, analogous to the MEST score in the Oxford classification, with special attention to defining clinically important categories of predictors. Awaiting further validation of a formal index, we suggest that at least fibrinoid necrosis, fibrous crescents, and IF/TA warrant explicit independent scoring to assess the risk of progressive renal dysfunction in conjunction with clinical findings.

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