

SERUM IL-6, sIL-6R, AND THE IFN- γ /IL-10 RATIO AS NOVEL PREDICTORS OF TREATMENT RESISTANCE IN PROLIFERATIVE LUPUS NEPHRITISSERUMSKI IL-6, sIL-6R I ODNOS IFN- γ /IL-10 KAO NOVI PREDIKTORI REZISTENCIJE NA TERAPIJU KOD PROLIFERATIVNOG LUPUS NEFRITISALiu Liu^{1*}, Yating Luo¹, Huiyu Yuan¹¹Department of Nephrology, First Affiliated Hospital of Nanhua University, Hengyang 421000, Hunan, China**Summary**

Background: Proliferative lupus nephritis (LN) exhibits heterogeneous treatment responses. While serum cytokines reflect inflammation, their prognostic utility for predicting long-term renal outcomes remains under-explored. We investigated whether a pre-treatment serum cytokine panel could identify patients at risk for treatment failure.

Methods: In this prospective observational cohort, we enrolled 112 treatment-naïve patients with biopsy-proven Class III/IV ($\pm$ V) LN. Serum levels of interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R), Interferon-gamma (IFN- γ), and interleukin-10 (IL-10) were measured at baseline (T0) and 6 months post-induction (T6) via high-sensitivity electrochemiluminescence. The primary composite endpoint was »treatment failure« by 24 months, defined as failure to achieve renal response (complete/partial) at 12 months or a $\geq$ 40% decline in estimated glomerular filtration rate (eGFR).

Results: Thirty-two patients (28.6%) met the treatment failure endpoint. At T0, non-responders exhibited significantly higher median levels of IL-6 (12.8 vs 5.1 pg/mL, $p < 0.001$), sIL-6R (45.3 vs. 28.7 ng/mL, $p = 0.002$), and the IFN- γ /IL-10 ratio (3.4 vs 1.1, $p < 0.001$). In multivariable Cox regression adjusted for histological indices and baseline eGFR, an elevated IFN- γ /IL-10 ratio (Hazard Ratio [HR] 3.8, 95% CI 1.9–7.6) and high IL-6 (HR 2.5, 95% CI 1.3–4.8) were independent predictors of treatment failure. A persistent high IFN- γ /IL-10 ratio at T6 conferred a 12-fold risk (HR 12.1, 95% CI 4.5–32.4) of progression to chronic kidney disease stage $\geq$ 3b.

Conclusion: A pre-treatment serum signature of elevated IL-6 and a high IFN- γ /IL-10 ratio identifies patients with proliferative LN at substantial risk for poor outcomes. This accessible biomarker panel offers prognostic stratification beyond standard parameters and could guide risk-adapted therapeutic strategies.

Kratak sadržaj

Uvod: Proliferativni lupus nefritis (LN) pokazuje heterogen odgovor na terapiju. Iako serumski citokini ukazuju na inflamaciju, njihova prognostička vrednost za predviđanje dugoročnih ishoda još uvek nije dovoljno ispitana. Ispitivano je da li preterapijski panel serumskih citokina može identifikovati pacijente kod kojih postoji rizik od neuspeha kod lečenja.

Metode: U ovu prospektivnu opservacionu kohortnu studiju uključeno je 112 prethodno nelečenih pacijenata sa biopsijom potvrđenim lupus nefritisom klase III/IV ($\pm$ V). Serumski nivoi interleukina-6 (IL-6), solubilnog receptora za IL-6 (sIL-6R), interferona-gama (IFN- γ) i interleukina-10 (IL-10) određivani su na početku studije (T0) i 6 meseci nakon indukcione terapije (T6) primenom visokoosetljive elektrohemiluminiscencije. Primarni kompozitni ishod bio je »neuspeh lečenja« do 24. meseca, definisan kao izostanak postizanja bubrežnog odgovora (potpunog/delimičnog) nakon 12 meseci ili pad procenjene brzine glomerulske filtracije (eGFR) $\geq$ 40%.

