CD163+ macrophage infiltration reflects disease activity and early treatment response in lupus nephritis

S.D. Crum[1,2,3], L. Kwant[1,4], G. Sela[1], N. Claessen[5], J.J.T.H. Roelofs[2,3,5], O. de Boer[5], A.J. Kwakernaak[1,2,6], L. Vogt[1,3], S. Florquin[5], M.W.P. Tsang-A-Sjoe[6,7], T.J. van der Putten[1,2,3], Y. Vegting[1,2], M. Hilhorst[1,2,3]

[1]Department of Internal Medicine and Department of Nephrology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam; [2]Amsterdam Institute for Immunology and Infectious Diseases, Amsterdam; [3]Amsterdam Cardiovascular Sciences, Amsterdam; [4]Department of Rheumatology, Leiden University Medical Center, Leiden; [5]Department of Pathology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam; [6]Department of Rheumatology and Clinical Immunology, Amsterdam University Medical Center, Amsterdam; [7]Amsterdam Rheumatology and Immunology Center, Amsterdam, The Netherlands.

Abstract

Objective

Lupus nephritis (LN) is a severe manifestation of systemic lupus erythematosus, potentially leading to progressive kidney damage and end-stage kidney disease. Emerging evidence suggests that macrophages play a key role in LN pathogenesis. The aim of this study was to investigate associations between renal macrophage infiltration and clinical, histopathological, and long-term renal outcomes in LN.

Methods

This retrospective cohort study included 58 biopsy-proven LN patients (2002–2018), with a mean follow-up of 77 months. Immunohistochemistry for CD68, CD163, CD206, and C5aR1 macrophage markers was performed on baseline kidney biopsies. Correlations with histological features and clinical parameters such as kidney function (eGFR), proteinuria and long-term renal outcomes were analysed using correlation matrices and linear mixed models.

Results

CD163+ macrophage infiltration in glomerular and tubulointerstitial compartments was significantly associated with clinical and histological activity, including lower baseline eGFR, lower complement levels, more leukocyturia/erythrocyturia and higher activity index scores. Patients with high CD163+ infiltration demonstrated significantly greater improvement of eGFR and proteinuria during the first 6 months of treatment post-diagnosis. Conversely, low CD163+ infiltration was associated with increased likelihood of requiring a repeat biopsy. These associations were not observed when stratifying by LN class. CD68+ macrophages showed a similar trend. CD206+ and C5aR1+ macrophages were not associated with markers of disease activity, although high glomerular C5aR1+ infiltration was linked to repeat biopsy. None of the macrophage markers were associated with long-term outcomes such as renal flares, dialysis, or transplantation.

Conclusion

CD163+ macrophage infiltration at baseline is associated with active disease and early treatment response in LN, independent of received treatment. While LN class guides treatment choice, CD163+ macrophage infiltration may offer additional prognostic value regarding early therapeutic response. These findings support further investigation of CD163+ macrophages as a biomarker not only providing prognostic information but also helping guide treatment choices beyond the current LN classification system.

Key words

CD163, immunohistochemistry, lupus nephritis, macrophage

*Suryanti D. Crum, MD**
*Lianne Kwant, MD**
Gal Sela
Nike Claessen
Joris J.T.H. Roelofs, MD, PhD
Onno de Boer, PhD
Arjan J. Kwakernaak, MD, PhD
Liffert Vogt, MD, PhD
Sandrine Florquin, MD, PhD
Michel W.P. Tsang-A-Sjoe, MD, PhD
Tim J. van der Putten, MD
*Yosta Vegting, MD, PhD***
*Marc Hilhorst, MD, PhD***

**Contributed equally as first authors.*
***Contributed equally as last authors.*

Please address correspondence to:
Suryanti D. Crum
Amsterdam UMC,
Meibergdreef 9,
1105az Amsterdam, The Netherlands.
E-mail: s.d.crum@amsterdamumc.nl
ORCID iD: 0009-0005-0747-5038

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Introduction

Lupus nephritis (LN) is a manifestation of systemic lupus erythematosus (SLE), a potentially destructive autoimmune disorder leading to loss of self-tolerance and subsequently immune complex deposition induced glomerulonephritis. This immune-mediated process causes progressive kidney damage and results in end stage kidney disease (ESKD) in 5–20% of affected patients (1). Novel immunosuppressive therapies target T- and B-cells, but clinical efficacy remains inconsistent (2, 3). This underscores the need to explore additional pathogenic mechanisms and develop improved therapeutic interventions for LN.

Historically, research on LN has predominantly focused on the adaptive immune system but emerging evidence highlights a role for innate immunity, particularly macrophages, in the pathogenesis (4). Renal chemokines promote recruitment of monocytes and macrophages to inflamed kidney tissue, resulting in marked macrophage infiltration in LN kidney biopsies (5-8). The functional heterogeneity of macrophages in LN remains obscure, especially whether these cells adopt pro-inflammatory phenotypes exacerbating tissue injury or anti-inflammatory profiles facilitating immune complex clearance and tissue repair. The traditional M1/M2 macrophage polarisation paradigm is being reconsidered, as recent studies reveal a diverse and dynamic spectrum of macrophage phenotypes within LN kidneys, with implications for disease progression and treatment response (4, 9, 10).

