Systemic lupus erythematosus : from diagnosis to prognosis

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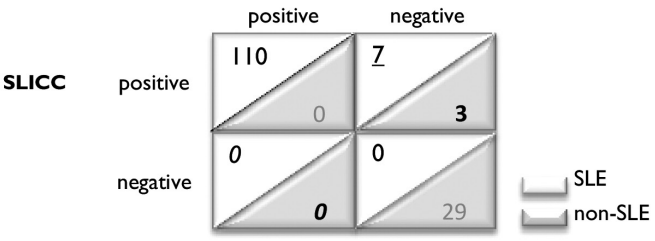
Appendix

A

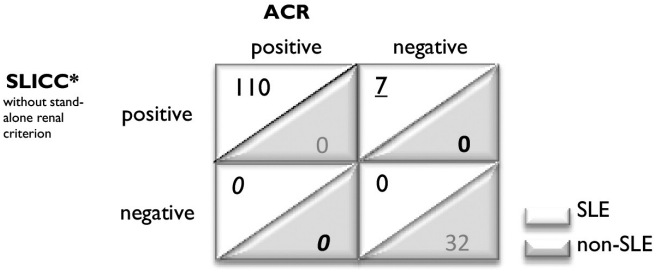
Chapter 2

Appendix 2.1. Reclassification tables

Reclassification tables of cases with and without SLE used for calculation of the net reclassification improvement (NRI)



NRI SLICC = (7-0)/117 + (0 -3)/32 = -0.034



NRI SLICC* = (7-0)/117 + (0 -0)/32 = 0.060

The NRI is based on reclassification tables constructed separately for patients with and without a reference standard clinical diagnosis of SLE ([SLE(+)] and [SLE(-)], respectively), and quantifies the correct movement in categories: [up|] for fulfilling classification criteria and [down|] for unfulfilling classification criteria as compared with reference classification criteria. The NRI can be expressed as follows:

$$NRI = \frac{\Sigma\, up|SLE(+) - down|SLE(+)}{\#SLE(+)} + \frac{\Sigma\, down|SLE(-) - up|SLE(-)}{\#SLE(-)}$$

Appendix 2.2. Prevalence of individual SLICC criteria

Prevalence of individual SLICC criteria in the full house cohort and the SLICC derivation cohort

Criterion	Patients with clinical SLE			Patients without clinical SLE		
	Full house cohort (n=117)	SLICC derivation cohort (n=310)	*P*	Full house cohort (n=32)	SLICC derivation cohort (n=392)	*P*
SLICC clinical criteria	n/total (%)	%		n/total (%)	%	
Acute cutaneous lupus	66/117 (56.4)	65.2	0.116	0/32 (0)	19.9	0.002
Chronic cutaneous lupus	14/117 (12.0)	19.7	0.065	0/32 (0)	6.4	0.242
Non-scarring alopecia	21/117 (17.9)	31.9	0.004	0/32 (0)	4.3	0.629
Oral/nasal ulcers	25/117 (21.4)	44.2	<0.001	3/32 (9.4)	7.9	0.734
Arthritis	86/117 (73.5)	79.0	0.243	1/32 (3.1)	56.4	<0.001
Serositis	44/117 (37.6)	35.2	0.652	1/32 (3.1)	2.8	1.000
Renal disorder	117/117 (100.0)	32.9	*	32/32 (100)	3.6	*
Neurologic disorder	18/117 (15.4)	5.5	0.002	0/32 (0)	1.0	1.000
Haemolytic anaemia	20/117 (17.1)	7.1	0.003	0/32 (0)	0.5	1.000
Leukopenia	36/117 (30.8)	46.4	0.004	0/32 (0)	5.2	0.385
Thrombocytopenia	28/117 (23.9)	13.5	0.013	0/32 (0)	2.0	1.000
SLICC immunologic criteria						
Antinuclear antibody	116/117 (99.1)	96.5	0.193	3/26 (11.5)	3.2	0.070
Anti-dsDNA	80/109 (73.4)	57.1	0.029	0/28 (0)	4.1	0.614
Anti-Sm	18/56 (32.1)	26.1	0.414	0/8 (0)	1.3	1.000
Antiphospholipid antibody	41/81 (50.6)	53.6	0.708	0/5 (0)	14.0	1.000
Hypocomplementaemia	94/109 (86.2)	59.0	<0.001	2/23 (8.7)	7.4	0.686
Direct Coombs' test	25/75 (33.3)	-	-	0/13 (0)	-	-

SLICC validation cohort derived from Petri *et al.*[1] Fulfilment of SLICC classification criteria in the full house cohort was registered up to and including the moment of renal biopsy. "Clinical SLE" refers to the reference standard clinicopathologic diagnosis of SLE. Anti-dsDNA, anti-double stranded DNA antibody. * Different by selection.

Chapter 3

Appendix 3.1. Clinical characteristics of patients with non-lupus full house nephropathy at the time of renal biopsy

Patient number	Clinicopathologic diagnosis	Serology at the time of biopsy	SLE criteria
Idiopathic non-lupus FHN			
1	Idiopathic minimal change-like nephropathy	ANA(-), ASO(-)	(-)
2	Idiopathic minimal change-like nephropathy	ANA(-), ASO(-)	(-)
3	Idiopathic mesangioproliferative nephropathy	ANA(-), ASO(-)	(-)
4	Idiopathic proliferative glomerulonephritis	ANA(-), HBsAg(-)	Hypocomplementaemia*
5	Idiopathic proliferative glomerulonephritis	ANA(dubious), anti-dsDNA(-), anti-GBM(-)	Nasal ulcers†
6	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), HBsAg(-)	Arthritis†, pleuritis*
7	Idiopathic proliferative glomerulonephritis	ANA(-), ASO(-)	(-)
8	Idiopathic proliferative glomerulonephritis	n/a	(-)
9	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), anti-GBM(-), ANCA(-), ASO(-)	(-)
10	Idiopathic proliferative glomerulonephritis	ANA(dubious), ANCA(-), anti-dsDNase B(-),	ANA (12)
11	Idiopathic proliferative glomerulonephritis	ANA(-)	(-)
12	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), ASO(-)	Arthritis (9)
13	Idiopathic MPGN + MN	n/a	(-)
14	Idiopathic proliferative glomerulonephritis	ANA(-), ANCA(-)	(-)
15	Idiopathic MN	ANA(-), HBsAg(-)	(-)
16	Idiopathic MN + TIN	ANA(-), HBsAg(-)	(-)
17	Idiopathic MN	ANA(-), ASO (-)	(-)
18	Idiopathic MN	n/a	(-)
19	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), HBsAg(-)	Serositis (2), arthritis (2)
20	Idiopathic focal segmental glomerulosclerosis	ANA(-), ASO(-)	(-)
Secondary non-lupus FHN			
21	IgA-like nephropathy	ANA(-), ASO(-)	(-)
22	Crescentic IgA-like nephropathy	HBV IgG(+)/IgM(-)/HBsAg(-)	(-)
23	Crescentic IgA-like nephropathy	ANA(-), HBsAg(-), anti-HCV(-), anti-HIV(-)	(-)
24	Crescentic IgA-like nephropathy	ANA(-), ASO(-)	(-)

Co-morbidity	Induction therapy	ESRD/death§	Follow-up, y
(-)	CS	(-)	34
DM II†	(-)	HD (2), deceased (13)	11
DM II (27), hypertension (27)	(-)	(-)	38
Alpha-thalassaemia*	CS	HD*, Tx (2)	24
Pulmonary silicosis†, nasal ulcers†	CS	CAPD*, Tx (1), deceased (11)	8
Seronegative RA*	CS	CAPD (1), deceased (11)	1
(-)	(-)	Tx (2), deceased (20)	20
Migraines (11 years of analgesic use)†, DM II (36)	(-)	CAPD (26), Tx (29)	41
Haemoptysis (9)	(-)	CAPD (15)	24
Pneumonia (Mycoplasma)*, gout*, pancytopenia (14)	Cyclosporin + CS	(-)	9
Malignant hypertension†, primary Raynaud's phenomenon (35)	(-)	Tx (39)	39
RA†, episcleritis*, cutaneous vasculitis*, pleuritis (4), pericarditis (7)	AZA + CS	Deceased (13)	13
Return of MPGN in kidney graft (5, 20), myocardial infarction (18)	AZA + CS	CAPD (4), Tx (5), deceased (28)	28
(-)	(-)	CAPD (3), Tx (7)	26
Migraine†, para-aortal lymphadenopathy (9)	(-)	Deceased (16)	15
Hypertensive retinopathy†, CAD (2)	CS	(-)	2
Coeliac disease (31), Hashimoto's thyroiditis (31)	(-)	(-)	34
Erysipelas*, hypogammaglobulinemia*, cocaine/cannabis abuse (6)	AZA + CS	CAPD (7), Tx (16)	21
Myocardial infarction†	(-)	Deceased (16)	7
Hashimoto's thyroiditis (13)	(-)	CAPD (16), Tx (20)	34
IgA nephropathy in transplanted kidney (14), prostate carcinoma and renal mass (35)	CS	Tx (14)	36
IgA nephropathy in transplanted kidney (16), CAD (17), DM II (23), Guillain-Barré syndrome (32)	Unknown	CAPD (14), Tx (16), deceased (36)	36
Preeclampsia†, malignant hypertension*, IgA nephropathy in transplanted kidney (3)	(-)	HD*, Tx (3)	12
IgA nephropathy in transplanted kidney (4, 13)	CS	Tx (4)	35

Appendix 3.1. Continued.