Rezultati: Kod trideset dva pacijenta (28,6%) zabeležen je neuspeh u lečenju. U trenutku T0, pacijenti bez odgovora na terapiju su imali značajno više medijalne vrednosti IL-6 (12,8 naspram 5,1 pg/mL, $p < 0,001$), sIL-6R (45,3 naspram 28,7 ng/mL, $p = 0,002$) i odnosa IFN- γ /IL-10 (3,4 naspram 1,1, $p < 0,001$). U multivarijantnoj Cox regresionoj analizi prilagođenoj histološkim indeksima i početnom eGFR-u, povišen odnos IFN- γ /IL-10 (hazard ratio [HR] 3,8; 95% CI 1,9–7,6) i visok IL-6 (HR 2,5; 95% CI 1,3–4,8) su bili nezavisni prediktori neuspeha lečenja. Perzistentno visok odnos IFN- γ /IL-10 u T6 je bio povezan sa 12 puta većim rizikom (HR 12,1; 95% CI 4,5–32,4) za progresiju u hroničnu bubrežnu bolest stadijuma $\geq$ 3b.

Zaključak: Preterapijski serumski profil koji karakterišu povišen IL-6 i visok odnos IFN- γ /IL-10 identifikuje pacijente sa proliferativnim LN sa značajno povećanim rizikom od

Keywords: systemic lupus erythematosus, lupus nephritis, biomarkers, cytokines, interleukin-6, interferon-gamma, interleukin-10, prognosis, treatment response
Apstrakt

Introduction

Proliferative lupus nephritis (LN), encompassing International Society of Nephrology/Renal Pathology Society (ISN/RPS) classes III and IV, is a severe organ manifestation of systemic lupus erythematosus (SLE) and a leading cause of morbidity and mortality (1). Despite standardised induction therapies, up to 30–40% of patients fail to achieve renal remission, with a significant subset progressing to chronic kidney disease (CKD) (2, 3). This heterogeneity underscores the critical unmet need for biomarkers that can accurately prognosticate outcomes at diagnosis, enabling personalised treatment approaches (4).

Current serological markers, including anti-double-stranded DNA (anti-dsDNA) antibodies and complement levels, correlate with global SLE activity but are imperfect predictors of renal-specific outcomes and treatment resistance in LN (5, 6). The renal biopsy remains the diagnostic gold standard, providing prognostic information via activity and chronicity indices (7). However, it is invasive, subject to sampling variability, and cannot be repeated serially to monitor the response dynamically.

Cytokines are pivotal mediators of the inflammatory and autoimmune processes in LN (8). Serum cytokine profiles offer a real-time, accessible snapshot of the systemic immune milieu. Interleukin-6 (IL-6), acting through the membrane-bound IL-6 receptor and the soluble IL-6 receptor (sIL-6R), is a pleiotropic cytokine that drives B-cell hyperactivity, acute inflammation, and, via trans-signalling, pro-fibrotic pathways implicated in renal damage progression (9, 10). In SLE, elevated serum IL-6 levels correlate with disease activity, but its specific prognostic value in LN remains controversial (11, 12).

The balance between T-helper type 1 (Th1) and regulatory/type 2 responses may be crucial in LN pathogenesis (13). Interferon-gamma (IFN- γ), a canonical Th1 cytokine, promotes macrophage activation and severe glomerulonephritis (14). Interleukin-10 (IL-10), while generally anti-inflammatory, has complex, often pathogenic roles in SLE, including promoting B-cell survival (15). The ratio of IFN- γ to IL-10 may thus better reflect the net pro-inflammatory drive than either cytokine alone, a concept not previously applied to LN prognosis (16).

lošeg ishoda. Ovaj lako dostupan panel biomarkera pruža prognostičku stratifikaciju izvan standardnih parametara i mogao bi da usmeri terapijske strategije prilagođene nivou rizika.

Ključne reči: sistemski eritemski lupus, lupus nefritis, biomarkeri, citokini, interleukin-6, interferon-gama, interleukin-10, prognoza, terapijski odgovor

We hypothesised that a pre-treatment serum signature, combining the IL-6 axis (IL-6 and sIL-6R) and the Th1/regulatory balance (IFN- γ /IL-10 ratio), would provide superior prognostic information for identifying patients with proliferative LN at high risk of treatment failure. This study aimed to validate these biomarkers as independent predictors of a composite renal outcome in a prospective, well-characterised cohort.

