

A NOVEL SERUM BIOMARKER PANEL (ICAM2, VSIG4, NRCAM, AND P-CADHERIN) FOR THE NON-INVASIVE PREDICTION OF ACTIVE LUPUS NEPHRITIS

NOVI PANEL SERUMSKIH BIOMARKERA (ICAM2, VSIG4, NRCAM I P-KADHERIN)
ZA NEINVAZIVNO PREDVIĐANJE AKTIVNOG LUPUS NEFRITISA

Ting Yu¹, Xiaohui Tang², Na Hu¹, Hongbo Li^{1*}

¹Department of Rheumatology, The Third Affiliated Hospital of Changchun University of Chinese Medicine, 130117, Changchun, Jilin Province, People's Republic of China

²Department of Nephrology, The Third Affiliated Hospital of Changchun University of Chinese Medicine, 130117, Changchun, Jilin Province, People's Republic of China

Summary

Background: Lupus nephritis (LN) is a serious and dynamic manifestation of systemic lupus erythematosus (SLE). The gold standard for diagnosis is the renal biopsy. However, it is invasive and not suitable for serial monitoring. Standard serological markers, such as anti-dsDNA and complement components, are not sufficiently sensitive or specific to accurately reflect intrarenal inflammation, underscoring the need for novel, non-invasive liquid biomarkers. We hypothesised that a panel of serum proteins indicative of endothelial activation (ICAM2), macrophage complement processing (VSIG4), podocyte integrity (NrCAM), and tubular epithelial stress (P-cadherin) could accurately predict active LN.

Methods: In this cross-sectional study, 168 SLE patients were prospectively enrolled and divided into 3 groups: active LN (n=68), inactive LN (n=50), and non-LN SLE controls (n=50). Serum concentrations of ICAM2, VSIG4, NrCAM, and P-cadherin were measured by specific, validated enzyme-linked immunosorbent assays (ELISAs). A combined biomarker panel score was derived by multivariate logistic regression. We analysed the discriminative power of active LN versus all non-active LN controls using receiver operating characteristic (ROC) curves and compared it with that of conventional biomarkers.

Results: A clear molecular profile was identified. Serum levels of ICAM2, VSIG4 and P-cadherin were significantly higher, and NrCAM was significantly lower in active LN patients compared to either the inactive LN or non-LN SLE groups ($p < 0.001$ for all). The novel four-biomarker panel showed excellent predictive accuracy for active LN,

Kratak sadržaj

Uvod: Lupus nefritis (LN) predstavlja ozbiljnu i dinamičnu manifestaciju sistemskog eritemskog lupusa (SLE). Zlatni standard za dijagnozu je biopsija bubrega. Međutim, ova metoda je invazivna i nije pogodna za serijsko praćenje. Standardni serološki markeri, kao što su anti-dsDNA i komponente komplementa, nisu dovoljno senzitivni niti specifični da precizno odraze intrarenalnu inflamaciju, što naglašava potrebu za novim, neinvazivnim tečnim biomarkerima. Pretpostavili smo da bi panel serumskih proteina koji ukazuju na aktivaciju endotela (ICAM2), procesuiranje komplementa od strane makrofaga (VSIG4), integritet podocita (NrCAM) i stres tubularnog epitela (P-kadherin) mogao tačno da predvidi aktivni LN.

Metode: U ovoj studiji preseka, prospektivno je uključeno 168 pacijenata sa SLE, podeljenih u 3 grupe: aktivni LN (n=68), neaktivni LN (n=50) i SLE kontrolni pacijenti bez LN (n=50). Koncentracije ICAM2, VSIG4, NrCAM i P-kadherina u serumu merene su specifičnim i validiranim ELISA testovima. Kombinovani skor biomarker panela dobijen je multivarijantnom logističkom regresijom. Analizirana je diskriminativna moć za aktivni LN u odnosu na sve neaktivne LN kontrole korišćenjem ROC krivih i upoređena sa konvencionalnim biomarkerima.

Rezultati: Identifikovan je jasan molekularni profil. Nivoi ICAM2, VSIG4 i P-kadherina u serumu bili su značajno viši, dok je NrCAM bio značajno niži kod pacijenata sa aktivnim LN u poređenju sa grupama neaktivnog LN ili SLE bez LN ($p < 0,001$ za sve). Novi četvorostruki biomarker panel je pokazao odličnu prediktivnu tačnost za aktivni LN, sa površinom ispod ROC krive (AUC) od

Address for correspondence:

Hongbo Li
Department of Rheumatology, The Third Affiliated Hospital of
Changchun University of Chinese Medicine,
130117, Changchun, Jilin Province, People's Republic of China
e-mail: 17519200570@163.com

with an area under the ROC curve (AUC) of 0.921 (95% CI 0.876–0.966). This panel was better than single novel biomarkers, anti-dsDNA (AUC 0.732) and complement C3 (AUC 0.695). With the optimal cutoff, the panel had a sensitivity of 85.3% and a specificity of 88.0%. The panel maintained strong discriminative performance in both proliferative class III/IV LN and membranous class V LN in the exploratory histology-stratified analysis, with no statistically significant difference in AUC between subgroups.

Conclusion: Serum biomarkers, including ICAM2, VSIG4, NrCAM, and P-cadherin, are potent and non-invasive predictors of active lupus nephritis. This panel covers several pathogenic pathways, including vascular inflammation, complement-mediated macrophage activation, podocyte injury, and tubular stress. It thus provides a comprehensive »molecular biopsy« of the kidney. This panel is a promising complementary tool to improve the monitoring and management of LN.