Patient number	Clinicopathologic diagnosis	Serology at the time of biopsy	SLE criteria
Secondary non-lupus FHN			
25	Infection-related glomerulonephritis	Anti-dsDNA(-), anti-amoeba(+), anti-malaria(-), HAV IgG(+)/IgM(-)	(-)
26	Infection-related glomerulonephritis	n/a	(-)
27	ANCA-associated glomerulonephritis	PR3-ANCA(+), ANA(-), anti-GBM(-), anti-dsDNase B(-), HBsAg(-), anti-HCV(-)	Hypocomplementaemia*, nasal ulcers*
28	ANCA-associated glomerulonephritis	ANA(-), anti-ENA(-), anti-cardiolipin(-), PR3-ANCA(+)	ANA† ([-] during FU), nasal ulcers*
29	MN (anti-PLA2R)	ANA(-), anti-PLA2R(+), aC1q(-)	(-)
30	MN (cancer-associated) + FSGS	ANA(-), ASO(-)	(-)
31	MN (cancer-associated) + secondary glomerular sclerosis	ANA(+), anti-dsDNA(-), HBsAg(-), anti-HCV(-)	ANA*, ANA (4)
32	MN (cancer-associated) + minimal endocapillary proliferation	ANA(+), anti-dsDNA(-), aSS-A(+)	ANA* (not tested during follow-up)

Numbers in parentheses represent years since renal biopsy showing full house immunofluorescence. * indicates that the phenomenon occurred at the time of renal biopsy showing full house immunofluorescence; † indicates that the phenomenon occurred >6 months before the renal biopsy showing full house immunofluorescence; § Patients 2, 12, 22, and 31 died of cardiovascular disease, patients 30 and 32 of malignancy, patient 7 of terminal renal insufficiency, patient 13 of sepsis, and patients 4, 5, 15, 19, and 26 of unknown causes.(+), present;(-), absent; n/a, not available; ESRD, end-stage renal disease; ANA, antinuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; ASO, anti-streptolysin O; AZA, azathioprine; CAD, coronary artery disease; CAPD, chronic

Co-morbidity	Induction therapy	ESRD/death§	Follow-up, y
Malaria†, amoebic liver abscess *, DM II (20)	(-)	(-)	20
Pyelourethral obstruction†, enterococcal sepsis*, cardiomyopathy (2), pericarditis (5)	(-)	Tx (3), deceased (13)	13
Nasal ulcers*, cerebral vasculitis*, cavitating pulmonary lesions (15)	CYC + CS	HD*, Tx (5)	19
ENT-limited PR3-ANCA-associated vasculitis*	CYC + CS	(-)	8
Multiple pulmonary emboli*	CS	(-)	4
Squamous cell carcinoma lung*	(-)	Deceased (8)	0.4
Prostate carcinoma*	(-)	Deceased (9)	20
Metastasised cervical carcinoma†	CS	Deceased (3)	3

ambulant peritoneal dialysis; CS, corticosteroids; CYC, cyclophosphamide; DM, diabetes mellitus; dsDNA, double stranded DNA; ENA, extractable nuclear antigen; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HD, haemodialysis; HIV, human immunodeficiency virus; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; PLA2R, anti-phospholipase A2 receptor; RA, rheumatoid arthritis; TIN, tubulointerstitial nephritis; Tx, transplantation.

Appendix 3.2. Electron microscopy findings in patients with non-lupus (full house nephropathy) FHN

Patient number		Electron microscopy
Idiopathic non-lupus FHN	Immune deposits	Podocyte foot process effacement
1	(–)	(+)
2	n/a	n/a
3	n/a	n/a
4	mes-sed-sep	(+)
5	n/a	n/a
6*	mes-sed-tm-sep	(+)
7	n/a	n/a
8	n/a	n/a
9	sep	(+)
10	sep	(+)
11	n/a	n/a
12	sep	(+)
13	mes-sed-sep	n/a
14	tm-sep	(+)
15	n/a	n/a
16	n/a	n/a
17	n/a	n/a
18	sep	(+)
19	n/a	n/a
20	n/a	n/a
Secondary non-lupus FHN		
21	n/a	n/a
22	n/a	n/a
23	sed	(+)
24	n/a	n/a
25	mes-sed	(+)
26	mes-sed-tm-sep (humps)	(+)
27	n/a	n/a
28	n/a	n/a
29	sep	(+)
30	mes-sep	(+)
31	sep	(+)
32*	sep	(+)

N/a, not available; mes, mesangial; sed, subendothelial; tm, transmembranous; sep, subepithelial. * Tubuloreticular inclusions were identified by electron microscopy.

Chapter 5

Appendix 5.1. Histopathology definitions

Definitions for glomerular lesions

Scorable glomeruli: Glomeruli that remained after excluding those which had less than 3 mesangial fields because they were too small, on the edge of the biopsy and therefore incomplete, or otherwise artefactually damaged.

Focal: Involving <50% of glomeruli.

Diffuse: Involving ≥50% of glomeruli.

Segmental: Lesion involving less than half of the glomerular area inside Bowman's capsule.

Global: Lesion involving more than half of the glomerular area inside Bowman's capsule.

Normal glomerulus: Glomerulus without a lesion. A normal glomerulus may show subtle changes as a result of ischemia.

Minimal leukocyte influx: Occurrence of <4 neutrophils, lymphocytes, or monocytes in an otherwise normal glomerulus, in the absence of endothelial cell swelling.

Global sclerosis: Sclerosis of the entire glomerulus (obliteration of the capillary lumen by increased extracellular matrix, with or without hyalinosis or foam cells).

Segmental sclerosis: Less than 100% of the glomerulus is sclerosed (obliteration of the capillary lumen by increased extracellular matrix, with or without hyalinosis or foam cells).

Ischemic glomerulus: A glomerulus showing one or more of the following lesions: wrinkling of the glomerular basement membrane (GBM), collapse of the capillary tuft, thickening/splitting of Bowman's capsule.

Mesangial hypercellularity: Four or more nuclei in the contiguous matrix of a peripheral mesangial segment. Note: mesangial hypercellularity is scored for each glomerulus by assessing the most cellular mesangial area. Mesangial areas immediately adjacent to the vascular stalk should not be scored. Scoring categories: (0) <4 nuclei; (1) 4–5 nuclei; (2) 6–7 nuclei; (3) >7 nuclei.

Mesangial matrix expansion: Width of extracellular matrix exceeding 2 mesangial cell nuclei in ≥2 glomerular lobules.

Endocapillary hypercellularity: Hypercellularity due to an increased number of cells within glomerular capillary lumina (leukocytes or endothelial cells), causing narrowing of the lumina. Indicate whether lesion is segmental or global.

Endocapillary inflammatory infiltrate: ≥4 inflammatory cells in the glomerulus – either granulocytes, lymphocytes, or monocytes.

Endothelial cell swelling: Prominence of endothelial cells in capillary lumens with narrowing of the lumen.

Wire loop: Capillary wall thickening characterised by subendothelial immune complex deposits as demonstrated in the PAS staining.

Adhesion: Area of continuity between glomerular tuft and Bowman's capsule separate from extracapillary lesion or from area of segmental sclerosis.