To characterise macrophage infiltration, we focused on specific macrophage markers that reflect distinct functional states and relevance to LN pathology. CD68 is a pan-macrophage marker expressed by all monocytes and macrophages, and its glomerular expression correlates with lupus disease activity (11-13). CD206, a mannose receptor expressed by alternatively activated macrophages, has been associated with protection against kidney damage in murine lupus models.(14-16). CD163, a scavenger receptor expressed on phagocytic macrophages, mediates anti-inflammatory and tissue remodelling functions and is enriched in LN kidney biopsies (17, 18). C5aR1, the receptor for the complement protein C5a, drives chemotaxis of macrophages to LN kidneys (5, 19) and is found to be upregulated in glomeruli and tubulointerstitial areas (20).

Few studies have systematically explored the relationship between macrophage infiltration and clinical parameters, histopathological features, and long-term renal outcomes in LN patients. We hypothesise that the presence and expression patterns of specific macrophage markers in baseline kidney biopsies might have associations with disease progression, including decline in renal function and proteinuria.

To test this hypothesis, we conducted a retrospective cohort study investigating whether infiltration by CD68+, CD163+, CD206+, and C5aR1+ macrophages correlates with clinical parameters, histological severity and long-term kidney outcomes in LN.

Methods

Study design

This retrospective monocentric cohort study included a multi-ethnic population in Amsterdam, the Netherlands. All patients diagnosed with LN from 2002-2018 were included. Informed consent was obtained via opt-out procedure after approval by the local ethics committee (W20_429#20.476).

Exclusion criteria were missing baseline kidney biopsy, no histological LN diagnosis, concurrent kidney disease in biopsy, follow-up <1 year and age <10 years at diagnosis. Baseline information included age, sex, self-reported ethnicity, biochemical disease activity (anti-dsDNA, complement, proteinuria) and medication use.

Follow-up data on kidney function was collected. 24-hour proteinuria and serum creatinine values were retrieved from the electronic health record (EHR). Primary outcomes were eGFR (CKD-EPI 2021 (21) formula) and 24-hour proteinuria change. Secondary outcomes were repeat biopsy, renal flares, start of kidney replacement therapy (haemo- or peritoneal dialysis), kidney transplantation and/or death. Data collection ended on 1-11-2022 or earlier if the patient died or was lost to follow-up.

Table I. Baseline patient characteristics.

Characteristic	Overall n=58	LN Class				*p*-value
		I and **II** n=4	**III** n=10	**IV** n=26	**pure V** n=8	
Age at baseline (years), mean (SD)	31.7 (13.3)	34.5 (6.8)	36.3 (17.4)	31.0 (12.6)	29.5 (13.2)	0.7
Sex, n (%)						0.3
Female	42 (72%)	3 (75%)	5 (50%)	19 (73%)	15 (83%)	
Ethnicity, n (%)						0.028
Non-White	46 (79%)	3 (75%)	7 (70%)	18 (69%)	18 (100%)	
White	12 (21%)	1 (25%)	3 (30%)	8 (31%)	0 (0%)	
eGFR (mL/min/1.73m^2), median (IQR)	98.9 (53.9–119.1)	70.0 (59.4–104.2)	94.2 (68.5–115.0)	88.4 (39.8–115.4)	115.4 (107.7–145.4)	0.049
Proteinuria (g/24h), median (IQR)	2.0 (1.0–5.0)	2.5 (0.8–5.6)	1.0 (0.8–2.8)	2.2 (1.4–5.3)	2.0 (1.3–7.2)	0.4
C3 (g/L), median (IQR)	0.6 (0.4–0.9)	1.2 (0.8–1.2)	0.7 (0.5–0.8)	0.4 (0.2–0.6)	0.8 (0.5–1.1)	<0.001
C4 (g/L), median (IQR)	0.1 (0.0–0.2)	0.1 (0.1–0.2)	0.1 (0.1–0.1)	0.1 (0.0–0.1)	0.1 (0.1–0.3)	0.046
Anti-dsDNA (klU/L), median (IQR)	136.5 (0.0-414.8)	136.0 (68.0-238.5)	151.0 (75.5-1218.5)	275.5 (49.3-725.8)	19.5 (0.0-327.5)	0.530
Induction therapy, n (%)						0.032
Mycophenolate mofetil (MMF)	29 (50%)	2 (50%)	3 (30%)	17 (65%)	7 (39%)	
Cyclophosphamide (CYC)	8 (14%)	0 (0%)	3 (30%)	4 (15%)	1 (5.6%)	
Switch CYC to/from MMF	2 (3.4%)	0 (0%)	0 (0%)	2 (7.7%)	0 (0%)	
None	18 (31%)	2 (50%)	3 (30%)	3 (12%)	10 (56%)	
Other	1 (1.7%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	
Corticosteroids, n (%)						0.2
None	5 (8.6%)	0 (0%)	0 (0%)	1 (3.8%)	4 (22%)	
Oral prednisone	48 (83%)	3 (75%)	10 (100%)	23 (88%)	12 (67%)	
Pulse methylprednisolone	5 (8.6%)	1 (25%)	0 (0%)	2 (7.7%)	2 (11%)	
Corticosteroids started before baseline, n (%)	18 (34%)	0 (0%)	6 (60%)	6 (24%)	6 (43%)	0.088
ACEi/ARB, n (%)	4 (15%)	0 (0%)	1 (25%)	3 (20%)	0 (0%)	0.8