Keywords: lupus nephritis, biomarkers, ICAM2, VSIG4, NrCAM, P-cadherin, diagnosis

Introduction

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease characterised by loss of self-tolerance and immune complex deposition, leading to multi-organ damage (1). Lupus nephritis (LN) is one of the most common and serious complications affecting up to 50% of SLE patients. It is a major cause of morbidity and end-stage renal disease (ESRD) (2, 3). However, a substantial proportion of patients will develop renal failure despite modern immunosuppressive regimes, highlighting the crucial limitations of current therapeutic and monitoring strategies (4).

The renal biopsy is a crucial tool for LN management, as it offers essential histopathological classification and prognostic information (5). However, a biopsy is an invasive procedure with a risk of morbidity, subject to sampling error. It provides only a single static snapshot of a highly dynamic inflammatory process. These features render it unsuitable for serial monitoring, early detection of renal flares or real-time assessment of therapeutic response (6). Currently, complementary non-invasive monitoring in clinical practice is based on proteinuria quantification, urinary sediment analysis and serological markers such as anti-double-stranded DNA (anti-dsDNA) antibodies and complement C3/C4 levels. Unfortunately, these standard markers have been shown to correlate poorly and inconsistently with intrarenal histological activity, and their sensitivity and specificity are suboptimal to guide complex therapeutic decisions (7, 8). This diagnostic gap highlights an unmet clinical need for novel liquid biomarkers that can reliably and non-invasively predict LN activity (9).

The pathogenesis of LN is highly complex, involving the interplay of circulating immune cells, the

0,921 (95% CI 0,876–0,966). Ovaj panel je bio bolji od pojedinačnih novih biomarkera, anti-dsDNK (AUC 0,732) i komplementa C3 (AUC 0,695). Pri optimalnoj graničnoj vrednosti, panel je imao senzitivnost od 85,3% i specifičnost od 88,0%. Panel je zadržao snažnu diskriminativnu sposobnost i kod proliferativnog LN klase III/IV i membranoznog LN klase V u eksplorativnoj analizi stratifikovanoj prema histologiji, bez statistički značajne razlike u AUC između podgrupa.

Zaključak: Serumski biomarkeri ICAM2, VSIG4, NrCAM i P-kadherin predstavljaju snažne i neinvazivne prediktore aktivnog lupus nefritisa. Ovaj panel pokriva više patogenetskih puteva, uključujući vaskularnu inflamaciju, komplementom posredovanu aktivaciju makrofaga, oštećenje podocita i tubularni stres. Na taj način pruža sveobuhvatnu »molekularnu biopsiju« bubrega. Panel predstavlja obećavajući komplementarni alat za unapređenje praćenja i lečenja LN.

Ključne reči: lupus nefritis, biomarkeri, ICAM2, VSIG4, NrCAM, P-kadherin, dijagnoza

renal microvascular endothelium, and resident renal parenchymal cells, including podocytes and tubular epithelial cells (10). We hypothesised that a powerful multi-dimensional biomarker panel could be obtained by measuring circulating proteins mechanistically linked to these specific pathogenic processes. We had four candidates for our hypothesis. Intercellular Adhesion Molecule 2 (ICAM2/CD102) is constitutively expressed on endothelial cells. It plays a critical, non-redundant role in mediating firm adhesion and the transendothelial migration of leukocytes into inflamed tissues, a key step in glomerular crescent formation (11, 12). V-set and immunoglobulin domain-containing 4 (VSIG4), also known as Complement Receptor of the Immunoglobulin superfamily (CRIg), is a specific receptor for C3b fragments that is expressed on tissue-resident macrophages. It has a dual role in phagocytosis and in the regulation of complement-driven inflammation. It is a strong candidate to assess intrarenal macrophage activity (13, 14). The neuronal cell adhesion molecule (NrCAM), first identified in the nervous system, is known to have an important non-neuronal function in the structural integrity of the glomerular filtration barrier, particularly at the podocyte foot process and slit diaphragm (15, 16). Finally, Placental-cadherin (P-cadherin), a classical cell adhesion molecule, is expressed at low levels in healthy adult kidney but is strongly upregulated in distal tubular epithelial cells under stress conditions such as hypoxia and inflammation (17, 18).

The main aim of this study was to test the hypothesis that serum levels of a novel, biologically integrated panel of four proteins (ICAM2, VSIG4, NrCAM, and P-cadherin) can serve as a highly accurate, non-invasive composite biomarker for predicting active LN.

Materials and Methods

Study design and patient population

This was a cross-sectional, single-centre observational study. We prospectively enrolled 168 adult patients who fulfilled the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for SLE (19) from the Nephrology and Rheumatology outpatient clinics between March 2023 and January 2024. Consecutive patients were stratified into three pre-defined groups based on renal involvement and disease activity at the time of enrolment:

- (i) Active LN group (n=68), defined by a renal biopsy performed within 8 weeks showing ISN/RPS Class III, IV (A or A/C), or V with a National Institutes of Health (NIH) activity index score >2, plus a urine protein-to-creatinine ratio (UPCR)>1.0 g/g (20);
- (ii) Inactive LN group (n=50), comprising patients with a prior biopsy-confirmed history of LN who were in sustained clinical renal remission. Remission was defined as UPCR<0.5 g/g and inactive urinary sediment (<5 RBCs/hpf and no cellular casts) for at least 6 consecutive months. During this remission period, patients were required to be receiving stable maintenance immunosuppressive therapy and/or tapering glucocorticoids, without treatment escalation. Treatment escalation was defined as initiation of induction therapy, increase in mycophenolate mofetil dose, administration of cyclophosphamide or rituximab, addition of a calcineurin inhibitor, or increase in systemic glucocorticoid dose during the preceding 6 months;
- (iii) Non-LN SLE group (n=50), comprising SLE patients with no historical or current clinical evidence of nephritis (UPCR<0.2 g/g, normal urinary sediment, normal serum creatinine). Exclusion criteria included: concomitant active infections (including HIV, Hepatitis B, and Hepatitis C), pregnancy, active malignancy, a history of non-SLE related renal disease, and an estimated glomerular filtration rate (eGFR)<15 mL/min/1.73m². The Institutional Review Board approved this study of [Institution Name] (Protocol No. 2023-RHE-01). All study procedures adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all participants.

Clinical and laboratory data collection

At enrolment, comprehensive demographic and clinical data were collected, including age, sex, self-reported ethnicity, SLE disease duration, and current prednisolone use. SLE disease activity was assessed using the validated Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (21). To avoid circularity and artificial inflation of associations between disease activity and circulating biomarkers, a modified clinical SLEDAI-2K score was calculated by excluding serological laboratory components, specifically anti-dsDNA positivity and hypocomplementemia, including low C3 and/or C4. Thus, the SLEDAI-2K score used in correlation and group-comparison analyses reflected clinical rather than conventional serological disease activity. Routine laboratory parameters were measured in the hospital's central clinical laboratory and included a complete blood count, serum creatinine, eGFR (calculated using the CKD-EPI 2021 equation), complement C3 and C4 (nephelometry), and anti-dsDNA antibody titers (by enzyme-linked immunosorbent assay, ELISA). For the active LN group, the renal biopsy reports were meticulously reviewed for ISN/RPS class and the individual components of the activity and chronicity indices.

This was an exploratory, single-centre biomarker study; therefore, the sample size was primarily determined by the number of eligible patients available during the predefined recruitment period from March 2023 to January 2024. Nevertheless, the final cohort size was assessed for adequacy with respect to the primary diagnostic objective. The primary ROC analysis compared active LN patients (n=68) with inactive LN and non-LN SLE controls (n=100). For ROC-based diagnostic studies, this sample size provides adequate power to detect an AUC substantially greater than the null value of 0.50. Assuming a two-sided α of 0.05, the available sample size provided greater than 90% power to detect an AUC of 0.75 or higher for the four-marker panel, while allowing estimation of the observed AUC with acceptable precision. Because the panel's observed AUC was 0.921, the enrolled cohort was deemed adequate for the primary exploratory diagnostic analysis.

Quantification of novel serum biomarkers

Sample collection and pre-analytical handling

Peripheral venous blood (10 mL) was collected from each participant into sterile, additive-free serum separator tubes (SST) (BD Biosciences, Franklin Lakes, NJ, USA). To minimise circadian variability, all samples were drawn between 8:00 AM and 10:00 AM following an overnight fast (22). Tubes were gently inverted five times and allowed to clot

at room temperature (20–25 °C) for exactly 30 minutes. Samples were then centrifuged at 3000×g for 15 minutes at 4 °C in a pre-cooled swinging-bucket centrifuge (Eppendorf 5810R, Hamburg, Germany). The supernatant was carefully aspirated and divided into 500 µL aliquots in sterile, DNase/RNase-free polypropylene cryovials (Corning Inc., Corning, NY, USA), then immediately flash-frozen at -80 °C. To eliminate the confounding effect of repeated freeze-thaw cycles on soluble adhesion molecule immunoreactivity, each aliquot was thawed only once, immediately before the assay (23). Haemolysed, lipemic, or icteric samples were identified by spectrophotometric indices and excluded (n=3), with the respective patients re-sampled.

General immunoassay methodology

All four biomarkers were measured using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). In this methodology, the target protein is captured between a pre-coated capture antibody and a biotinylated detection antibody, followed by horseradish peroxidase (HRP)-conjugated streptavidin and a colourimetric substrate. The optical density (OD) is directly proportional to the analyte concentration (24). All standards, blanks, and serum samples were run in duplicate. A single manufacturer's lot was used for each kit. Two trained technicians, blinded to all clinical data, performed the assays.

Specific assay protocols

Quantification of human ICAM2 (CD102)

Soluble ICAM2 was quantified with the Quantikine Human ICAM-2/CD102 Immunoassay (R&D Systems, Minneapolis, MN, USA; Cat# DIC200). This assay has a lower limit of detection (LLOD) of 0.033 ng/mL and a calibrated range of 0.8–50 ng/mL. The intra- and inter-assay CVs are <6% and <8%, respectively. Serum samples were diluted 1:20 in the provided diluent. The standard curve was generated from a 7-point, 2-fold serial dilution of a recombinant human ICAM2 standard. The plate was incubated for 2 hours at room temperature on an orbital shaker at 500 rpm. Colour was developed with tetramethylbenzidine (TMB) substrate and stopped with 2N sulfuric acid. Absorbance was read at 450 nm with a 570 nm correction. No significant cross-reactivity with ICAM-1, ICAM-3, VCAM-1, or PECAM-1 is reported.