Crescent: one of the following lesions involving >10% of circumference of Bowman's capsule:

- **Cellular crescent**: Extracapillary cell proliferation of ≥3 cell layers with ≥50% of the lesion occupied by cells.
- **Fibrocellular crescent**: Extracapillary lesion comprising cells and extracellular matrix, with <50% cells and <90% matrix.
- **Fibrous crescent**: Extracapillary lesion composed of ≥90% matrix.
- **Segmental crescent:** Lesion occupying less than 50% of the circumference of Bowman's capsule.
- **Circumferential crescent:** Lesion occupying 50% or more of the circumference of Bowman's capsule.

Fibrinoid necrosis: Disruption of the GBM with fibrin exudation and karyorrhexis.
Karyorrhexis: Presence of fragmented nuclei including apoptosis.
Microthrombus: A microscopic clump of fibrin, platelets, and red blood cells.
Pseudothrombus: Eosinophilic, rounded aggregates in glomerular capillaries due to immune complex precipitates rather than fibrin, also known as hyaline thrombi.
Double contour/tram track: Double layer of GBM separated by clear zone on silver or PAS stains.
Spikes/vacuoles: Extensions of glomerular basement membrane between deposits (egg racks).

Definitions of tubulointerstitial lesions

Interstitial infiltration: Inflammatory cells within the cortical interstitium in more than 5% of the cortical area. Specify dominant cell type of infiltrate: either lymphocytes, granulocytes, or other. Scoring categories: (0) <5%; (1) 5–24%; (2) 25–49%; (3) ≥50% of the cortical area.
Interstitial fibrosis: Extracellular matrix separating tubules in more than 5% of the cortical area. Scoring categories: (0) <5%; (1) 5–24%; (2) 25–49%; (3) ≥50% of the cortical area.
Focal cortical atrophy: Subcapsular ischemic cortical atrophy, sharply demarcated from normal cortex.
Tubular atrophy: Loss of cytoplasmic organelles, accompanied by a decreased diameter of tubules and thick irregular tubular basement membrane (TBM). Scoring categories: (0) <5%; (1) 5–24%; (2) 25–49%; (3) ≥50% of tubules.
Acute tubular injury: Necrosis of tubular epithelial cells (coagulation necrosis, karyorrhexis, pyknosis), swelling and clear vacuolation of tubular epithelium; can be accompanied by separation/detachment of tubular epithelium from TBM.
Tubular casts: Presence of proteinaceous structures within the lumen of the tubules; may contain cellular debris; only scored when present in nonatrophic tubuli.
Tubular luminal macrophages: Presence of macrophages in tubular lumina; to be distinguish from sloughed epithelial cells.
Tubular regeneration: Regeneration following acute tubular injury usually characterised by the presence of mitotic figures.
Tubular reabsorption droplets: PAS/silver-positive resorption droplets in the proximal tubular epithelium.
Tubulitis: Lymphocytes/other inflammatory cells within the epithelial layer of tubules.

Definitions of vascular lesions

Vasculitis: Inflammation in an arterial/arteriolar wall, characterised by the presence of inflammatory cells and/or fibrinoid necrosis.

Fibrinoid necrosis: Homogeneous, fibrin-like, deeply eosinophilic area with disruption of the architecture of the arterial/arteriolar vascular wall.

Thrombosis: Total occlusion of vessel with fibrin.

Hyaline arteriolosclerosis: Accumulation of glassy, refractive, strongly PAS-positive material in the arteriolar intima and/or media.

Fibrous intimal hyperplasia: Cellular and fibroelastic intimal thickening with a fibrous intimal projection or cushion bulging into the lumen.

Arterial intimal fibrosis: Concentric thickening of the intima by deposition of collagen.

Appendix 5.2. Detailed study outcomes and statistical methods

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 variables 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. The models described below make no assumptions about the distribution of independent variables and the dependent variable, eGFR, was normally distributed; therefore, the variables were entered in the models without transformation.

Renal flare and ESRD

The outcomes "renal flare" and "ESRD" relate to time-to-event analyses. We studied the time to a first LN flare in patients who achieved (partial) remission after induction therapy. Time to first renal flare was calculated from the date of biopsy until the date of flare for patients who reached this endpoint. For patients who did not, the follow-up time was from the time of biopsy until the last follow-up or until the patient reached ESRD. The definition of ESRD was dialysis-dependence for >3 months or renal transplantation. Time to ESRD was calculated analogous to time to renal flare. Patients who reached ESRD before the last follow-up were regarded as having eGFR=0 mL/min/1.73 m^2 at the remaining time points. Outcomes were assessed using Kaplan-Meier analysis and Log-Rank tests. To ascertain independent predictors of ESRD and renal flare, multivariable Cox proportional-hazards models were designed including the prespecified variables. The multivariable models were simplified by stepwise removal of the least significant variables. Hazard ratios and 95% confidence intervals were estimated.

eGFR during follow-up

The extent by which variables were associated with irreversible nephron loss was investigated by modelling eGFR during follow-up. An adjusted average level (intercept) and rate (slope) of decline of renal function during follow-up were modelled. Baseline variables were tested for their potential to predict a change in the intercept of this adjusted average level of decline in random intercept/slope linear mixed-effects models. Variables were tested in univariable models in the complete cohort (**Table A5.2.1**). A full model including the prespecified variables was designed and simplified by removing the least significant variables (Wald test) and comparing the goodness of fit of nested models (maximum likelihood ratio test). The distribution of data and the homogeneity of variance were assessed using graphical evaluation of residuals.

Progressive eGFR decline

To investigate progressive eGFR decline that did not necessarily result in ESRD and/or renal flare, variables were analyzed in association with progressive eGFR decline over 1, 5, and 10 years relative to its linear prediction based upon $eGFR_0$. The linear relationship between $eGFR_0$ and the predicted eGFR at time t ($eGFR_{Predicted(t)}$) was defined as: $eGFR_{Predicted(t)} = eGFR_0 * \beta_{(t)} + constant$. Progressive eGFR decline relative to the $eGFR_{Predicted(t)}$ was assessed by calculating the corrected eGFR ($eGFR_{CORR(t)}$), which was defined as the difference between the observed eGFR(t) and predicted eGFR(t).[2] This procedure created a corrected value that was independent of the starting value. Variables were first tested in univariable linear regression models for the outcomes $eGFR_{CORR(1)}$, $eGFR_{CORR(5)}$, and $eGFR_{CORR(10)}$ in the complete cohort (**Table A5.2.2**). For the complete cohort and the selected subset, a prediction model for these outcomes was designed using automated backward linear regression starting with the prespecified variables.

Table A5.2.1 Univariable predictions of eGFR during follow-up up in 105 LN patients.

eGFR in mL/min/1.73 m² during follow-up†		
Clinical variables		β (95% CI)
Female sex		−5.5 (−21.5; 10.4)
Age_0, y		−0.8 (−1.3; −0.4)*
Race	*Caucasian*	0.5 (−13.1; 14.1)
	Asian	−0.8 (−16.3; 14.7)
	Afro-Caribbean	0.3 (−19.3; 20.0)
Years since diagnosis SLE_0		−1.2 (−2.3; 0.0)*
$Proteinuria_0$, g/24h		−0.4 (−2.1; 1.4)
$Erythrocyturia_0$ >1+		−11.9 (−42.3; 18.5)
MAP_0, mm Hg		−0.5 (−0.9; −0.1)*
ACE inhibitor (after Bx)		−2.2 (−19.1; 14.7)
Cytotoxic immunosuppressive (after Bx)		−13.1 (−39.0; 12.8)
Biopsied after 2000		15.4 (2.5; 28.3)*

Table A5.2.1 Continued.