[1] Kruskal-Wallis rank sum test; Fisher's exact test

Kidney histology

Kidney biopsies were preserved in paraffin sections. Sections with Periodic Acid Schiff (PAS) and haematoxylin-eosin (HE) staining were retrieved. All HE and PAS stained biopsies were examined using light microscopy. All biopsies were reviewed by a single experienced nephropathologist (JR). Global sclerotic glomeruli, crescentic glomeruli, Interstitial fibrosis and tubular atrophy (IFTA), arterial fibrosis, interstitial infiltration, National Institutes of Health (NIH) activity/chronicity indices and LN classification using the 2018 ISN/RPS classification (22) were evaluated. Total, cellular, and fibrocellular/fibrous crescent scores were assessed as % of total glomeruli. IFTA score was assessed as a percentage of total tubulointerstitial area. Biopsies were classified as minimal damage (class I + II), proliferative (III or IV, with or without V) or membranous (pure class V).

Immunohistochemistry for macrophages

Immunohistochemical staining was performed to quantify macrophage infiltration and subtypes. In brief, 4µm sections were deparaffinised, antigen retrieval was performed, and staining applied with primary and secondary antibodies (*Supplementary Methods*).

Biopsy staining was semi-automatically assessed with Fiji (ImageJ2, NIH) (23, 24) . Representative sections containing at least five glomeruli were analysed at 20 × magnification. Glomeruli were manually outlined around Bowman's capsule to indicate the glomerular compartment. The tubulointerstitial compartment was calculated as total biopsy area minus glomerular area. Positively stained area was quantified and expressed as % of the total glomerular and tubulointerstitial areas.

Specificity of macrophage marker staining

Staining of CD206 and C5aR1 resulted in unspecific tubular staining (consistent with prior reports of renal staining patterns in the Human Protein Atlas) (25). The tubulointerstitial quantification was therefore excluded from further analysis; the glomerular quantification was included.

Statistical analyses

Statistical analyses were performed in R v. 4.1, with $p<0.05$ considered significant. Associations between baseline clinical/histological parameters and macrophage infiltration were tested using two-tailed Spearman's rank correlation with Benjamini-Hochberg multiple testing correction. Differences in macrophage infiltration between LN classes were assessed with Kruskal-Wallis tests, and Bonferroni-adjusted Mann-Whitney U-tests for *post-hoc* pairwise comparisons. Additional specific statistic tests are described in the online Supplementary file.

Linear mixed models (LMM) were used to determine associations between macrophage infiltration in baseline biopsy and longitudinal kidney function/proteinuria. Real-time eGFR data were used, excluding values during flares to preserve linearity. Based on the tertile distribution of the raw macrophage infiltration values, patients were classified into three groups: low, medium, and high infiltration. Outcomes were eGFR and 24-hour proteinuria, with slopes calculated for 0–6 months after diagnosis. Treatment was classified into three groups: MMF or CYC in combination with corticosteroids, corticosteroids only and no treatment. The chosen time period provided the greatest number of

Table II. Baseline biopsy characteristics.

Characteristic	Overall n=58	LN Class I and II n=4	III n=10	IV n=26	pure V n=8	*p*-value
Total glomeruli, median (IQR)	20 (12–28)	14 (6–31)	14 (9–25)	19 (12–30)	24 (15–31)	0.2
Global glomerulosclerosis (%), median (IQR)	0 (0–0)	0 (0–2)	2 (0–12)	0 (0–0)	0 (0–0)	0.12
Cellular crescents (%), median (IQR)	0 (0–15)	0 (0–0)	9 (0–20)	9 (0–36)	0 (0–0)	<0.001
Fibrous crescents (%), median (IQR)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–13)	0 (0–0)	0.015
Activity index, median (IQR)	4 (1–8)	1 (1–2)	4 (3–7)	8 (5–13)	0 (0–1)	<0.001
Chronicity index, median (IQR)	2 (0–3)	1 (0–3)	2 (2–3)	2 (0–4)	2 (0–2)	0.4
Arterial intimal fibrosis, n (%))	11 (19%)	1 (25%)	4 (40%)	5 (19%)	1 (5.6%)	0.12
Interstitial infiltration, n (%)	34 (59%)	4 (100%)	4 (40%)	20 (77%)	6 (33%)	0.004
IFTA (%), median (IQR)	10 (0–10)	5 (0–10)	10 (1–10)	8 (0–20)	8 (0–10)	0.8

[1] Kruskal-Wallis rank sum test; Fisher's exact test

retrospectively available eGFR measurements, maximising statistical power. Slopes were tested for their association with macrophage presence by comparing the medium and high tertiles to the low tertile. Unadjusted baseline models were fitted with fixed effects for time, macrophage tertiles, and their interaction. A random intercept for each patient was included to account for inter-individual differences in baseline eGFR/proteinuria. In adjusted models, baseline eGFR (for the eGFR model) or baseline proteinuria (for the proteinuria model), treatment and treatment-time interaction were included as fixed effects.