Quantification of human VSIG4 (CRlg)

VSIG4 was quantified using Human VSIG4 ELISA Kit (MyBioSource, San Diego, CA, USA;

Cat# MBS762022). The LLOD was 0.12 ng/mL, and the dynamic range was 0.312–20 ng/mL. Intra- and inter-assay CVs were <8% and <10%, respectively. Serum samples were assayed without dilution. The protocol consisted of a 90-minute incubation of the sample, a 60-minute incubation with the detection antibody, and a 30-minute incubation with HRP-streptavidin. TMB substrate development was carried out for 15 minutes in the dark.

Quantification of human NrCAM

Serum NrCAM levels were measured with a Human NrCAM ELISA Kit (LifeSpan BioSciences, Seattle, WA, USA; Cat# LS-F27275). The LLOD is <0.1 ng/mL with a detection range of 0.156–10 ng/mL. Intra- and inter-assay CVs are <6% and <8%. Samples were run undiluted. The sample incubation phase was set to 2 hours to ensure high-affinity binding of this large glycoprotein, and the TMB incubation was extended to 20 minutes. Cross-reactivity with L1CAM, CHL1, or Neurofascin is negligible.

Quantification of human P-cadherin

Soluble P-cadherin was quantified using the Human P-Cadherin DuoSet ELISA Development System (R&D Systems, Minneapolis, MN, USA; Cat# DY861). The in-house optimised assay had an LLOD of 45 pg/mL. Intra- and inter-assay CVs were 5.1% and 9.2%. Serum samples were diluted 1:2. The standard curve was generated with a recombinant human P-cadherin/Fc chimera. Cross-reactivity was minimal with E-cadherin, N-cadherin, VE-cadherin, or R-cadherin.

Data reduction and quality control

OD values were imported into a five-parameter logistic (5-PL) curve-fitting software (SoftMax Pro 7.1, Molecular Devices, San Jose, CA, USA) (25). Standard curves were accepted only if $R^2 > 0.99$. The back-calculated concentration for each standard was required to be within 15% of the nominal value. Sample duplicates with a CV of 15% were re-assayed. Two kit-provided quality controls (high and low) on each plate had to fall within 2 SD of the established laboratory mean. Final concentrations were expressed in ng/mL.

Statistical analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY) and GraphPad Prism 9.0 (San Diego, CA). The normality of distribution for continuous variables was assessed using

the Shapiro-Wilk test. Accordingly, demographic and clinical data were presented as mean $\pm$ standard deviation (SD) for normally distributed variables, or median (interquartile range, IQR) for non-normally distributed variables. Comparisons across the three groups were performed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test, or the Kruskal-Wallis test with Dunn's post-hoc test, respectively. Categorical variables were compared using the Chi-square test. Binary logistic regression was used to develop a composite four-biomarker model for the primary outcome of active LN. The regression equation generated a linear predictor, which was converted into an individual predicted probability of active LN using the logistic transformation: predicted probability = $1/[1 + \exp(-\text{linear predictor})]$. This predicted probability, ranging from 0 to 1, was used as the composite panel score for ROC analysis. Because the panel score represents a probability rather than a biomarker concentration, it is unitless. The diagnostic performance was evaluated using ROC curve analysis, and the area under the curve (AUC) with 95% confidence intervals (CI) was calculated. The optimal cutoff for the panel score was determined by maximising the Youden Index (Sensitivity + Specificity – 1). A two-tailed p-value < 0.05 was considered statistically significant for all tests.

Results

Baseline clinical characteristics of the study population

We summarised the demographic and clinical characteristics of the 168 participants in *Table I*. The three groups were well-matched for age, sex, and SLE disease duration. As expected, the active LN group exhibited significantly higher clinical disease activity based on the SLEDAI-2K score (median 16 [IQR 12–20]) compared to the inactive LN (2 [1–4]) and non-LN SLE (4 [2–6]) groups ($p < 0.001$). Similarly, anti-dsDNA titers were significantly higher, while C3 and C4 levels were significantly lower, in active LN patients. The most frequent histological class in the active LN group was Class IV (45.6%), followed by Class III (29.4%).

Differential serum levels of candidate biomarkers

The serum concentrations of the four novel biomarkers across the three study groups are detailed in *Table II*. A highly distinct and statistically significant molecular signature was observed. Serum ICAM2 levels in active LN patients (median 28.5 ng/mL) were significantly elevated compared

Table I Baseline demographic and clinical characteristics of the study groups.

Variable	Active LN (n=68)	Inactive LN (n=50)	Non-LN SLE (n=50)	p-value
Age, years	34.5 $\pm$ 10.2	36.8 $\pm$ 9.5	35.2 $\pm$ 11.1	0.48
Female, n (%)	60 (88.2)	44 (88.0)	44 (88.0)	0.99
SLE duration, years	6.2 (3.1–10.5)	7.5 (4.2–11.8)	6.8 (3.5–10.0)	0.35
Modified SLEDAI-2K score	16 (12–20)	2 (1–4)	4 (2–6)	<0.001
Full SLEDAI-2K score	20 (16–24)	4 (2–6)	6 (4–8)	<0.001
UPCR, g/g	2.8 (1.6–4.5)	0.3 (0.1–0.4)	0.1 (0.08–0.15)	<0.001
eGFR, mL/min/1.73m ²	78 (52–98)	88 (72–105)	94 (82–108)	0.02
Anti-dsDNA, IU/mL	215 (85–450)	28 (15–65)	45 (18–110)	<0.001
C3, mg/dL	55 (38–72)	90 (75–105)	85 (70–100)	<0.001
C4, mg/dL	10 (6–15)	18 (13–24)	16 (12–22)	<0.01
Prednisone use, n (%)	58 (85.3)	35 (70.0)	32 (64.0)	0.02
ISN/RPS class, n (%)				
Class III	20 (29.4)			
Class IV	31 (45.6)			
Class V	17 (25.0)			

Data are presented as mean $\pm$ SD, median (IQR), or n (%). LN, lupus nephritis; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; UPCR, urine protein-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

Table II Serum Concentrations of the novel biomarker panel across study groups.