Glomerular variables		β (95% CI)
% Normal glomeruli/minimal leukocyte influx§		0.4 (0.2; 0.7)*
% Global sclerosis		−0.8 (−1.2; −0.4)*
% Segmental sclerosis		3.3 (−0.3; 6.9)
% Ischemic glomeruli		−0.7 (−1.1; −0.2)*
% Mesangial hypercellularity		0.0 (−0.1; 0.1)
% Mesangial matrix expansion		0.1 (−0.3; 0.4)
% Endocapillary hypercellularity	*Any*	0.0 (−0.2; 0.2)
	Segmental	0.1 (−0.2; 0.4)
	Global	−0.1 (−0.3; 0.1)
% Endocapillary infiltration	*Lymphocytes*	0.0 (−0.2; 0.2)
	Monocytes	0.0 (−0.3; 0.2)
	Granulocytes	0.1 (−0.4; 0.6)
% Crescents	*Cellular/fibrocellular§*	−0.4 (−0.6; −0.1)*
	Fibrous	−1.6 (−2.8; −0.4)*
% Wire loops		0.0 (−0.3; 0.2)
% Adhesions		–0.4 (−1.1; 0.3)
% Fibrinoid necrosis		−0.2 (−1.1; 0.6)
% Karyorrhexis		−0.1 (−0.6; 0.3)
% Double contours		−0.1 (−0.5; 0.2)
% Spikes/vacuoles		0.1 (−0.2; 0.3)
Tubulointerstitial variables		β (95% CI)
IF/TA	5−24%	−3.9 (−14.0; 11.1)
	25−49%	−37.5 (−60.8; −14.2)*
	≥50%	−50.1 (−82.4; −17.7)*
Interstitial infiltration	5−24%	−11.8 (−25.2; 1.6)
	25−49%	13.1 (−20.7; 46.9)
	≥50%	−34.1 (−57.8; −10.5)*
Tubular casts		−19.5 (−32.0; −7.1)*
Tubular macrophages		−15.7 (−32.4; 0.9)
Tubular reabsorption droplets		−10.2 (−23.2; 2.8)
Arterial intimal fibrosis		−44.7 (−67.6; −21.7)*

β represents the mean change in the level of eGFR decline over time with one unit change of the variable.
* $P<0.05$. † univariable random intercept/random slope mixed-effects models. § The composite variables "normal glomeruli/minimal leukocyte influx" and "extracapillary 2" were used rather than their individual components, as effect sizes of components were in strong accordance (data not shown).
Abbreviations: Bx, biopsy; IF/TA, interstitial fibrosis or tubular atrophy; MAP, mean arterial pressure.

Table A5.2.2 Univariable predictions of progressive eGFR decline (in mL/min/1.73 m^2) over 1, 5, and 10 years follow-up in 105 LN patients.

		eGFR decline over 1 year ($eGFR_{CORR(1)}$)‡	eGFR decline over 5 years ($eGFR_{CORR(5)}$)‡	eGFR decline over 10 years ($eGFR_{CORR(10)}$)‡
		β	β	β
Clinical variables				
Female sex		−9.0 (−22.1; 4.1)	6.1 (−13.1; 25.2)	−6.2 (−32.5; 20.1)
Age_0, y		−0.5 (−0.9; −0.1)*	−0.9 (−1.5; −0.4)*	−1.2 (−1.9; −0.4)*
Race	*Caucasian*	10.2 (−0.8; 21.3)	11.2 (−7.1; 29.6)	13.7 (−11.2; 38.6)
	Asian	−2.9 (−15.7; 9.8)	5.3 (−10.8; 21.3)	3.5 (−18.6; 25.5)
	Afro-Caribbean	−16.6 (−32.6; −0.7)*	−28.5 (−50.9; −6.1)*	−28.5 (−59.1; 2.2)
Years since diagnosis SLE_0		−1.0 (−2.0; −0.1)*	−2.4 (−3.6; −1.2)*	−2.2 (−3.9; −0.5)*
$Proteinuria_0$, g/24h		0.2 (−1.3; 1.6)	−0.5 (−2.5; 1.5)	1.2 (−1.5; 3.8)
$Erythrocyturia_0$ >1+		1.6 (−23.5; 26.8)	−2.8 (−41.1; 35.5)	−10.2 (−98.6; 78.2)
MAP_0, mm Hg		−0.1 (−0.4; 0.2)	−0.3 (−0.8; 0.2)	−0.9 (−1.4; −0.3)*
ACE inhibitor (after Bx)		0.0 (−13.9; 14.0)	2.1 (−17.9; 22.1)	9.9 (−18.9; 38.7)
Cytotoxic immunosuppressive (after Bx)		1.2 (−20.3; 22.7)	4.4 (−27.2; 36.0)	13.4 (−27.3; 54.1)
Biopsied after 2000		7.6 (−3.3; 18.5)	10.2 (−5.2; 25.6)	7.0 (−14.3; 28.2)
Glomerular variables				
% Normal glomeruli/minimal leukocyte influx§		0.0 (−0.2; 0.2)	0.1 (−0.2; 0.4)	0.1 (−0.3; 0.5)
% Global sclerosis		−0.5 (−0.8; −0.2)*	−0.7 (−1.1; −0.2)*	−0.7 (−1.3; −0.1)*
% Segmental sclerosis		−0.8 (−3.9; 2.3)	2.3 (−2.3; 6.8)	3.0 (−2.5; 8.5)
% Ischemic glomeruli		−0.2 (−0.6; 0.2)	−0.3 (−0.9; 0.3)	−0.6 (−1.5; 0.3)
% Mesangial hypercellularity		−0.1 (−0.2; 0.1)	−0.1 (−0.2; 0.1)	0.0 (−0.2; 0.2)
% Mesangial matrix expansion		−0.1 (−0.3; 0.2)	−0.1 (−0.4; 0.3)	0.0 (−0.5; 0.5)
% Endocapillary hypercellularity	*Any*	0.2 (0.0; 0.3)	0.2 (−0.1; 0.4)	0.3 (0.0; 0.6)
	Segmental	0.0 (−0.3; 0.2)	0.1 (−0.3; 0.4)	0.4 (−0.1; 0.8)
	Global	0.2 (0.0; 0.4)	0.2 (−0.1; 0.5)	0.1 (−0.3; 0.5)
% Endocapillary infiltration	*Lymphocytes*	0.1 (−0.1; 0.3)	0.1 (−0.2; 0.3)	0.4 (0.0; 0.7)
	Monocytes	0.2 (0.0; 0.4)	0.2 (0.0; 0.5)	0.4 (0.0; 0.7)*
	Granulocytes	0.4 (0.0; 0.8)*	0.6 (0.0; 1.1)*	0.7 (−0.1; 1.5)
% Crescents	*Cellular/fibrocellular§*	−0.1 (−0.3; 0.2)	−0.1 (−0.5; 0.2)	−0.1 (−0.6; 0.3)
	Fibrous	−0.6 (−1.6; 0.5)	−0.9 (−2.4; 0.7)	−1.0 (−3.2; 1.2)
% Wire loops		0.2 (−0.1; 0.4)	0.3 (0.0; 0.6)	0.3 (−0.1; 0.8)
% Adhesions		−0.1 (−0.8; 0.5)	−0.4 (−1.2; 0.5)	−0.1 (−1.3; 1.0)
% Fibrinoid necrosis		−1.0 (−1.7; −0.3)*	−1.0 (−2.0; 0.1)	−0.2 (−1.8; 1.5)
% Karyorrhexis		0.2 (−0.2; 0.5)	0.0 (−0.7; 0.6)	−0.6 (−1.7; 0.5)
% Double contours		0.0 (−0.3; 0.3)	−0.2 (−0.7; 0.3)	−0.1 (−0.7; 0.4)
% Spikes/vacuoles		0.0 (−0.3; 0.2)	−0.1 (−0.5; 0.2)	0.0 (−0.4; 0.4)

Table A5.2.2 Continued.

		eGFR decline over 1 year ($eGFR_{CORR(1)}$)‡	eGFR decline over 5 years ($eGFR_{CORR(5)}$)‡	eGFR decline over 10 years ($eGFR_{CORR(10)}$)‡
		β	β	β
Tubulointerstitial variables				
IF/TA	*5–24%*	3.2 (−9.3; 15.6)	−6.4 (−23.8; 10.9)	−13.5 (−37.0; 10.0)
	25–49%	−17.1 (−37.0; 2.8)	−26.5 (−57.6; 4.6)	−35.5 (−79.9; 9.0)
	≥50%	−27.9 (−55.4; −0.5)*	−33.7 (−71.4; 3.9)	−48.2 (−98.7; 2.3)
Interstitial infiltration	*5–24%*	−2.5 (−13.8; 8.8)	0.7 (−15.2; 16.5)	−5.5 (−27.0; 16.0)
	25–49%	9.3 (−18.7; 37.2)	4.7 (−33.6; 42.9)	35.5 (−26.9; 98.0)
	≥50%	−11.7 (−31.8; 8.3)	−22.5 (−53.8; 8.7)	−30.2 (−81.6; 21.1)
Tubular casts		−9.8 (−20.4; 0.9)	−14.1 (−29.1; 0.9)	−11.3 (−32.5; 9.8)
Tubular macrophages		8.4 (−5.8; 22.5)	−3.6 (−23.6; 16.4)	10.6 (−16.3; 37.6)
Tubular reabsorption droplets		−5.9 (−16.7; 5.0)	−17.0 (−32.2; −1.9)*	−15.9 (−37.1; 5.3)
Arterial intimal fibrosis		−34.5 (−53.7; −15.3)*	−38.0 (−64.7; −11.3)*	−31.1 (−63.5; 1.4)

Interpretation $eGFR_{CORR}^{1/5/10}$ (in mL/min/1.73 m^2): β represents the change in $eGFR_{CORR}$ with one unit change of the variable. * $P<0.05$. ‡ Univariable linear regression models. § The composite variables "normal glomeruli/minimal leukocyte influx" and "extracapillary 2" were used rather than their individual components, as effect sizes of components were in strong accordance (data not shown).
Abbreviations: Bx, biopsy; IF/TA, interstitial fibrosis or tubular atrophy; MAP, mean arterial pressure.