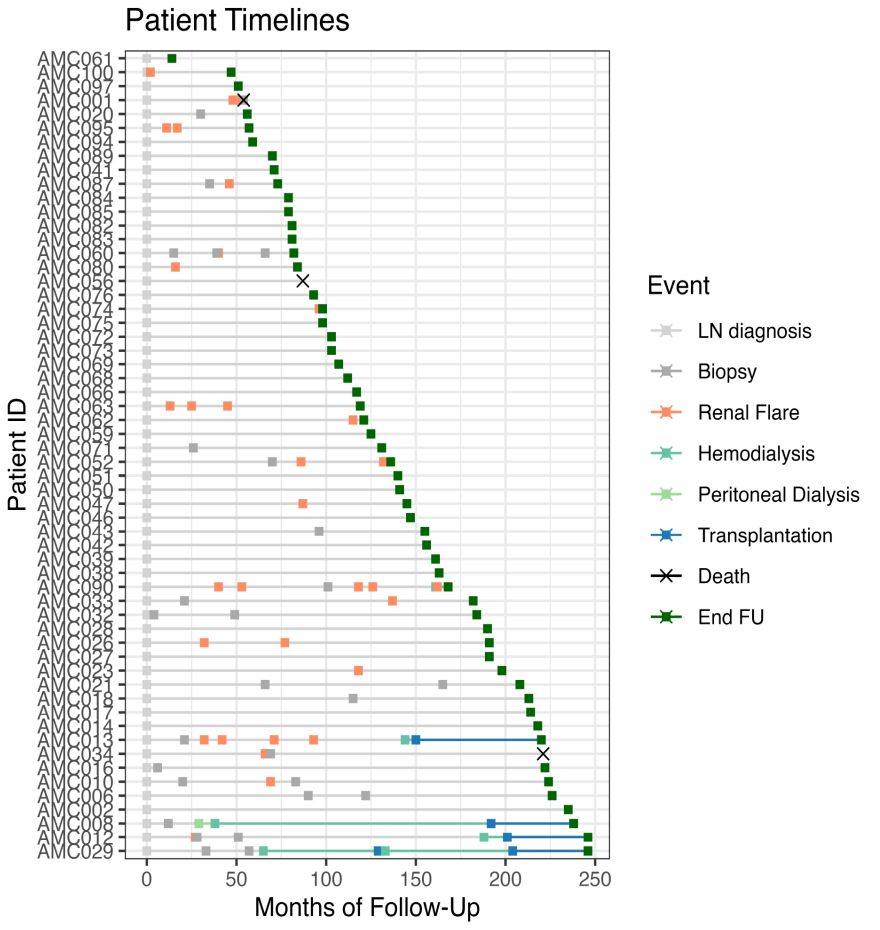


Fig. 1. Event overview through time (in months) of the full cohort.
Each horizontal grey line represents a patient, starting with a diagnostic biopsy at baseline. Squares represent events: biopsy, renal flare, start dialysis, renal transplant or death. Line colour changes when patients receive renal replacement therapy. LN: lupus nephritis; FU: follow-up.

Patient and public involvement statement

This study was supported by grants from the Dutch Kidney Foundation (DKF). As part of the funding process, the full grant proposals were reviewed by an independent advisory board of patient reviewers from the Dutch Kidney Patients Association (NVN). Patients were not directly involved in the design or conduct of this study.

Results

Amsterdam LN cohort

58 of 103 screened patients were included (Supplementary Fig. 1). From all patients the original kidney biopsy report was available. For additional immunohistochemical staining, all available diagnostic (n=51) biopsies were collected. Baseline clinical data are shown in Table I, categorised by LN classification.

Most patients were females (72%). Mean age was 31.7 (SD 13.3). This cohort was multi-ethnic, with 79% of the included patients self-reporting as non-white race or ethnic group (*i.e.* Surinamese, African, Asian etc.). Classes IV and pure V were most common in this cohort. Patients presented with a median proteinuria of 2.0 (IQR 1.0–5.0) g/24h and a median eGFR of 98.9 (IQR 53.9–119.1) mL/min/1.73m^2. The majority of patients received induction treatment with mycophenolate mofetil (MMF) in combination with glucocorticoids following diagnosis.