Biomarker	Active LN (n=68)	Inactive LN (n=50)	Non-LN SLE (n=50)	Overall p-value	Effect size: Active LN vs inactive LN
ICAM2 (ng/mL)	28.5 (22.1–35.8)	19.8 (15.5–23.1)	17.2 (14.1–20.5)	<0.001	Cliff's d=0.66, large
VSIG4 (ng/mL)	15.2 (10.8–20.5)	9.1 (6.2–12.5)	8.5 (5.9–11.0)	<0.001	Cliff's d=0.58, large
NrCAM (ng/mL)	1.8 (1.2–2.5)	4.2 (3.1–5.6)	4.5 (3.4–5.8)	<0.001	Cliff's d=-0.74, large
P-cadherin (ng/mL)	3.6 (2.4–5.1)	1.9 (1.3–2.8)	1.7 (1.1–2.5)	<0.001	Cliff's d=0.61, large

All data are presented as median (IQR). *Kruskal-Wallis test. Dunn's post-hoc test showed significant differences between the Active LN group and both the Inactive LN and Non-LN SLE groups for all four biomarkers ($p<0.001$). No significant difference was observed between the Inactive LN and Non-LN SLE groups for any marker.

Table III Spearman correlation matrix among the four serum biomarkers.

Biomarker	ICAM2	VSIG4	NrCAM	P-cadherin
ICAM2	1.00	0.42***	-0.41***	0.39***
VSIG4	0.42***	1.00	-0.34***	0.36***
NrCAM	-0.41***	-0.34***	1.00	-0.38***
P-cadherin	0.39***	0.36***	-0.38***	1.00

Values represent Spearman's rank correlation coefficients calculated across the full study cohort. *** $p<0.001$. Correlations were considered weak when $|\rho|<0.30$, moderate when $|\rho|=0.30-0.59$, and strong when $|\rho|\geq 0.60$.

to inactive LN (19.8 ng/mL) and non-LN SLE controls (17.2 ng/mL) ($p<0.001$). A similar, significant upward trend was observed for VSIG4 (active LN: 15.2 ng/mL vs inactive LN: 9.1 ng/mL vs non-LN SLE: 8.5 ng/mL, $p<0.001$) and P-cadherin (active LN: 3.6 ng/mL vs inactive LN: 1.9 ng/mL vs non-LN SLE: 1.7 ng/mL, $p<0.001$). In striking contrast, serum NrCAM concentrations were profoundly suppressed in the active LN group (median 1.8 ng/mL) compared to both the inactive LN (4.2 ng/mL) and non-LN SLE groups (4.5 ng/mL) ($p<0.001$). Post-hoc analysis confirmed no significant difference between the inactive LN and non-LN SLE control groups for any of the four markers, indicating that the changes are specifically associated with active nephritic disease rather than a history of nephritis or SLE itself.

To assess the clinical magnitude of these differences, we calculated non-parametric effect sizes for the active LN versus inactive LN contrasts. All four biomarkers demonstrated large effect sizes. ICAM2 showed a large positive effect size, indicating substantially higher levels in active LN compared with inactive LN (Cliff's $\delta=0.66$). VSIG4 also demonstrated a large positive effect size (Cliff's $\delta=0.58$), as did P-cadherin (Cliff's $\delta=0.61$). In contrast, NrCAM showed a large negative effect size (Cliff's

$\delta=-0.74$), consistent with markedly lower levels in active LN. These findings indicate that the observed biomarker differences are not only statistically significant but also clinically meaningful in magnitude.

Inter-biomarker correlation matrix

To determine whether the four biomarkers represented redundant or complementary biological signals, we performed pairwise Spearman correlation analysis across the full study cohort. The resulting correlation matrix is shown in *Table III*. Overall, the biomarkers demonstrated weak-to-moderate intercorrelations. ICAM2 correlated moderately with VSIG4 ($\rho=0.42$, $p<0.001$) and P-cadherin ($\rho=0.39$, $p<0.001$). VSIG4 also showed a moderate positive correlation with P-cadherin ($\rho=0.36$, $p<0.001$). In contrast, NrCAM showed inverse correlations with ICAM2 ($\rho=-0.41$, $p<0.001$), VSIG4 ($\rho=-0.34$, $p<0.001$), and P-cadherin ($\rho=-0.38$, $p<0.001$). Importantly, no pairwise correlation exceeded $|\rho|=0.60$, suggesting that the four biomarkers are not highly collinear. These findings support the multi-compartment rationale of the panel, in which each analyte contributes partially distinct information related to active LN biology (*Table III*).

Table IV Receiver operating characteristic (ROC) curve analysis for predicting active LN.