Appendix 5.3 Correlations between histopathologic variables

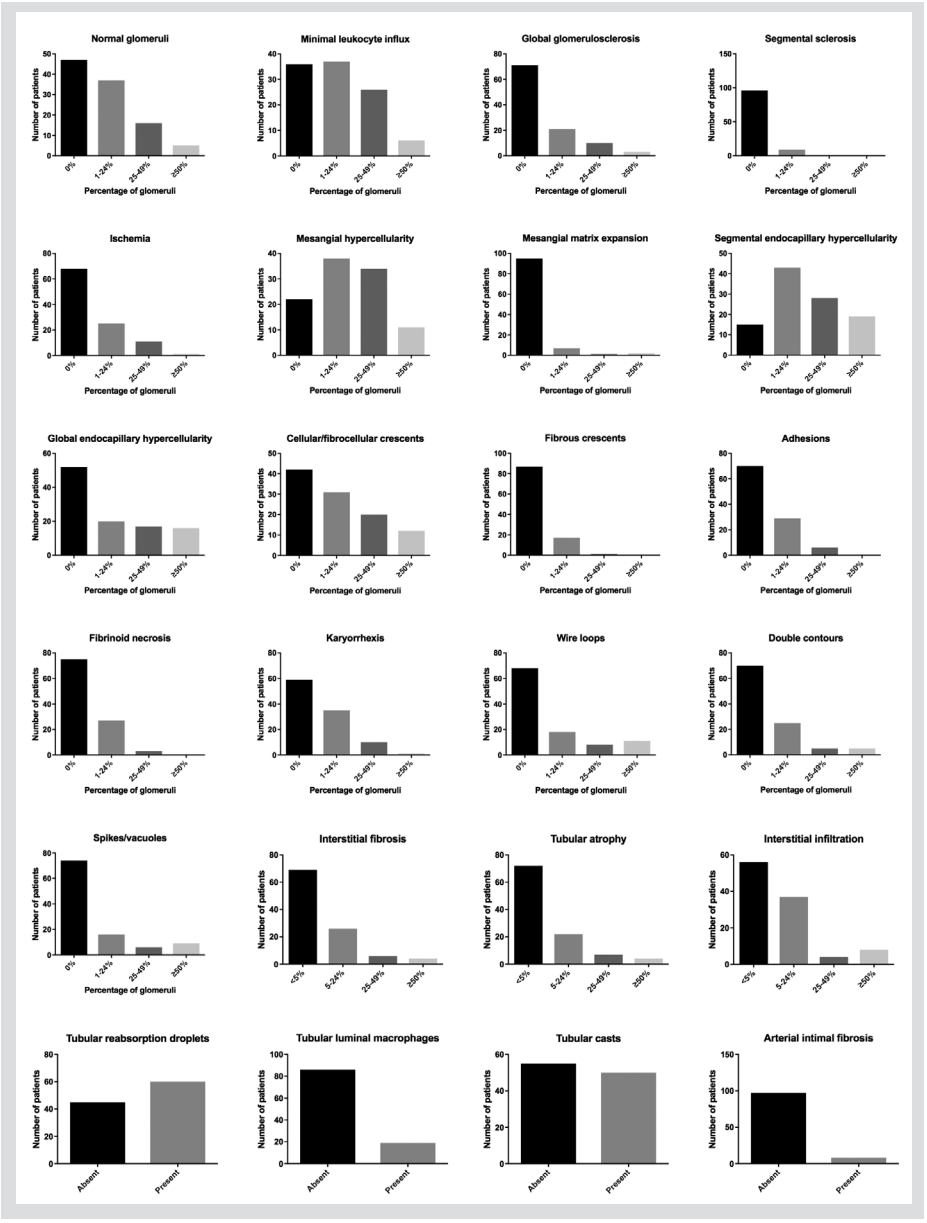
To prevent the inclusion of strongly correlated variables in our analyses of the various outcomes, we assessed correlations between 29 glomerular and 9 tubulointerstitial variables (occurring in >5 patients), excluding normal glomeruli. Significant correlation coefficients between histopathologic variables after Bonferroni correction are shown in the table below. The following variables that were strongly correlated with other variables (r/ρ >0.8) were dropped: "mesangial 1" and "extracapillary 3/5" because of relatively laborious scoring; "endocapillary 3" because "minimal leukocyte influx" was encompassed by "normal glomeruli/minimal leukocyte influx"; "endothelial swelling", "endocapillary inflammatory infiltrate", and "endocapillary monocytes" because they were encompassed by "endocapillary 1/2/4"; "extracapillary 1" because cellular crescents were encompassed by "extracapillary 2"; and "interstitial lymphocytes" because they were encompassed by "interstitial infiltration". "Interstitial fibrosis" and "tubular atrophy" were combined (whichever was the higher value) into a composite variable: "interstitial fibrosis/tubular atrophy" (IF/TA). Thus, 29 histopathologic variables remained to be tested in relation to outcomes.

	Mes2	End2	End3	EndSw	EndInf	EndGran	EndLym	EndMon	WL	Extr1
GlobGS			-0.5							
Isch										
Mes1	**0.9**									
Mes3										0.3
MinLeu		-0.4			-0.4					
End1		0.5	0.6	0.5	0.5					
End2		—	**0.8**	**0.99**	**0.9**	0.5	0.7	**0.8**	0.5	
End3			—	**0.8**	**0.8**		0.6	0.6	0.5	
EndSw				—	**0.9**	0.5	0.7	**0.8**	0.5	
EndInf					—	0.5	**0.8**	**0.8**	0.4	
EndGran						—		0.4		
EndLym							—	0.6		
EndMon								—	0.5	
Extr1										
Extr2										
Extr3										
Extr4										
TA										
IntInf										

Because 666 comparisons were assessed, the significance was set to $P=8\cdot10^{-5}$ by Bonferroni correction ($P=0.05/666$). Only correlation coefficients with $P<8\cdot10^{-5}$ are shown. Numbers in the cells represent Pearson/Spearman correlation coefficients. Correlation coefficients >0.8 are bold-printed. Abbreviations: DC, double contours; End1/2/3: endocapillary 1/2/3; EndInf, endocapillary inflammatory infiltrate; EndGran, endocapillary granulocytes; EndLym, endocapillary lymphocytes; EndMon, endocapillary monocytes; EndSw: endothelial cell swelling; Extr1/2/3/4/5: extracapillary 1/2/3/4/5; GlobGS, global sclerosis; IF, interstitial fibrosis; ArtIF, arterial intimal fibrosis; IntInf, interstitial infiltration; IntLym, interstitial lymphocytes; IntGran, interstitial granulocytes; Mes1/2/3: mesangial 1/2/3; MinLeu, minimal leukocyte influx; TA, tubular atrophy; WL, wire loops.

Extr2	Extr3	Extr5	Necr	DC	TubAt	IntInf	IntLym	IntGran	IF	ArtIF
					0.5				0.5	0.4
					0.5				0.4	
				0.4						
0.4	0.4									
			0.3							
0.4										
0.9	**0.8**					0.4				
—	**0.9**					0.4				
	—					0.4				
		0.97								
					—				**0.8**	
						—	**0.9**			

Appendix 5.4 Distribution of histopathologic lesions across patients with LN



Correlations between histopathologic variables and the number of scorable glomeruli
None of the glomerular or tubulointerstitial variables were correlated with the number of scorable glomeruli. Patients who did not have more (≥) than 10 scorable glomeruli in their renal biopsies – as is the minimum biopsy requirement according to the ISN/RPS – had 5 (n=5), 6 (n=3), 7 (n=2), 8 (n=6), or 9 (n=3) scorable glomeruli. A comparison between patients with fewer (<) or more (≥) than 10 scorable glomeruli revealed that only the distribution of karyorrhexis, endocapillary granulocytes, and monocytes was different between the groups (all $P<0.05$; higher scores in patients with ≥10 scorable glomeruli). Following these results, we decided to uphold our initial threshold of ≥5 scorable glomeruli. Karyorrhexis, endocapillary granulocytes, and endocapillary monocytes were not univariably associated with any of the outcomes studied. These variables were not incorporated in our statistical models.