Baseline kidney biopsies (Table II) contained a median of 20 (IQR 12–28) glomeruli per slide. Median histological activity index (AI) was 4 (IQR 1–8) and

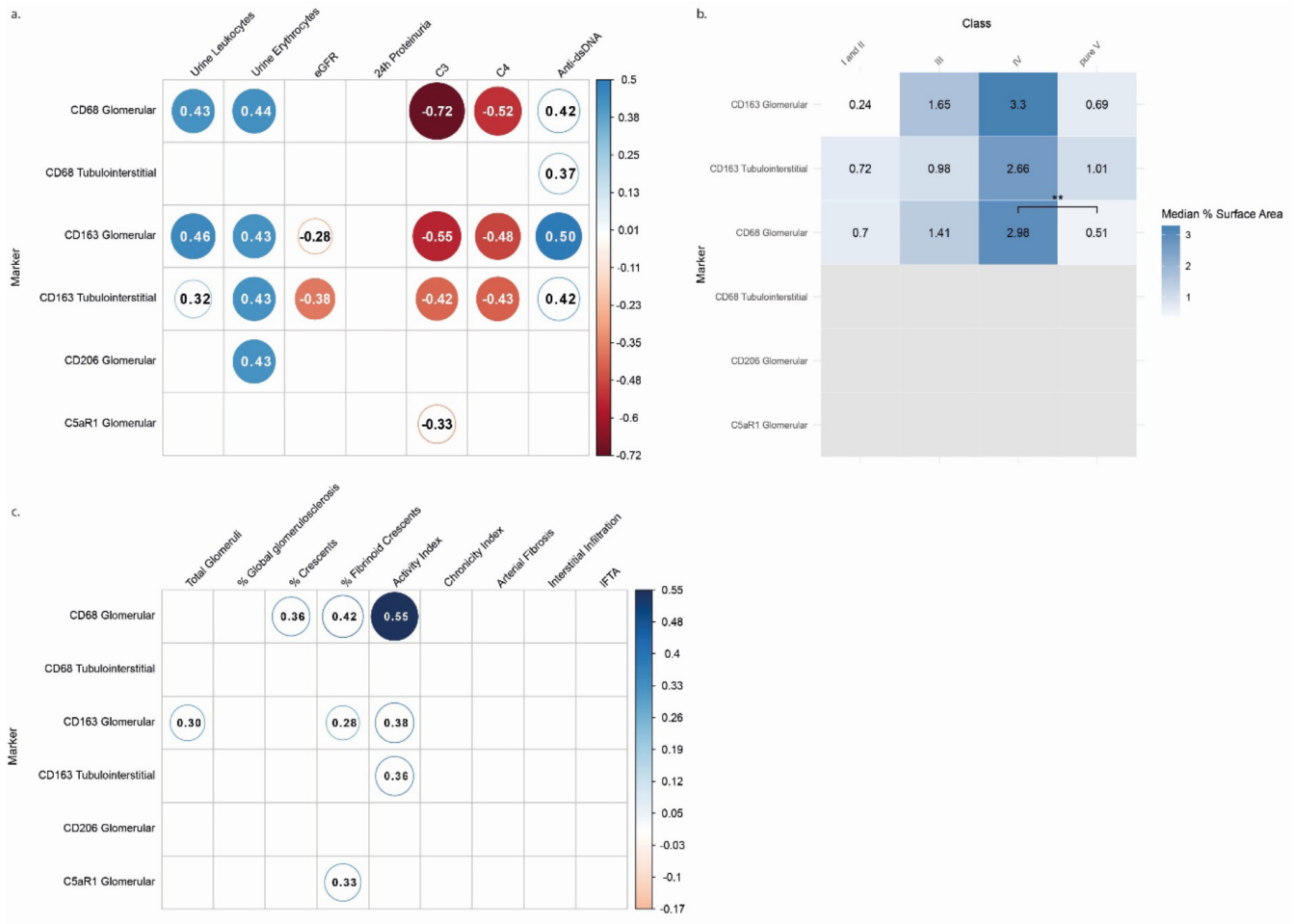


Fig. 2. Associations between macrophage markers and baseline characteristics. Filled circles indicate statistically significant associations (p<0.05). Outlined circles represent associations that were no longer significant after correction for multiple testing (Benjamini-Hochberg), highlighting trends that may reach significance in a larger cohort.
a: Spearman correlation plot of macrophage markers and clinical baseline characteristics.
b: Heatmap showing the median percentage of infiltrated surface area per marker and per LN class. *Post-hoc* testing using Mann-Whitney U-tests revealed a significant difference in glomerular CD68+ infiltration between class IV and class V LN (p=0.008).
c: Spearman correlation plot of macrophage markers and baseline histological characteristics.
eGFR: estimated glomerular filtration rate; 24h: 24-hour; c3: Complement C3; c4: Complement C4; Anti-dsDNA: anti-double stranded DNA; IFTA: interstitial fibrosis and tubular atrophy.

10 out of 58 patients (17%) had an AI >10. Median chronicity index (CI) was 2 (IQR 0–3) at baseline. Patients with class IV LN showed the highest activity scores including crescentic lesions (p<0.001). *Post-hoc* analysis revealed significantly higher AI scores in class IV biopsies compared to non-proliferative LN (class I+II and pure class V).
Median follow-up time was 133.5 months (IQR 82.5–191.0). Repeat biopsies, renal flares, the need for dialysis or a renal transplant and death, were recorded (Fig. 1).
20 out of 58 patients (34.5%) underwent a repeat biopsy during follow-up, with a median of 31 months (IQR 20.7–69.3) after baseline biopsy. In all cases, the indication for repeat biopsy was worsening eGFR, proteinuria, or both.
Patients experienced 0.76 flares on average during total follow-up time. Renal flares occurred in 19 (33%) patients, with a combined total of 31 flares (Fig. 1). Five (9%) patients started dialysis, and 4 of these patients later received a kidney transplant. The number of renal flares during follow-up time was significantly correlated with the risk of kidney replacement (therapy) and tended to have higher flare counts (p=0.018).
During follow-up, 3 (5%) patients died. No deaths were directly related to LN and deceased patients had not received renal replacement therapy at any timepoint.