Materials and Methods

Study design and participants

We conducted a prospective, single-centre, observational cohort study at the University Medical Centre Nephrology Department from January 2018 to December 2023. The study was approved by the Institutional Ethics Committee (Ref: IMM-LN-2017-05), and all participants provided written informed consent.

We enrolled consecutive adult patients (≥ 18 years) meeting the following criteria: 1) diagnosis of SLE according to the 2019 EULAR/ACR classification criteria (17); 2) first biopsy-proven active proliferative LN (ISN/RPS Class III or IV, with or without concomitant Class V) with no prior history of biopsy-proven LN of any class; 3) naive to induction immunosuppression for the current episode; and 4) provision of serum samples at the time of biopsy (T0). Key exclusion criteria were: severe CKD (eGFR < 15 mL/min/1.73 m²) or dialysis, active severe infection, pregnancy, or other concomitant autoimmune diseases (18).

Clinical and laboratory data

Demographic, clinical, and standard laboratory data (including serum creatinine, eGFR by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2021 formula (19), urine protein-to-creatinine ratio [UPCR], anti-dsDNA, C3, and C4) were collected at T0, month 6 (T6), month 12, and month 24. All patients received standard-of-care induction therapy per contemporary guidelines (mycophenolate mofetil or cyclophosphamide, combined with corticosteroids) (20). Baseline and induction medication exposure was recorded, including hydroxychloroquine use, renin-angiotensin-aldosterone

system blockade, intravenous methylprednisolone pulses, initial oral prednisone dose, induction regimen with mycophenolate mofetil or cyclophosphamide, and use of add-on belimumab or calcineurin-inhibitor therapy when applicable.

Serum cytokine measurements

Peripheral venous blood was collected in serum-separating tubes at the time of renal biopsy (T0), before the administration of any immunosuppressive therapy, including intravenous methylprednisolone pulses or oral corticosteroids, or within 2 hours of the first pulse dose in patients where urgent clinical presentation necessitated immediate steroid initiation. In all cases, blood was drawn before the completion of the first 24-hour steroid exposure. Samples were processed within 2 hours and stored at -80°C until batch analysis. Serum concentrations of IL-6, sIL-6R, IFN- γ , and IL-10 were quantified in batch analysis using a validated, high-sensitivity V-PLEX Plus Proinflammatory Panel 2 Human Kit (Meso Scale Discovery, USA). The IFN- γ /IL-10 ratio was calculated. All samples were measured in duplicate, and the analyst was blinded to clinical outcomes. The lower limits of detection were: IL-6: 0.1 pg/mL; sIL-6R: 1 ng/mL; IFN- γ : 0.2 pg/mL; IL-10: 0.1 pg/mL.

Renal histopathology

All renal biopsies were reviewed by two expert renal pathologists blinded to clinical and cytokine data. The ISN/RPS class was assigned, and the NIH Activity Index (AI) and Chronicity Index (CI) were scored [7].

Outcome definitions

The primary endpoint was a composite of »treatment failure« within 24 months, defined as:

Failure to achieve renal response at 12 months: Not meeting criteria for either complete response (CR: eGFR $\geq 90\%$ of baseline and UPCr < 0.5 g/g) or partial response (PR: $\geq 50\%$ reduction in UPCr to < 1.0 g/g if baseline ≤ 3.0 g/g, or to < 3.0 g/g if baseline > 3.0 g/g, with stable eGFR [decline $< 20\%$]). Renal response definitions were based on established EULAR/ERA-EDTA response criteria. They were considered in the context of the 2023 EULAR recommendations for SLE management, given that patient follow-up extended through 2023 [21].

Renal function decline: A sustained $\geq 40\%$ reduction in eGFR from baseline, confirmed over 3 months, by 24 months [22].

Secondary outcomes included: 1) time to treatment failure; 2) progression to CKD stage $\geq 3b$ (eGFR < 45 mL/min/ 1.73 m 2); and 3) correlation of cytokine levels with histological indices.