Appendix 5.5. Correlations between histopathologic variables and mean arterial pressure (MAP), eGFR, and proteinuria at the time of renal biopsy$_0$ in 105 patients with LN

		$eGFR_0$, mL/min/1.73 m^2		$Proteinuria_0$, g/24h		MAP_0, mm Hg	
Glomerular variables		r	*P*	r	*P*	r	*P*
% Normal glomeruli		0.3	<0.001*	−1.1	0.31	−0.2	0.02*
% Minimal leukocyte influx		0.3	<0.001*	−0.2	0.06	−0.3	0.007*
% Normal glomeruli/minimal leukocyte influx		0.4	<0.001*	−0.2	0.05*	−0.3	0.001*
% Global sclerosis		−0.2	0.02*	0.0	0.78	0.1	0.27
% Ischemic glomeruli		−0.2	0.01*	0.0	0.82	0.0	0.78
% Endocapillary hypercellularity	*Any*	−0.2	0.11	0.2	0.02*	0.3	<0.001*
	Segmental	0.1	0.36	0.0	0.85	0.2	0.08
	Global	−0.3	0.008*	0.3	0.007*	0.3	0.009*
% Wire loops		−0.1	0.20	0.2	0.11	0.2	0.02*
% Cellular/fibrocellular crescents		−0.2	0.03*	0.2	0.10	0.2	0.02*
Tubulointerstitial/vascular variables		ρ	*P*	ρ	*P*	ρ	*P*
IF/TA		−0.3	0.001*	0.1	0.67	0.2	0.03*
Interstitial infiltration		−0.3	0.002*	0.4	<0.001*	0.1	0.23
Tubular reabsorption droplets		−0.1	0.34	0.2	0.07	0.2	0.03*
Tubular casts		−0.3	0.007*	0.2	0.10	0.2	0.14
Tubular macrophages		−0.3	0.008*	0.3	0.005*	0.1	0.18
Arterial intimal fibrosis		−0.2	0.03*	0.0	0.78	0.2	0.04*

Only variables with any significant correlation with $eGFR_0$, $proteinuria_0$, and/or MAP_0 are shown.*$P<0.05$. IF/TA, interstitial fibrosis or tubular atrophy; r, Pearson's correlation coefficient; ρ, Spearman's correlation coefficient.

Appendix 5.6. Analysis of progressive eGFR decline

Results

Results for the complete cohort and the subset were comparable. In the complete cohort, a decline of eGFR over 1 and 5 years was independently predicted by non-Caucasian race (1 year: −17.7 mL/min/1.73 m^2; 5 years: −17.8 mL/min/1.73 m^2), age_0 (1 year: −0.5 mL/min/1.73 m^2/year; 5 years: −0.9 mL/min/1.73 m^2/year), fibrinoid necrosis (1 year: −1.4 mL/min/1.73 m^2/%glomeruli; 5 years: −1.5 mL/min/1.73 m^2/%glomeruli), fibrous crescents (1 year: −1.2 mL/min/1.73 m^2/%glomeruli; 5 years: −1.6 mL/min/1.73 m^2/%glomeruli), and the presence of IF/TA ≥25% (1 year: −21.7 mL/min/1.73 m^2; 5 years: −30.3 mL/min/1.73 m^2). Over 10 years follow-up, MAP_0 was associated with eGFR decline (−1.1 mL/min/1.73 m^2/mm Hg), and endocapillary lymphocytes and wire loops were associated with eGFR recovery (both +0.4 mL/min/1.73 m^2/%glomeruli).

Multivariable prediction models for progressive eGFR decline in mL/min/1.73 m^2.

	ISN/RPS Class I–V (n=105)	ISN/RPS Class III/IV (±V)‡ (n=91)
Progressive eGFR decline over 1 year ($eGFR_{CORR(1)}$)† (n=99)		
Variables	β (95% CI)	β (95% CI)
(Constant)	30.6 (17.0; 44.1)	33.1 (18.2; 47.9)
Non-Caucasian	−17.7 (−27.7; −7.7)	−21.1 (−32.2; −10.0)
Age_0, y	−0.5 (−0.9; −0.2)	−0.5 (−0.9; -0.1)
% Fibrinoid necrosis (glomerular)	−1.4 (−2.0; −0.8)	−1.4 (−2.0; −0.8)
% Fibrous crescents	−1.2 (−2.1; −0.2)	−1.3 (−2.3; −0.2)
IF/TA ≥25%	−21.7 (−36.2; −7.2)	−25.2 (−40.8; −9.5)
Tubular macrophages present	9.7 (−2.6; 21.9)	11.1 (-2.1; 24.4)
Progressive eGFR decline over 5 years ($eGFR_{CORR(5)}$)† (n=98)		
Variables	β (95% CI)	β (95% CI)
(Constant)	45.7 (26.2; 65.2)	45.2 (24.4; 66.0)
Non-Caucasian	−17.8 (−33.0; −2.7)	−22.9 (−39.1; −6.6)
Age_0, y	−0.9 (−1.5; −0.4)	−0.8 (−1.4; −0.3)
% Fibrous crescents	−1.6 (−3.0; −0.2)	−1.7 (−3.3; −0.1)
% Fibrinoid necrosis (glomerular)	−1.5 (−2.5; −0.5)	−1.5 (−2.6; −0.5)
IF/TA ≥25%	−30.3 (−52.5; −8.1)	−31.7 (−54.4; −9.1)
Progressive eGFR decline over 10 years ($GFR_{CORR(10)}$)† (n=71)		
Variables	β (95% CI)	β (95% CI)
(Constant)	91.1 (40.4 ; 141.8)	93.6 (35.6; 151.6)
MAP_0, mm Hg	−1.1 (−1.6; −0.6)	−1.1 (−1.7; −0.6)
% Endocapillary lymphocytes	0.4 (0.0; 0.7)	0.4 (0.0; 0.7)
% Wire loops	0.4 (0.0; 0.9)	0.5 (0.0; 0.9)

$eGFR_{CORR(t)}$ is the renal function deterioration (or improvement) in mL/min/1.73 m^2 at t years relative to the expected value based on the eGFR at baseline and the unadjusted mean decline of eGFR over t years. $eGFR_{CORR(t)} = eGFR_{Observed(t)} - eGFR_{Predicted(t)}$. For a given patient, the eGFR at time t is given by: $eGFR_{Predicted(t)} + eGFR_{CORR(t)}$. Estimations of $eGFR_{Predicted(t)}$ were: $eGFR_{Predicted(1\ year)} = 29.9 + 0.67*eGFR_0$; $eGFR_{Predicted(5\ years)} = 42.0 + 0.53*eGFR_0$; and $eGFR_{Predicted(10\ years)} = 33.9 + 0.50*eGFR_0$. Estimations of $eGFR_{CORR(t)}$ are given in the table; e.g. $eGFR_{CORR1}$ = Constant + $\beta_{Age0}*Age_0$ + $\beta_{\%fibrous\ crescents}$*%fibrous crescents + $\beta_{\%fibrinoid\ necrosis}$*%fibrinoid necrosis + $\beta_{non\text{-}Caucasian}$ (if Non-Caucasian) + $\beta_{IF/TA\ \geq 25\%}$ (if IF/TA ≥25%).

A

Appendix 5.7

Interactions between histopathologic variables and race

Compared with patients with Afro-Caribbean race, spikes/vacuoles were more positively associated with eGFR in patients with Caucasian or Asian race (β=0.9 mL/min/1.73 m^2 for each percent of glomeruli [95% CI, 0.2 to 1.5]). Moreover, the relationship between tubular reabsorption droplets on eGFR was different between ethnicities: Caucasian and Asian patients with tubular reabsorption droplets had a higher average eGFR than Afro-Caribbean patients (β=55.6 mL/min/1.73 m^2 [95% CI, 16.8 to 94.4]).