Association between macrophage markers and clinical data

Baseline clinical parameters

Kidney macrophage infiltration was associated with clinical symptoms of LN activity (Fig. 2a).
Complement C3 and C4 levels at baseline were negatively correlated with glomerular CD68+ and glomerular and tubulointerstitial CD163+ infiltration (C3: r= -0.72, p<0.001, r= -0.55, p=0.004 and r=-0.42, p=0.02, respectively. C4: r= -0.52, p=0.006, r= -0.48, p=0.02 and r= -0.43, p=0.02, respectively). Anti-dsDNA levels were significantly correlated to glomerular CD163+ infiltration (r=0.5, p=0.02).
Leukocyturia and erythrocyturia, mark-

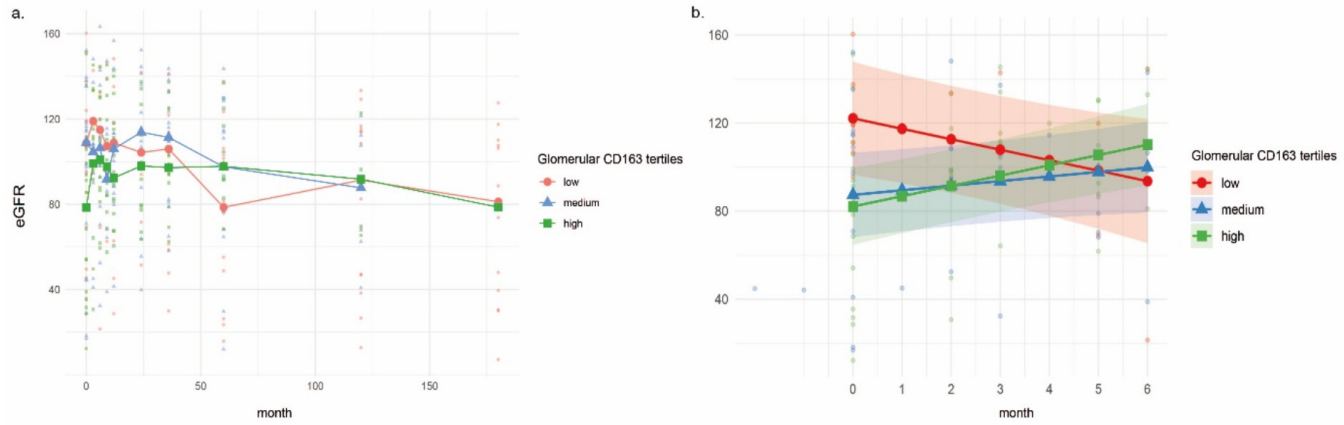


Fig. 3. Kidney function (eGFR) over time, grouped by glomerular CD163+ tertiles.
a: Kidney function of all included patients during total follow-up time. Individual dots are single observations, clustered per month to compute accurate medians. Lines are drawn in between median eGFR per timepoint to visualise kidney function course over years. Long-term follow-up shows kidney function decline over time independent of tertile group.
b: Visualisation of predicted eGFR during the first 6 months after baseline biopsy, estimated using a linear mixed model with 95% confidence intervals, stratified by glomerular CD163 tertiles.

ers of glomerular inflammation, were both positively associated with glomerular CD68+ macrophage infiltration (r=0.43, p=0.02 and r=0.44, p=0.02, respectively) and with both CD163+ macrophage infiltration in glomerular (r=0.46, p=0.02 and r=0.43, p=0.02) and tubulointerstitial (r=0.32, p=0.13 and r=0.43, p=0.02) compartments. Erythrocyturia also showed a positive correlation with glomerular CD206+ macrophage infiltration (r=0.43, p=0.02).

A negative correlation was found between eGFR and CD163+ macrophages in both glomerular (r= -0.28, p=0.13) and tubulointerstitial (r =-0.38, p=0.02) compartments, meaning that CD163+ macrophage infiltration was correlated with lower kidney function at baseline.

No significant correlations were found between 24-hour proteinuria at diagnosis and macrophage infiltration.

Baseline histological data

Associations between histological parameters and macrophage infiltration were explored. Between LN classes, significant differences in positively stained surface area (median %) were seen for glomerular CD68, glomerular CD163 and tubulointerstitial CD163 (H=14.46, p=0.002, H=9.86, p=0.02 and H=8.12 p=0.044, respectively; Fig. 2b). All significant markers showed a trend of increasing median infiltration from minimal change to proliferative LN classes. *Post-hoc* analysis showed that glomerular CD68 was significantly higher in LN class IV compared to pure class V LN (p=0.008).