Marker/Panel	AUC	95% CI	Sensitivity (%)	Specificity (%)	Cutoff Value	p-value (vs Panel)
4-Marker Panel	0.921	0.876–0.966	85.3	88.0	>0.62 (Score)	Reference
ICAM2	0.845	0.785–0.905	76.5	79.0	>22.5 ng/mL	<0.01
VSIG4	0.798	0.728–0.867	72.1	75.0	>12.0 ng/mL	<0.001
NrCAM	0.825	0.760–0.890	79.4	74.0	<2.8 ng/mL	<0.001
P-cadherin	0.812	0.745–0.879	69.1	85.0	>2.9 ng/mL	<0.01
Anti-dsDNA	0.732	0.655–0.809	80.9	56.0	>80 IU/mL	<0.001
C3	0.695	0.615–0.775	67.6	65.0	<78 mg/dL	<0.001

Diagnostic performance of the novel biomarker panel

To create a unified diagnostic tool, a composite biomarker panel score was derived from the four analytes using binary logistic regression. The linear predictor from the logistic regression model was calculated as follows: linear predictor = $-3.2 + (0.08 \times \text{ICAM2}) + (0.15 \times \text{VSIG4}) - (0.45 \times \text{NrCAM}) + (0.28 \times \text{P-cadherin})$. This value was then transformed into a predicted probability of active LN using the logistic function: panel score = $1/[1 + \exp(-\text{linear predictor})]$. Thus, the panel score is a unitless probability ranging from 0 to 1, with higher values indicating a greater probability of active LN. The diagnostic performance of this panel and its individual components was assessed using ROC curve analysis, as detailed in Table IV. The four-marker panel demonstrated outstanding discriminative power for predicting active LN, with an AUC of 0.921 (95% CI: 0.876–0.966). At the optimal cutoff value, the panel achieved a sensitivity of 85.3% and a specificity of 88.0%. This performance was significantly superior to any of the four individual biomarkers, which had AUCs ranging from 0.798 (VSIG4) to 0.845 (ICAM2). Crucially, the panel dramatically outperformed the conventional clinical markers, anti-dsDNA (AUC 0.732; sensitivity 80.9%, specificity 56.0%) and complement C3 (AUC 0.695; sensitivity 67.6%, specificity 65.0%), which both showed only modest predictive ability in our cohort.

Because membranous class V LN differs pathophysiologically from proliferative class III/IV LN, we performed an exploratory histologic subgroup analysis within the active LN cohort. Among active LN patients, 51 had proliferative class III/IV LN, and 17 had pure class V membranous LN. The four-marker panel retained strong discriminatory performance in both subgroups. The AUC was 0.934 for proliferative class III/IV LN and 0.876 for pure class V LN. Although the AUC was numerically lower in class V LN, the difference between the proliferative and membranous subgroups was not statistically significant by DeLong's test ($p=0.28$). These findings

suggest that the panel is not restricted to proliferative LN. However, the class V subgroup analysis should be considered exploratory because of the smaller sample size.

The four-marker panel score represents the predicted probability of active LN derived from the logistic regression model. The model linear predictor was calculated as:

$$-3.2 + (0.08 \times \text{ICAM2}) + (0.15 \times \text{VSIG4}) - (0.45 \times \text{NrCAM}) + (0.28 \times \text{P-cadherin}),$$

and then converted to a probability using the logistic transformation:

$$\text{predicted probability} = 1/[1 + \exp(-\text{linear predictor})].$$

The score is unitless and ranges from 0 to 1.

Exploratory correlations between serum biomarkers and histologic activity features

To further examine the biological relevance of the candidate biomarkers, we performed exploratory Spearman correlation analyses of individual serum biomarker levels with biopsy activity-index components in the active LN group. ICAM2 positively correlated with endocapillary hypercellularity ($\rho=0.46$, $p<0.001$) and cellular crescents ($\rho=0.39$, $p=0.001$), consistent with its posited role as a marker of endothelial activation and leukocyte trafficking. VSIG4 correlated with interstitial inflammation ($\rho=0.43$, $p<0.001$), endocapillary hypercellularity ($\rho=0.36$, $p=0.003$) and overall NIH activity index ($\rho=0.41$, $p<0.001$), consistent with its association with complement-linked macrophage activation. NrCAM showed inverse correlations with cellular crescents ($\rho=-0.34$, $p=0.005$), NIH activity index ($\rho=-0.38$, $p=0.002$) and UPCR ($\rho=-0.42$, $p<0.001$), consistent with lower levels in circulation in patients with more active glomerular injury. P-cadherin correlated most strongly with interstitial inflammation ($\rho=0.48$, $p<0.001$) and tubular injury/tubulointer-

stitial activity ($p=0.44$, $p<0.001$), supporting its interpretation as a marker of tubular epithelial stress. These exploratory histology-linked associations suggest that the four biomarkers reflect partially distinct but complementary renal injury compartments.

Exploratory analysis of biomarker levels according to immunosuppressive treatment

Because immunosuppressive therapy could influence circulating biomarker concentrations, we performed exploratory treatment-stratified analyses. At enrolment, active LN patients were receiving heterogeneous treatment regimens, including glucocorticoids, mycophenolate mofetil, cyclophosphamide, and rituximab. Within the active LN group, biomarker levels did not show consistent differences by treatment. ICAM2 levels were similar in patients receiving cyclophosphamide compared with those not receiving cyclophosphamide (median 29.1 [IQR 23.0–36.5] vs 27.8 [21.6–35.0] ng/mL, $p=0.48$) and in patients receiving rituximab compared with those not receiving rituximab (27.4 [22.0–34.8] vs 28.9 [22.4–36.1] ng/mL, $p=0.62$). VSIG4 levels were also comparable according to cyclophosphamide exposure (15.8 [11.2–21.0] vs 14.9 [10.5–20.1] ng/mL, $p=0.55$) and rituximab exposure (14.5 [10.1–19.8] vs 15.4 [11.0–20.7] ng/mL, $p=0.59$). Similar non-significant patterns were observed for NrCAM and P-cadherin. These exploratory findings suggest that a single immunosuppressive treatment exposure did not drive the biomarker differences observed in active LN. However, residual confounding by disease severity and treatment indication cannot be excluded.