Interactions between histopathologic variables and age

Age at the time of renal biopsy was significantly correlated with endocapillary granulocytes ($r=-0.22$, $P=0.026$), IF/TA ($\rho=0.21$, $P=0.04$), tubular casts ($\rho=0.26$, $P=0.008$) and arterial intimal fibrosis ($\rho=0.31$, $P=0.001$). A comparison between children (age <18 years) and adults revealed that children showed significantly less global glomerulosclerosis ($P<0.001$) and ischemia ($P=0.03$), and more endocapillary granulocytes ($P<0.001$). No differences in tubulointerstitial/vascular parameters were noted. No interactions between age and histopathologic variables were found.

Interactions between histopathologic variables and therapy

To study whether cytotoxic immunosuppressive therapy influenced the predictive value of histopathologic lesions, induction therapy was divided in three categories: (i) no therapy/prednisolone only; (ii) guideline-recommended therapy[3-5] including intravenous cyclophosphamide or mycophenolate mofetil (MMF); and (iii) azathioprine with prednisolone. No differences in the predictive value of histopathologic variables were noted between the treatment categories in mixed models for eGFR and in linear regression models for renal function recovery/deterioration in the complete cohort. In the subset of patients with class III or IV LN treated with cytotoxic drugs, analysis by treatment category for the outcomes ESRD and renal flare revealed no differences between predictive values of pathology variables, with one exception. Global glomerulosclerosis was significantly associated with ESRD for patients treated with azathioprine (HR 1.03 per %glomeruli, $P=0.01$), whereas in patients who received cyclophosphamide or MMF it was not. Time to first renal flare was not different between patients in each treatment category during 10 years follow-up ($P=0.5$). Twenty-five of 53 patients with ≥10 years follow-up did not experience a first renal flare during 10 years follow-up: of these, 3 patients who received induction immunosuppression with azathioprine (n=10) and none of the patients in the other treatment categories (n=11) experienced a first renal flare after this period ($P=0.2$).

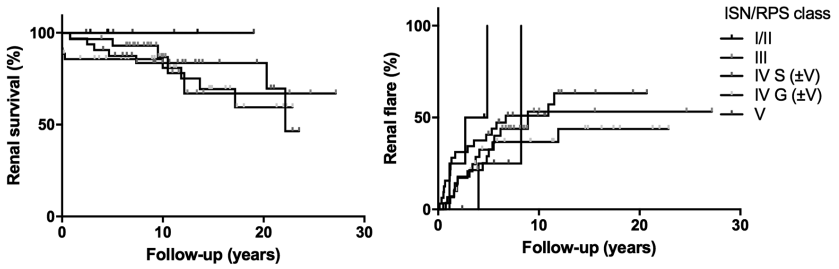
Appendix 5.8. ISN/RPS classes in relation to outcome

Renal flare and ESRD

ISN/RPS classes were not significantly associated with overall renal survival (Log Rank test, *P*=0.7) and renal flare (Log Rank test, *P*=0.3) by Kaplan-Meier analysis (Figures below).

eGFR during follow-up

The ISN/RPS class of LN (either I/II, III, IV-S [±V], IV-G [±V], or V) was significantly associated with the mean eGFR during follow-up (*P*<0.05). The lowest mean eGFR during follow-up was found in class IV-S LN.



eGFR during follow-up in mL/min/1.73 m^2 adjusted for ISN/RPS class (Mixed model analysis).

Model	β (95% CI)
(Intercept)	105.1 (76.3; 114.0)
(Time, y)	−0.7 (−1.5; 0.02)
ISN/RPS class	
I/II	−10.2 (−53.4; 33.0)
III	−18.3 (−49.5; 12.9)
IV-S	−36.8 (−67.6; −6.0)
IV-G	−21.9 (−52.9; 9.1)
V*	–

*Class V is the reference category; e.g. eGFR in class IV-S LN is on average 36.8 mL/min/1.73 m^2 lower than eGFR in class V LN (which is 105.1 mL/min/1.73 m^2 at the time of biopsy).

Chapter 6

Appendix 6.1. Case histories

Patient 1. NP-SLE, antiphospholipid syndrome, and cerebrovascular disease
This 57-year-old female had a 28-year history of SLE complicated by arthritis, endocarditis, epilepsy, cerebral infarctions, and antiphospholipid syndrome. She suffered an epileptic insult at home and was admitted to the hospital in a confusional state. An MRI scan (1.5-T) two weeks before her death revealed diffuse cortical atrophy, multiple previous cortical infarctions, and diffuse white-matter hyperintensities. The patient developed a myocardial infarction, severe pulmonary embolism, acute renal failure, and multiple cerebral infarctions. Antinuclear, anti-dsDNA, and antiphospholipid antibodies were repeatedly positive. The patient showed no sign of a central nervous system infection. The patient died in a coma from multiorgan failure due to active SLE and diffuse thrombotic complications. Autopsy revealed atrophy of the cerebral cortex, laminar cortical necrosis, old and recent micro-infarctions, macroinfarctions, and diffuse vasculopathy.

Patient 2. NP-SLE, acute neurologic deterioration and vasculitis
This 38-year-old male had a 10-year history of SLE. He was admitted in a sub-comatose condition, a state that had developed the previous night. His SLE was associated with skin lesions, pleuritis, pericarditis, arthritis, and hypocomplementaemia. Antinuclear and anti-dsDNA antibodies were positive, whereas antiphospholipid antibodies were negative. All cerebrospinal fluid cultures at admission were negative. Ante-mortem CT and MRI (1.5-T) scans did not reveal any abnormalities. Upon the clinical diagnosis of NP-SLE, the patient was treated with high-dose immunosuppressive therapy (cyclophosphamide and prednisolone). However, the clinical course was complicated by the development of an opportunistic pulmonary infection with *Klebsiella pneumoniae*, and the patient died from respiratory distress in the intensive care unit. Autopsy revealed venous abnormalities consistent with venous vasculitis (invasion of lymphocytes within the vascular wall and fibrinoid necrosis) and diffuse vasculopathy.

Patient 3. SLE, acute myocardial infarction, no neuropsychiatric symptoms
This 63-year-old female had a 30-year history of SLE complicated by arthritis, glomerulonephritis, pleuritis, and skin lesions. During the course of her disease, she did not develop neuropsychiatric symptoms. The patient was positive for antinuclear antibodies and anti-dsDNA antibodies, but negative for antiphospholipid antibodies. Autopsy revealed a myocardial infarction and no apparent cerebral abnormalities.

Appendix 6.2. Post-mortem neuroimaging and evaluation of the acquired images

Formalin-fixed brains were sectioned into approximately 1-cm-thick coronal sections and stored according to standard protocols. Remnants of the dura and vasculature were removed from the pial surface, and residual formalin was removed by immersion in phosphate-buffered saline for at least one day to partially restore the transverse relaxation parameter.[6] The brain specimens were placed between polymethyl methacrylate plates (170 mm long; 80 mm wide) and immersed in proton-free fluid (Fomblin LC55, Solvay). Post-mortem MRI scans were acquired using a whole-body 7-Tesla system (Philips Healthcare, Best, the Netherlands) fitted with a Nova Medical transmit coil with a 16-channel receiver array.

Images were acquired as described previously,[7] with slight modifications; echo times (TE) ranged from 20–40 ms. After visual inspection, a TE time of 35 ms was found to provide the optimum combination of image quality and contrast; this TE was subsequently used for imaging all remaining brain specimens. Scan parameters were as follows: voxel resolution 0.3 x 0.3 x 0.3 mm for a 3D T_2^*-weighted gradient echo sequence, with repetition time/TE/flip angle = 60 ms/35 ms/10°. The number of slices was adjusted to match specimen size and ranged from 60−80 slices, resulting in scan duration of approximately 2.5 hours; seven signal averages were acquired to obtain sufficient image quality.

Appendix 6.3. Relationship between C1q, C4d, and C5b-9 in SLE and NP-SLE

Twenty-five of 34 (74%) patients with SLE and/or NP-SLE had concurrent positive (either focal or diffuse) vascular staining of C1q, C4d, and C5b-9. Twenty-nine of the 30 patients with positive C4d staining (either focal or diffuse) had concurrent C1q deposits and 25 had concurrent C5b-9 deposits (an overlap of 97% and 83%, respectively). Conversely, 29 of the 33 patients with positive C1q staining (either focal or diffuse) had concurrent C4d, and 28 had concurrent C5b-9 staining (an overlap of 89% and 85%, respectively). Of the 28 patients with positive C5b-9 staining, 25 (89%) had concurrent C1q and C4d staining.