Statistical analysis

Data were analysed using R version 4.3.0. Continuous variables are presented as median (interquartile range [IQR]) and compared using the Mann-Whitney U test. Categorical variables are presented as n (%) and compared using Chi-square or Fisher's exact test. Optimal cut-off values for cytokine levels were determined using Youden's index from receiver operating characteristic (ROC) analysis. Correlations were assessed using Spearman's rank coefficient. Survival analysis for time-to-treatment-failure was performed using Kaplan-Meier curves with the log-rank test. Univariate and multivariable Cox proportional hazards models were used to identify independent predictors, adjusting for prespecified clinical confounders (baseline eGFR, UPCr, NIH-AI, NIH-CI, and induction therapy type). A two-sided p-value < 0.05 was considered statistically significant. To address potential confounding by baseline and induction medications, we performed a prespecified medication-adjusted sensitivity analysis. Given the number of treatment-failure events, medication covariates were incorporated into a parsimonious model that included induction regimen, intravenous methylprednisolone pulse therapy, baseline oral prednisone dose, hydroxychloroquine use, RAAS-inhibitor use, and add-on belimumab or calcineurin-inhibitor therapy. The primary biomarker estimates were compared with those from the main multivariable model.

Results

Baseline characteristics of the cohort

A total of 112 patients with proliferative LN were included. The median age was 32 years (IQR 25–42), and 95 (84.8%) were female. Baseline median eGFR was 78 mL/min/ 1.73 m 2 (IQR 52–98) and median UPCr was 4.1 g/g (IQR 2.3–6.8). Histologically, 61 patients (54.5%) had pure proliferative LN (Class III/IV), and 51 (45.5%) had mixed membranous and proliferative disease (Class III/IV+V). The median NIH Activity and Chronicity Indices were 8 (IQR 6–10) and 2 (IQR 1–3), respectively.

Cytokine levels and association with treatment failure

By 24 months, 32 patients (28.6%) met the composite endpoint of treatment failure. As shown

Table 1 Baseline characteristics stratified by treatment outcome.

Characteristic	Total Cohort (n=112)	Responders (n=80)	Non-Responders (n=32)	p-value
Demographics				
Age, years	32 (25–42)	31 (24–41)	34 (27–45)	0.23
Female, n (%)	95 (84.8)	69 (86.3)	26 (81.3)	0.55
Clinical/Lab				
Baseline eGFR, mL/min/1.73 m ²	78 (52–98)	82 (58–102)	65 (43–85)	0.04
Baseline UPCR, g/g	4.1 (2.3–6.8)	3.9 (2.2–6.5)	4.5 (2.7–7.5)	0.31
Low C3, n (%)	89 (79.5)	61 (76.3)	28 (87.5)	0.21
Histology				
NIH Activity Index	8 (6–10)	8 (5–10)	9 (7–11)	0.08
NIH Chronicity Index	2 (1–3)	2 (1–3)	2 (1–4)	0.15
Serum Biomarkers (T0)				
IL-6, pg/mL	6.8 (3.2–14.5)	5.1 (2.5–9.3)	12.8 (8.1–22.4)	<0.001
sIL-6R, ng/mL	32.1 (24.5–44.8)	28.7 (22.1–38.9)	45.3 (35.2–58.1)	0.002
IFN- γ , pg/mL	8.4 (4.1–15.0)	6.5 (3.5–11.2)	14.8 (9.0–21.5)	<0.001
IL-10, pg/mL	5.2 (2.8–9.1)	5.8 (3.4–10.1)	4.3 (2.1–7.0)	0.03
IFN- γ / IL-10 Ratio	1.5 (0.7–2.8)	1.1 (0.5–1.9)	3.4 (2.1–5.2)	<0.001
baseline and induction medications				
Hydroxychloroquine use, n (%)	95 (84.8)	70 (87.5)	25 (78.1)	0.21
RAAS inhibitor use, n (%)	68 (60.7)	50 (62.5)	18 (56.3)	0.54
IV methylprednisolone pulses, n (%)	83 (74.1)	58 (72.5)	25 (78.1)	0.54
Initial oral prednisone dose, mg/day	40 (30–50)	40 (30–50)	45 (35–60)	0.08
MMF induction, n (%)	76 (67.9)	56 (70.0)	20 (62.5)	0.44
Cyclophosphamide induction, n (%)	36 (32.1)	24 (30.0)	12 (37.5)	0.44
Add-on belimumab or CNI during induction, n (%)	8 (7.1)	5 (6.3)	3 (9.4)	0.68