Discussion

In this cross-sectional study, we identified and internally validated a novel composite panel of four serum biomarkers that differentiate active LN from inactive and non-renal SLE with remarkable precision. The panel's AUC of 0.921, with sensitivity of 85.3% and specificity of 88.0%, is a substantial improvement over the conventional clinical markers used in daily practice, anti-dsDNA and C3, which demonstrated only modest discriminative ability. Our findings strongly support the idea that a multi-analyte approach, intentionally designed to reflect the complex and heterogeneous pathology of LN, can provide a more holistic »liquid biopsy« than any single analyte.

The biological rationale for each biomarker is key to the panel's strong performance. It provides pathophysiological insight into the active LN state. The marked increase in soluble ICAM2 likely reflects endothelial activation and/or injury

in the renal microvasculature. Unlike ICAM-1, which is upregulated by inflammation, ICAM-2 is constitutively expressed at high levels on endothelial cells and functions as an important docking molecule for the integrin LFA-1 on circulating leukocytes to mediate their arrest and diapedesis into the glomerulus (11, 26). Its leakage into the serum directly reflects injury to the »vascular gateway,« a feature typical of active proliferative and crescentic forms of LN (27). With the increase of VSIG4, the centre of this panel is the complement system and tissue-resident macrophages. VSIG4 is a phagocytic receptor for C3b-opsonised particles and is essential for the clearance of deposited immune complexes (13, 28). The high levels of active LN in serum may reflect macrophage hyperactivation or exhaustion in response to overwhelming intrarenal complement activation and immune-complex load, consistent with the resurgence of complement-targeted therapeutics in nephrology (29). Of particular interest is the marked decrease in serum NrCAM. NrCAM is a structural component of the slit diaphragm complex. Low circulating levels probably reflect loss from injured podocytes, either through suppressed production or shedding upon cell detachment, as seen for nephrin in other proteinuric diseases (16, 30). This highlights a crucial, previously unrecognised podocytopathic aspect of the inflammatory milieu in active LN. Finally, the up-regulation of P-cadherin is likely to be a marker of distal tubular epithelial cell stress and a partial epithelial-to-mesenchymal transition (EMT) program. P-cadherin is normally low in healthy kidneys, but is strongly upregulated by TGF- β and hypoxia (17, 31). Its presence in serum is therefore a potential surrogate of active tubulointerstitial damage, a lesion of ultimate prognostic relevance, notoriously difficult to monitor non-invasively (32).

The main strength of this study is its biology-based, hypothesis-driven panel design, in contrast to untargated proteomic approaches that often yield non-specific acute-phase reactants. The composite score integrates independent signals from four distinct nephron compartments, including the endothelium (ICAM2), infiltrating macrophages (VSIG4), podocytes (NrCAM) and tubular epithelium (P-cadherin), providing a high-fidelity molecular snapshot of the kidney. This explains its significant advantage over the single-marker approach. The data's internal validity is ensured by the rigorous pre-analytical and analytical quality control measures implemented.

However, our study has limitations that pave the way for future studies. The single-centre and cross-sectional design limits causal inference and external generalizability. The true clinical utility of the panel needs to be tested in large, multi-ethnic, prospective longitudinal cohorts. Such studies

are needed to validate the panel's ability to predict future renal flares before a rise in proteinuria, to monitor response to therapy in real time, and, ultimately, to predict long-term renal survival. Additionally, although ELISA remains the gold standard for validation of a single analyte, development of a rapid, cost-effective, single-reaction multiplex assay (e.g. bead-based flow cytometry assay, such as Luminex, or an electrochemiluminescence platform) that could provide results within a clinically actionable time frame would be necessary for clinical translation of a 4-marker panel (33). Fourth, our cohort included a range of ISN/RPS classes. Still, the sample size was insufficient to perform robust subgroup analysis by histological class. The distinct pathogenic mechanisms of proliferative (III/IV) and membranous (V) LN, and their impact on the panel's performance, remain to be elucidated.

Conclusion

In conclusion, this study introduces and validates a novel, biologically integrated serum biomarker panel of ICAM2, VSIG4, NrCAM, and P-cad-

herin as a superior non-invasive predictor of active lupus nephritis. The panel's high accuracy, which reflects the multifaceted pathology of the disease, has the potential to improve clinical monitoring of LN. However, this requires confirmation in prospective longitudinal studies.

Funding

Study on the Mechanism of Si Miao Wan Combined with Bi Xie Shen Shi Decoction in Treating Gouty Arthritis Based on the TLR4/NLRP3 Pathway (JJKH20250626KJ).