C1q staining pattern	C5b-9 staining pattern			C4d staining pattern
SLE (n=18)		No C4d staining	Focal C4d staining	Diffuse C4d staining
No C1q staining	No C5b-9	0	1	0
	Focal C5b-9	0	0	0
	Diffuse C5b-9	0	0	0
Focal C1q staining	No C5b-9	0	2	0
	Focal C5b-9	2	4	1
	Diffuse C5b-9	0	1	0
Diffuse C1q staining	No C5b-9	0	0	0
	Focal C5b-9	0	4	1
	Diffuse C5b-9	0	2	0
NP-SLE (n=16)		No C4d staining	Focal C4d staining	Diffuse C4d staining
No C1q staining	No C5b-9	0	0	0
	Focal C5b-9	0	0	0
	Diffuse C5b-9	0	0	0
Focal C1q staining	No C5b-9	1	0	0
	Focal C5b-9	1	6	1
	Diffuse C5b-9	0	1	1
Diffuse C1q staining	No C5b-9	0	2	0
	Focal C5b-9	0	1	0
	Diffuse C5b-9	0	1	1

Chapter 8

Appendix 8.1. Primers

Marker name	Position	5' Primer 3'
S01a	F	GGT ACC GGG TCT CCA CAT GA
S01b	F	GTA CCG GGT CTC CAC CAG G
S01a/b	R*	GGG AAA GTC ACT CAC CCA AGG
S03	F	CTT TTG CTT TCT GTT TCT TAA GGG C
S03	R	TCA ATC TTT GGG CAG GTT GAA
S04a/b	F*	CTG GTG CCC ACA GTT ACG CT
S04a	R	AAG GAT GCG TGA CTG CTA TGG
S04b	R	AGG ATG CGT GAC TGC TCC TC
S05b	F	AGT TAA AGT AGA CAC GGC CTC CC
S05b	R	CAT CCC CAC ATA CGG AAA AGA
S07b	F	GGT ATT GGC TTT AAA ATA CTC AAC C
S07b	R	CAG CTG CAA CAG TTA TCA ACG TT
S08b	F	GCT GGA TGC CTC ACT GAT GTT
S08b	R	TGG GAA GGA TGC ATA TGA TCT G
S09b	F	GGG CAC CCG TGT GAG TTT T
S09b	R	CAG CTT GTC TGC TTT CTG CTG
S10a	F	GCC ACA AGA GAC TCA G
S10b	F	TTA GAG CCA CAA GAG ACA ACC AG
S10a/b	R*	TGG CTT CCT TGA GGT GGA AT
S11a	F	TAG GAT TCA ACC CTG GAA GC
S11b	F	CCC TGG ATC GCC GTG AA
S11a/b	R*	CCA GCA TGC ACC TGA CTA ACA
GSTM1	F	GAA CTC CCT GAA AAG CTA AAG CT
GSTM1	R	GTT GGG CTC AAA TAT ACG GTG G
GSTT1	F	TCC TTA CTG GTC CTC ACA TCT C
GSTT1	R	TCC CAG CTC ACC GGA TCA T
RhD	F	GCC TGC ATT TGT ACG TGA GA
RhD	R	CAA AGA GTG GCA GAG AAA GGA
Xq28	F	TGG GTT CCA ACC AGC A
Xq28	R	ACT GAC AAT TAT CAC AGC TT
R27I	F	AGA GGA TTG ACT CGG G
R27I	R	GTT ACG TCT TAG ATG CCA G
SRY	F	TGG CGA TTA AGT CAA ATT CGC
SRY	R	CCC CCT AGT ACC CTG ACA ATG TAT T

F= forward, R= reverse, *common primer.

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Curriculum vitae

Emilie Christine Rijnink werd op 25 februari 1992 geboren in Leiden. In 2010 behaalde zij *cum laude* haar gymnasiumdiploma en het International Baccalaureate English diploma (tweetalig onderwijs) aan het Rijnlands Lyceum Oegstgeest. Datzelfde jaar begon zij met de studie Geneeskunde aan de Universiteit Leiden in het Leids Universitair Medisch Centrum. In 2011 werd zij toegalaten tot het Excellente Studententraject van het Honours College van de Universiteit Leiden. Zij startte toen met onderzoek naar chimerisme en lupus naast haar studie Geneeskunde onder begeleiding van dr. Ingeborg Bajema, dr. Onno Teng en prof. dr. Jan Anthonie Bruijn. Naast haar studie was zij ook actief bij haar studentenvereniging L.S.V. Minerva, waar zij lid was van de "Groene Genen" duurzaamheidscommissie van 2013 tot 2014. Na het *cum laude* behalen van haar bachelorsdiploma Geneeskunde en het Honours College certificaat in 2013, verrichtte zij fulltime promotieonderzoek op de afdeling Pathologie van het Leids Universitair Medisch Centrum (hoofd: prof. dr. V.T.H.B.M. Smit). In 2013 ontving zij hiervoor de Kolff studentonderzoeker beurs van de Nierstichting en in 2014 ontving zij een beurs voor een tweejarige aanstelling in het kader van het Excellente Studententraject. De resultaten van dit onderzoek zijn beschreven in dit proefschrift. Tijdens haar onderzoek bezocht Emilie verschillende congressen waar zij haar onderzoek presenteerde, onder andere op de *American Society of Nephrology Kidney Week* in Atlanta (2013), Philadelphia (2014), San Diego (2015) en Chicago (2016). In Philadelphia ontving Emilie de Pirani Award van de *Renal Pathology Society* en in San Diego nam zij deel aan het "Kidney Stars" programma van de *American Society of Nephrology.* Verder nam zij deel aan diverse cursussen, waaronder de "Leiden Oxford Transplantation Summerschool". Haar eerste publicatie over chimerisme tijdens de zwangerschap (dit proefschrift) kreeg internationale media-aandacht, met verhandelingen in onder andere de "New York Times" (Verenigde Staten), "National Geographic" (Spanje), en "El Pais" (Spanje). In September 2016 startte zij met haar co-schappen, waaronder drie co-schappen in het Academisch Ziekenhuis Paramaribo (Suriname). Begin 2019 hoopt zij het artsexamen te behalen.

Emilie Christine Rijnink was born on 25 februari 1992 in Leiden, The Netherlands. In 2010, she graduated from the Rijnlands Lyceum Oegstgeest with honours and received the International Baccalaureate English certificate concluding her bilingual education. The same year, she started medical school at Leiden University at the Leiden University Medical Center. In 2011, she was accepted to participate in the MD/PhD program of the Honours College at Leiden University. At the time, she started doing part-time research on chimerism and lupus next to her medical studies under supervision of dr. Ingeborg Bajema, dr. Onno Teng, and prof. dr. Jan Anthonie Bruijn. Alongside attending her regular curriculum, she was also active at her students' society L.S.V. Minerva, where she was part of the "Green Genes" committee. After completing her Bachelor's degree with honours and receiving the Honours College degree in 2013, she started performing fulltime PhD research at the department of Pathology of the Leiden University Medical Center (head: prof. dr. V.T.H.B.M. Smit). In 2013, she received the Kolff student researcher grant of the Dutch Kidney Foundation and in 2014 she received a PhD grant for 2 years as part of the MD/PhD program of the Leiden University Honours College. The results of the PhD research are described in this thesis. During her PhD research, Emilie visited various international conferences where she presented her work, including the *American Society of Nephrology Kidney Week* in Atlanta (2013), Philadelphia (2014), San Diego (2015), and Chicago (2016). In Philadelphia Emilie received the Pirani Award assigned by the *Renal Pathology Society* and in San Diego she participated in the "Kidney Stars" program of the *American Society of Nephrology.* Futhermore, she attended various courses during her PhD research, including the "Leiden Oxford Transplantation Summerschool". Her first publication on chimerism during pregnancy received worldwide media attention, including exposure in the "New York Times" (United States), "National Geographic" (Spain), and "El Pais" (Spain). In September 2016, she started her clinical rotations, three of which she did in Academic Hospital Paramaribo (Surinam). In the beginning of 2019 she plans to attain her medical degree.

List of publications

1. **Rijnink EC,** Teng YKO, Kraaij T, Dekkers OM, Bruijn JA, Bajema I. Validation of Systemic Lupus International Collaborating Clinics Classification Criteria in a Cohort of Patients with Full House Glomerular Deposits. Kidney Int. 2017, *In Print*.

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