Activity Indices (AI) in baseline biopsy were correlated with glomerular CD68 (r=0.55, p=0.002) and CD163 in glomerular (r=0.38, p=0.12) and tubulointerstitial (r=0.36, p=0.12) compartments (Fig. 2c). Chronicity indices (CI) were not correlated with any subset of macrophage infiltration at baseline.

Total crescent score was positively correlated with glomerular CD68 (r=0.36, p=0.12). Combined score for fibrous and fibrocellular crescents positively correlated with glomerular CD68 (r=0.42, p=0.07), CD163 (r=0.28, p=0.33) and C5aR1 (r=0.33, p=0.23). Interstitial fibrosis and tubular atrophy (IFTA) presence in the baseline biopsy was not correlated with macrophage infiltration in glomerular or tubulointerstitial compartments.

Longitudinal eGFR and proteinuria analysis

Longitudinal changes in eGFR and proteinuria following biopsy were assessed. A schematic display of median eGFR during total follow-up time is shown in Fig. 3a.

The LMM's showed that baseline eGFR levels and change over the first 6 months differed significantly between low, medium, and high glomerular and tubulointerstitial CD163 groups (Fig. 3b). Patients with medium and high glomerular CD163 in their baseline biopsy had a significantly lower eGFR at baseline (eGFR=87, p=0.033 and eGFR=82, p=0.012) compared to the low tertile group (eGFR=122, p<0.001). During the first 6 months after diagnosis however, medium and high glomerular CD163 groups showed a significant increase in eGFR (+3.7/month, p<0.001 and +5.5/month, p<0.001) per month while the low tertile group showed a significant decrease (-5.6/month, p<0.001) independent of treatment group.

Tubulointerstitial CD163 showed similar results, but only the high tertile group had a significantly lower eGFR at baseline (eGFR=73, p<0.001) in comparison with the low tertile group (eGFR=125, p<0.001). A significant eGFR increase was seen in the medium and high groups (+4.7/month, p<0.001 and +5.9/month, p<0.001), in contrast with a significant decrease in eGFR in the low tertile group (-6.0/month, p<0.001) independent of chosen treatment.

Glomerular CD68 showed only the high tertile group having a significantly eGFR increase over time (+2.3/month, p<0.001) in comparison to a decrease in the low tertile group (3.4/month, p<0.001). For other markers, eGFR course did not significantly differ between all tertiles. Similarly, modelling by LN class only showed class IV to be associated with significant slope differ-

ence (+3.8/month, p=0.002) compared to class I/II (-4.7/month, p<0.001).

When modelling eGFR course from 6 months to total follow-up time, only CD163 in glomerular and tubulointerstitial compartments showed similar results – a significantly increasing eGFR slope with increasing infiltration group (Suppl. Table 2a). Notably, when dividing by LN class, pure class V had a significantly higher eGFR at 6 months (eGFR = 120, p=0.02) than class I+II (eGFR=77, p<0.001), but showed a significantly stronger decrease in eGFR over time (-0.2/month, p<0.001) as opposed to class I+II (-0.1/month, p<0.001), indicating long-term renal function loss despite absence of proliferative lesions.

Similar analyses were done for 24-hour proteinuria change during follow-up. In the first 6 months after baseline and during total follow-up time, most medium and high tertile groups of markers seemed to indicate a slow decrease of proteinuria over time, however results were often not significant and not consistent between macrophage tertiles. Only C5aR1 showed a markedly significant increase of proteinuria during the first 6 months in both medium (+0.2 g/month, p=0.005) and high (+0.6 g/month, p<0.001) in comparison to a decrease in the low group (-0.2 g/month, p=0.03) however this effect did not remain significant during total follow-up time.

Modelling by LN class showed no consistently significant differences, except for a clear significant independent effect of (especially CYC/MMF in combination with prednisolone) treatment leading to both short- and long-time decrease in proteinuria. Raw data of all LMM's are summarised in Supplementary Tables 1 and 2.

Clinical outcomes

No significant associations between macrophage infiltration in the baseline biopsy and renal events such as flares, dialysis and kidney transplantation could be found. Since death was unrelated to LN in all cases, the analysis was repeated excluding death as an outcome, which also did not reveal any significant associations.

A second biopsy was performed in 34% of patients during follow-up (median time to second biopsy was 31.5 months). Patients who underwent a second biopsy had significantly lower glomerular and tubulointerstitial CD163 (p=0.01 and p=0.001), tubulointerstitial CD68 (p=0.006), and glomerular CD206 (p=0.022) infiltration at baseline (data not shown), whereas higher glomerular C5aR1 infiltration was associated with a higher likelihood of repeat biopsy (p=0.006). Additionally, no association was found between patients having to undergo a repeat biopsy and LN class at baseline.

Discussion

In this study, LN patients with high CD163+ macrophage infiltration in diagnostic kidney biopsies showed significantly lower baseline eGFR, but a markedly steeper improvement in renal function within six months after diagnosis independent of treatment received. This suggests a better treatment response, possibly mediated by reduced CD163+ macrophage infiltration in kidney tissue. Of note, our multi-ethnic LN cohort with long follow-up showed ESKD and flare rates consistent with prior reports (26-28).