in *Table 1*, baseline characteristics were comparable between responders and non-responders, except for a lower baseline eGFR in the failure group ($p=0.04$). Strikingly, non-responders had significantly higher pre-treatment levels of all three biomarker candidates. Baseline and induction medication exposure was also broadly balanced between outcome groups. Hydroxychloroquine was used in 95 patients (84.8%), RAAS inhibitors in 68 patients (60.7%), and intravenous methylprednisolone pulses in 83 patients (74.1%). The induction regimen consisted of MMF in 76 patients (67.9%) and cyclophosphamide in 36 patients (32.1%), without a significant difference between responders and non-responders. Add-on belimumab or calcineurin-inhibitor therapy

was used infrequently and was similarly distributed between groups.

AI, Activity Index; CI, Chronicity Index; CNI, calcineurin inhibitor; CR, complete response; CRS, Cytokine Risk Score; CYC, cyclophosphamide; eGFR, estimated glomerular filtration rate; IFN- γ , interferon-gamma; IL, interleukin; MMF, mycophenolate mofetil; PR, partial response; RAAS, renin-angiotensin-aldosterone system; sIL-6R, soluble interleukin-6 receptor; UPCR, urine protein-to-creatinine ratio.

Data are presented as median (interquartile range [IQR]) for continuous variables and n (%) for categorical variables. Medication variables re-

Table II Multivariable Cox proportional hazards model for treatment failure.

Predictor	Hazard Ratio (HR)	95% Confidence Interval	p-value
Baseline eGFR (per 10 mL/min decrease)	1.25	1.05–1.48	0.01
Baseline UPCR (per 1 g/g increase)	1.12	0.97–1.30	0.12
NIH Activity Index (per 1 point)	1.15	0.98–1.35	0.09
Induction Therapy (MMF vs. CYC)	0.85	0.41–1.78	0.67
IL-6 > 8.5 pg/mL	2.5	1.3–4.8	0.006
IFN- γ /IL-10 Ratio >2.0	3.8	1.9–7.6	<0.001

MMF, mycophenolate mofetil; CYC, cyclophosphamide.

fer to treatments prescribed at baseline or during induction therapy. RAAS inhibitors included angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Add-on therapy included belimumab or calcineurin inhibitors, including tacrolimus or voclosporin, when used in addition to standard induction therapy. MMF, mycophenolate mofetil; CNI, calcineurin inhibitor; RAAS, renin-angiotensin-aldosterone system.

ROC analysis identified optimal prognostic cut-offs: IL-6 >8.5 pg/mL (AUC 0.82), sIL-6R >38 ng/mL (AUC 0.75), and IFN- γ /IL-10 ratio >2.0 (AUC 0.88). Kaplan-Meier analysis revealed significantly shorter time-to-treatment-failure for patients above these cut-offs (all log-rank p-values <0.001).

Multivariable analysis and cytokine dynamics

In univariate Cox analysis, baseline IL-6 >8.5 pg/mL (HR 3.8, 95% CI 2.1–6.9), sIL-6R >38 ng/mL (HR 2.9, 95% CI 1.5–5.6), and IFN- γ /IL-10 ratio >2.0 (HR 5.2, 95% CI 2.7–9.9) predicted treatment failure. *Table II* presents the final multivariable model. After adjustment for baseline eGFR, UPCR, NIH-AI, and induction therapy, both a high IFN- γ /IL-10 ratio and high IL-6 remained independent predictors. The IFN- γ /IL-10 ratio was the strongest predictor (HR 3.8).

Analysis of 6-month (T6) samples from 98 patients revealed that biomarker persistence was highly prognostic. Patients who normalised their IFN- γ /IL-10 ratio by T6 (n=15 from the high-risk group) had a 90% renal survival rate, identical to the low-risk group. Conversely, patients with a persistently high T6 ratio (n=17) had a dramatically increased risk of progressing to CKD stage $\geq 3b$ (HR 12.1, 95% CI 4.5–32.4).

In medication-adjusted sensitivity analyses, the associations of IL-6 and the IFN- γ /IL-10 ratio with treatment failure remained materially unchanged. After additional adjustment for induction regimen, glucocorticoid exposure, hydroxychloroquine use,

RAAS-inhibitor use, and add-on belimumab or calcineurin-inhibitor therapy, IL-6 >8.5 pg/mL remained associated with treatment failure (HR 2.38, 95% CI 1.20–4.72), as did an IFN- γ /IL-10 ratio >2.0 (HR 3.61, 95% CI 1.76–7.41). These findings suggest that measured differences in baseline or induction medication exposure did not explain the prognostic associations.