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Conflict of interest statement

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

References

1. Tsokos GC. Systemic lupus erythematosus. *N Engl J Med* 2011; 365(22): 2110–21.
2. Anders HJ, Saxena R, Zhao MH, Parodis I, Salmon JE, Mohan C. Lupus nephritis. *Nat Rev Dis Primers* 2020; 6(1): 7.
3. Hanly JG, O'Keeffe AG, Su L, Urowitz MB, Romero-Diaz J, Gordon C, et al. The frequency and outcome of lupus nephritis: results from an international inception cohort study. *Rheumatology (Oxford)* 2016; 55(2): 252–62.
4. Tektonidou MG, Dasgupta A, Ward MM. Risk of End-Stage Renal Disease in Patients With Lupus Nephritis, 1971-2015: A Systematic Review and Bayesian Meta-Analysis. *Arthritis Rheumatol* 2016; 68(6): 1432–41.
5. Bajema IM, Wilhelmus S, Alpers CE, Bruijn JA, Colvin RB, Cook HT, et al. Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: clarification of definitions, and modified National Institutes of Health activity and chronicity indices. *Kidney Int* 2018; 93(4): 789–96.
6. Moroni G, Vercelloni PG, Quaglini S, Gallelli B, Sacchi L, Raffiotta F, et al. The changing role of biopsy in the management of lupus nephritis. *Nephrol Dial Transplant* 2021; 36(1): 13–22.
7. Pisetsky DS, Lipsky PE. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. *Nat Rev Rheumatol* 2020; 16(10): 565–79.
8. Birmingham DJ, Irshaid F, Nagaraja HN, Zou X, Tsao BP, Wu H, et al. The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. *Lupus* 2010; 19(11): 1272–80.
9. Mok CC. Biomarkers in lupus nephritis: A need for better ones. *J Rheumatol* 2019; 46(5): 431–3.
10. Davidson A, Aranow C, Mackay M. Lupus nephritis: challenges and progress. *Curr Opin Rheumatol* 2019; 31(6): 669–75.
11. Lyck R, Enzmann G. The physiological roles of ICAM-1 and ICAM-2 in neutrophil migration into tissues. *Curr Opin Hematol* 2015; 22(1): 53–9.
12. Sumagin R, Sarelius IH. Intercellular adhesion molecule-1 is required for a slow rolling step during firm adhesion under shear flow. *Am J Physiol Cell Physiol* 2006; 290(5): C1356–64.
13. Helmy KY, Katschke KJ Jr, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, et al. CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cel* 2006; 124(5): 915–27.
14. Liesmaa I, Paavonen T, Valmu L, Törnblom H, Nilsson CG, Kontinen YT. VSIG4 expression on macrophages correlated with inflammatory activity in rheumatoid arthritis synovial tissue. *Arthritis Rheum* 2012; 64(11): 3565–74.
15. Sakurai T. The role of NrCAM in neural development and disorders--beyond a simple glue in the brain. *Mol Cell Neurosci* 2012; 49(3): 351–63.

16. Hahm K, Lukashev ME, Luo Y, Yang WJ, Dolinski BM, Weinreb PH, et al. $\alpha\beta6$ integrin regulates renal fibrosis and inflammation in Alport mouse. *Am J Pathol* 2007; 170(1): 110–25.
17. Vieira AF, Paredes J. P-cadherin and the journey to cancer metastasis. *Mol Cancer* 2015; 14: 178.
18. Prozialeck WC, Edwards JR. Cell adhesion molecules in chemically-induced renal injury. *Pharmacol Ther* 2007; 114(1): 74–93.
19. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis* 2019; 78(9): 1151–9.
20. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004; 65(2): 521–30.
21. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29(2): 288–91.
22. Diver MJ, Imtiaz KE, Ahmad AM, Vora JP, Fraser WD. Diurnal rhythms of serum total, free and bioavailable testosterone and of SHBG in middle-aged men compared with those in young men. *Clin Endocrinol (Oxf)* 2003; 58(6): 710–7.
23. de Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol* 2009; 10: 52.
24. Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, Kuchel GA. ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. *J Gerontol A Biol Sci Med Sci* 2008; 63(8): 879–84.
25. Findlay JW, Dillard RF. Appropriate calibration curve fitting in ligand binding assays. *AAPS J* 2007; 9(2): E260–7.
26. Smith A, Stanley P, Jones K, Svensson L, McDowall A, Hogg N. The role of the integrin LFA-1 in T-lymphocyte migration. *Immunol Rev* 2007; 218: 135–46.
27. Parikh SV, Malvar A, Song H, Alberton V, Lococo B, Vance J, et al. Characterising the immune cell landscape of acute tubular necrosis by renal biopsy imaging transcriptomics. *Ann Rheum Dis* 2022; 81(2): 268–76.
28. He JQ, Wiesmann C, van Lookeren Campagne M. A role of macrophage complement receptor CR1g in immune clearance and inflammation. *Mol Immunol* 2008; 45(16): 4041–7.
29. Rovin BH, Solomons N, Pendergraft WF 3rd, Dooley MA, Tumlin J, Romero-Diaz J, et al. A randomised, controlled double-blind study comparing the efficacy and safety of obinutuzumab in proliferative lupus nephritis. *Ann Rheum Dis* 2022; 81(1): 100–107.
30. Kandasamy Y, Smith R, Lumbers ER, Rudd D. Nephritin - a biomarker of early glomerular injury. *Biomark Res* 2014; 2: 21.
31. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; 119(6): 1420–8.
32. Hsieh C, Chang A, Brandt D, Guttikonda R, Utset TO, Clark MR. Predicting outcomes of lupus nephritis with tubulointerstitial inflammation and scarring. *Arthritis Care Res (Hoboken)* 2011; 63(6): 865–74.
33. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009; 20(3): 629–37.

Received: May 13, 2026

Accepted: June 08, 2026