Glomerular and tubulointerstitial CD68+ and CD163+ macrophages were significantly associated with clinical markers of active disease, including decreased kidney function, decreased complement C3/C4 levels, anti-dsDNA levels, erythrocyturia and leukocyturia. CD163+ and CD68+ macrophage infiltration also correlated with AI and crescent formation, reflecting histological disease activity. Prior studies identified CD163+ macrophages as the predominant subtype in LN kidneys (18), with single-cell data showing a highly pro-inflammatory intrarenal phenotype (29). Similarly, urinary CD163 levels have been correlated with clinical severity, AI and LN class, likely reflecting glomerular macrophage infiltration (30-32). These findings challenge previous assumptions that CD163 expression is largely restricted to anti-inflammatory M2c macrophages (33).

Importantly, patients with high CD163+ infiltration were less likely to undergo a repeat biopsy. Since repeat biopsies in LN are typically performed due to suspected renal flare, partial treatment response or progression of chronic kidney disease (34), this may indicate persistent disease activity and therefore poorer treatment response in patients with low CD163+ infiltration at baseline. Thus, CD163+ infiltration not only reflects disease activity but is also associated with initial therapy response. Notably, similar eGFR trajectories and biopsy associations were not observed when patients were stratified by LN class, suggesting that CD163+ macrophage infiltration is more strongly associated with treatment response than ISN/RPS classification.

Furthermore, both CD163+ and CD68+ infiltration increased with advancing LN class, with lower infiltration in pure class V compared to class IV. Since membranous LN (class V) is characterised by subepithelial immune complex deposits and a relatively muted inflammatory response, often limited to proteinuria (35), the lower macrophage infiltration observed in class V biopsies compared to the more proliferative classes (III and IV) is expected. Only patients with high or medium CD163+ infiltration showed a significant reduction in proteinuria during the first six months after treatment. Conversely, patients with pure class V or low CD163+ infiltration exhibited persistent proteinuria, resulting in long-term eGFR decline not observed in other classes. These findings highlight mechanistic differences and the limited benefit of conventional therapy in membranous LN and in patients with low CD163+ infiltration, supporting macrophage-guided treatment strategies based on infiltration patterns rather than LN class alone.

We found no associations between glomerular CD206+ infiltration and clinical or histological markers of LN injury, contrasting with earlier studies linking CD206+ to decreased eGFR, AI, CI, and hypertension (18, 36, 37). Similarly, no associations were observed between C5aR1+ macrophages and disease activity. Interestingly, higher glomerular C5aR1+ macrophage infiltration was associated with a signifi-

cant increase in proteinuria during the first 6 months after diagnosis and an increased likelihood of repeat biopsy. While Tampe *et al*. reported an association between glomerular C5aR1 and favourable treatment response (38), Ye *et al*. observed upregulation of C5aR1 in LN kidney tissue and a strong correlation between C5aR1+ podocytes and proteinuria severity (39). Therefore, glomerular C5aR1+ cells may indicate disease severity, potentially reflecting both macrophage infiltration and podocyte expression. The role of C5aR1 in LN remains unclear and warrants further investigation.

No baseline macrophage marker was associated with long-term kidney function, proteinuria or renal outcomes, including flare frequency and kidney replacement therapy. This lack of association may partly reflect limited statistical power due to the relatively small cohort size. In addition, macrophage infiltration is dynamic and a biopsy reflects only a single time point, which may explain why baseline infiltration is more closely related to short-term treatment response than to long-term outcomes. Previous research has emphasised the role of chronic histopathological damage, as reflected by the CI (40), as well as the predictive value of repeat biopsy findings (41), in assessing the risk of progression to end-stage kidney disease in patients with lupus nephritis.

Our study has several limitations. As a retrospective analysis, nearly half of screened patients were excluded due to missing data or other reasons, which may introduce selection bias, particularly since the absence of baseline biopsies could be associated with disease severity and/or access to treatment. Immunohistochemical staining of kidney biopsies may also have captured signals from non-macrophage cells, such as podocytes or tubular cells. Although double staining was performed on a small subset of samples to address this issue, it was not applied to all biopsies. Additionally, unspecific tubular staining led to exclusion of tubulointerstitial CD206 and C5aR1, resulting in missing data. The relatively small cohort also limits statistical power, and some correlations lost significance after multiple testing correction. Despite these limitations, the study benefits from a cohort with long follow-up and high-quality tissue staining.

In conclusion, this study demonstrates a strong association between glomerular and tubulointerstitial CD163+ macrophage infiltration and baseline disease activity, reflected by both clinical and histological parameters. Moreover, CD163+ infiltration may reflect early treatment response, as indicated by kidney function recovery within the first six months after diagnosis, and appears to be more strongly associated with eGFR course than the current LN classification system. Patients with low CD163+ infiltration at baseline may lack histological signs of active disease, are less likely to respond to treatment and are more likely to require repeat biopsies during follow-up. These findings highlight the potential for CD163+ macrophage infiltration to guide more personalised treatment strategies, allowing therapy to be tailored based on infiltration patterns rather than LN class alone. The predictive value of CD163+ macrophage infiltration in kidney tissue merits further investigation and validation in future research.

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