Correlation with histology and proposed risk score

sIL-6R showed a significant positive correlation with the NIH Chronicity Index ($r=0.41$, $p<0.001$), but not with the Activity Index. IL-6 and the IFN- γ /IL-10 ratio correlated weakly with the Activity Index ($r=0.28$ and $r=0.32$, respectively). We developed a simple Cytokine Risk Score (CRS) that awards 1 point each for IL-6 >8.5 pg/mL, sIL-6R >38 ng/mL, and an IFN- γ /IL-10 ratio >2.0. The 24-month treatment failure rates were: CRS 0 (n=45): 4.4%; CRS 1–2 (n=48): 29.2%; CRS 3 (n=19): 73.7% ($p<0.001$).

Discussion

This prospective study provides robust clinical evidence that a pre-treatment serum cytokine signature powerfully predicts resistance to standard therapy in proliferative LN. Our key novel findings are: 1) the IFN- γ /IL-10 ratio showed the strongest independent association with treatment failure among the evaluated cytokine biomarkers, supporting its potential value as a prognostic marker in proliferative LN, 2) persistence of this ratio at 6 months portends an exceptionally poor renal prognosis, and 3) a simple 3-parameter Cytokine Risk Score stratifies patients into distinct risk categories.

The prognostic superiority of the IFN- γ /IL-10 ratio over individual cytokines underscores the importance of immune context (23). A high ratio may reflect a net inflammatory phenotype in which IFN- γ -associated effector pathways domi-

nate relative to IL-10-linked counter-regulatory and B-cell-supportive signals (24, 25). This interpretation is particularly important because IL-10 has dual and context-dependent effects in SLE. Although IL-10 can suppress inflammatory cytokine production in some settings, it may also promote B-cell survival, plasma-cell differentiation, and autoantibody production, thereby contributing to lupus immunopathology. Accordingly, the IFN- γ /IL-10 ratio should not be interpreted as a pure Th1/regulatory balance. Rather, it may serve as a composite marker of immune polarisation and net inflammatory tone in proliferative LN.

This aligns with murine LN models, in which IFN- γ is critical for severe nephritis (26), and with studies showing an association between a Th1-skewed transcriptome and poor LN outcomes (27). The persistence of this imbalance after 6 months of therapy is a particularly alarming sign, suggesting intrinsic biological resistance and identifying a group needing urgent therapeutic re-evaluation (28).

Mechanistically, a high IFN- γ /IL-10 ratio may represent a Th1-skewed inflammatory phenotype in which IFN- γ -driven effector pathways dominate over counter-regulatory cytokine signals. IFN- γ is a central Th1 cytokine that promotes macrophage activation, antigen presentation, chemokine production, and amplification of tissue inflammation. In lupus nephritis, infiltration of activated T cells and inflammatory immune cells within renal tissue has been described, supporting the biological plausibility that a Th1-dominant cytokine environment may contribute to persistent glomerular and tubulointerstitial injury. Conversely, IL-10 has complex and context-dependent effects in SLE: although classically anti-inflammatory, it may also support B-cell survival, differentiation, and autoantibody production. Therefore, the IFN- γ /IL-10 ratio may better capture the net direction of immune activation than either cytokine alone, particularly in patients with proliferative LN who fail to respond to conventional induction therapy (29).

Our findings on IL-6 and sIL-6R reinforce their role in active LN pathogenesis (9, 30). More importantly, we specifically link sIL-6R to histological chronicity, supporting the hypothesis that IL-6 trans-signalling promotes renal fibrosis and progressive damage (10, 31). This positions sIL-6R not only as an activity marker but also as a potential progression marker, warranting its use in trials of anti-IL-6 therapies in LN (32). The link between sIL-6R and chronic tissue changes may point to IL-6 trans-signalling, not just classic signalling. In classic signalling, IL-6 acts through the membrane-bound IL-6R, which is expressed on fewer cell types. In trans-signalling, IL-6 binds to soluble IL-6R and activates cells with gp130, making more kidney and immune cells responsive to IL-6, even if they have little or

no membrane-bound IL-6R. This process matters for chronic inflammation because IL-6 trans-signalling is linked to ongoing immune cell recruitment, STAT3 activation, fibroblast accumulation, extracellular matrix remodelling, and kidney scarring. So, the observed link between sIL-6R in the blood and the NIH Chronicity Index in our group may reflect a broader IL-6/sIL-6R pathway that leads to lasting kidney damage (33).

These mechanistic interpretations remain hypothesis-generating, and causality cannot be inferred from the present observational cohort. These ideas are still hypotheses, and we cannot prove cause and effect from this observational study. Future research should combine long-term blood and urine cytokine measurements with immune cell analysis of kidney tissue to determine whether blood cytokine patterns match immune activity in the kidney. Techniques such as single-cell RNA sequencing and spatial transcriptomics on kidney biopsies could reveal whether high-risk patients have more IFN- γ -responsive macrophages, T-bet-positive Th1 cells, IL-6/sIL-6R-responsive tubular or stromal cells, and STAT3-driven fibrosis programs. To test these findings, researchers could stimulate patient blood or kidney cells outside the body with IFN- γ , IL-10, IL-6, and IL-6/sIL-6R complexes, then block these pathways using anti-IFN- γ antibodies, IL-6R inhibitors, selective IL-6 trans-signalling blockers like sgp130/Fc, or JAK/STAT inhibitors. These studies could help determine whether the IFN- γ /IL-10 ratio and the IL-6/sIL-6R pathway actually cause treatment resistance or are merely signs of an already established inflammatory and fibrotic kidney environment (34).

The strengths of this study include its prospective design, the use of a clinically meaningful composite endpoint, longitudinal sampling, and rigorous adjustment for established prognostic factors. If externally validated, the CRS may offer a pragmatic framework for early risk stratification at the time of diagnostic biopsy. Patients classified as CRS 0 may be suitable for standard guideline-based induction and routine monitoring, while those with CRS 1–2 could benefit from closer surveillance of proteinuria, eGFR, and serological activity during the initial 6–12 months. Patients with CRS 3, who exhibit the highest observed treatment-failure rate, may constitute a biologically enriched, high-risk subgroup warranting early multidisciplinary review, protocolised response assessment, consideration of therapy intensification in line with contemporary LN guidelines, or enrollment in clinical trials of targeted agents. However, the CRS should not be used as a stand-alone tool to escalate immunosuppression until it is validated in independent and ethnically diverse cohorts (35, 36).

Exhibitum cytokine panel should be regarded as complementary to, rather than competitive with,

other LN biomarker candidates. Conventional serological markers, such as anti-dsDNA antibodies and complement levels, remain valuable in predicting systemic disease activity but have limited specificity for renal outcomes. Several urinary biomarkers, including MCP-1, NGAL, ALCAM, VCAM-1, TWEAK, CXCL10, and soluble CD163, have demonstrated potential as non-invasive indicators of intrarenal inflammation, histological activity, or renal injury. In contrast, the current panel captures systemic immune polarisation and the IL-6-axis a priori to treatment integration. Integrating serum immune context markers with urinary biomarkers of kidney-compartment injury may yield a more comprehensive biomarker for systemic assessment, reflecting intrarenal inflammatory activity and structural damage (37, 38).

Limitations include the single-centre design and the need for validation in larger, multi-ethnic cohorts. Furthermore, while we measured total sIL-6R, future studies should assess levels of the IL-6/sIL-6R complex. While our protocol specified blood collection before or within 2 hours of the first corticosteroid dose, we cannot completely exclude a minor,

transient effect of ultra-early steroid exposure on cytokine levels in a small subset of patients. However, any such effect would be expected to bias our results toward the null, leading to conservative estimates of the observed associations.

Conclusion

We define a novel, accessible serum cytokine signature that provides critical prognostic information at the diagnosis of proliferative LN. By identifying patients with a high pre-treatment IFN- γ /IL-10 ratio and elevated IL-6, clinicians can move beyond a »one-size-fits-all« approach toward a risk-stratified management paradigm, ultimately aiming to improve renal survival in this challenging patient population.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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