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Neutrophil gelatinase-associated lipocalin as a predictor of treatment outcomes in primary glomerulonephritis

Lipokalin udružen sa neutrofilnom gelatinazom kao prediktor ishoda lečenja primarnog glomerulonefritisa

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Abstract

Background/Aim. Biomarkers for predicting disease course could facilitate treatment selection in primary glomerulonephritis (PGN). Data on the role of neutrophil gelatinase-associated lipocalin (NGAL) in PGN are limited. The aim of this study was to evaluate the significance of NGAL in predicting treatment outcomes in PGN. **Methods.** The study included a total of 60 PGN patients followed between 2012 and 2024. At diagnosis, serum NGAL (sNGAL) and urinary NGAL (uNGAL) were measured. Renal function parameters (serum creatinine, proteinuria) and disease outcome–development of end-stage renal disease (ESRD)–were assessed at baseline, after 5 years, and at the end of the follow-up period. The association between NGAL and clinical outcomes was analyzed using appropriate statistical tests. **Results.** At baseline, median sNGAL and uNGAL levels were 154.71 ng/mL and 13.94 ng/mL, respectively. During a median follow-up of 112 months, 8.33% of patients were lost to follow-up, and 20% developed ESRD. Patients who developed ESRD had higher sNGAL levels (p = 0.027). Using sNGAL, ESRD was fairly predictive (AUC = 0.709; p = 0.036), and sNGAL was associated with time-to-ESRD (Kaplan-Meier analysis, $p < 0.001$). The group with the highest sNGAL showed the lowest 5-year renal survival. Patients with stable or improved estimated glomerular filtration rate (eGFR) had higher uNGAL/creatinine ratio values (p = 0.049). Changes in proteinuria correlated negatively with sNGAL ($p < 0.001$) and uNGAL (p = 0.005). **Conclusion.** In PGN, sNGAL could be a predictor of ESRD development, potentially reflecting its pro-fibrotic activity. In contrast, the correlation between NGAL levels and change in proteinuria, as well as the associations between higher uNGAL/creatinine ratios and improved eGFR, suggest a complex and potentially protective role of NGAL in disease progression.

Keywords:
biomarkers; glomerulonephritis; kidney failure, chronic; lipocalins; treatment outcome.

Apstrakt

Uvod/Cilj. Biomarkeri za predviđanje toka bolesti mogli bi da olakšaju izbor terapije primarnog glomerulonefritisa (PGN). Podaci o ulozi lipokalina udruženog sa neutrofilnom gelatinazom (*neutrophil gelatinase-associated lipocalin* – NGAL) u PGN su oskudni. Cilj rada bio je da se proceni značaj NGAL u predviđanju ishoda lečenja PGN. **Metode.** Istraživanjem je obuhvaćeno ukupno 60 obolelih od PGN, praćenih od 2012. do 2024. godine. Pri postavljanju dijagnoze određivani su serumski NGAL (sNGAL) i urinarni NGAL (uNGAL). Parametri bubrežne funkcije (serumski kreatinin, proteinurija) i ishod bolesti–razvoj terminalnog stadijuma bubrežne slabosti (*end-stage renal disease* – ESRD)–procenjivani su na početku praćenja, posle 5 godina i posle kompletnog perioda praćenja. Povezanost NGAL i kliničkih ishoda analizirana je korišćenjem odgovarajućih statističkih testova. **Rezultati.** Na početku, medijane nivoa za sNGAL i uNGAL iznosile su 154,71 ng/mL i 13,94 ng/mL, redom. Tokom medijane praćenja od 112 meseci, 8,33% bolesnika je izgubljeno iz praćenja, a 20% je razvilo ESRD. Bolesnici koji su razvili ESRD imali su više nivoe sNGAL (p = 0,027). Korišćenjem sNGAL, razvoj ESRD bio je solidno predvidljiv (AUC = 0,709; p = 0,036), a sNGAL je bio značajno povezan sa vremenskim periodom do razvoja ESRD (Kaplan-Meier analiza, $p < 0,001$). U grupi sa najvišim sNGAL petogodišnje preživljavanje bubrega bilo je najniže. Bolesnici sa stabilnom ili poboljšanom procenjenom stopom glomerularne filtracije (*estimated*

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glomerular filtration rate – eGFR) imali su više vrednosti odnosa uNGAL/kreatinin (p = 0,049). Promene u vrednostima proteinurije bile su u negativnoj korelaciji sa koncentracijama sNGAL (p < 0,001) i uNGAL (p = 0,005). **Zaključak.** Kod obolelih od PGN, sNGAL može biti prediktor razvoja ESRD, potencijalno odražavajući njegovu profibroznu aktivnost. Nasuprot tome, korelacija između nivoa NGAL i promene vrednosti proteinurije, kao i povezanost između viših vrednosti odnosa uNGAL/kreatinin i poboljšane eGFR, ukazuju na složenu i potencijalno protektivnu ulogu NGAL u progresiji bolesti.

Ključne reči:
biomarkeri; glomerulonefritis; bubreg, hronična insuficijencija; lipokalini; lečenje, ishod.

Introduction

Primary glomerulonephritis (GN) – PGN comprises a heterogeneous group of inflammatory kidney diseases. The main therapeutic goals are to prevent the onset and progression of renal damage and to reduce proteinuria. Despite current treatments, up to 60% of patients progress to end-stage renal disease (ESRD) within ten years [1]. The 5-year mortality rate ranges from 6% to 33% among patients with nephrotic syndrome [2]. Although percutaneous renal biopsy remains the diagnostic gold standard for GN, it is an invasive procedure with limited applicability in certain cases. There is a need to identify reliable biomarkers that can facilitate GN diagnosis. Biomarkers capable of predicting PGN course could enable individualized treatment, avoiding unnecessary exposure to intensive therapies while ensuring timely intervention for high-risk patients. Most biomarkers studied in PGN are not routinely used in clinical practice [3]. Neutrophil gelatinase-associated lipocalin (NGAL), a 25-kilodalton low-molecular-weight glycoprotein, is a well-established nephrology biomarker [4]. In humans, NGAL is primarily detected in neutrophils [5, 6]. It is also found in various tissues, including the renal tubules, which release NGAL following injury [6, 7]. Beyond glomerular damage, tubulointerstitial changes significantly influence PGN outcome. Renal mesangial cells possess NGAL receptors and can produce NGAL in response to inflammation [8]. NGAL regulates cellular processes, including differentiation, proliferation, migration, and apoptosis [6, 9].

The blood of healthy individuals contains small amounts of NGAL [10]. Serum NGAL (sNGAL) and urinary NGAL (uNGAL) increase during PGN, through multiple mechanisms [4, 5, 8, 11, 12]. Neutrophils, the main source of NGAL, infiltrate the kidneys during active disease. Glomerular basement membrane (GBM) damage leads to increased filtration and loss of sNGAL [5, 12]. Prolonged proteinuria saturates the cubilin-megalin transport system, responsible for NGAL uptake, thereby reducing reabsorption. NGAL production is increased in renal and extrarenal tissues under chronic stress induced by proteinuria and cytokine stimulation [4, 5]. Published data suggest correlations of NGAL with residual renal function, serum creatinine (Cr), and proteinuria [4, 5, 11], supporting its potential role in PGN diagnosis and monitoring.

The aim of this study was to evaluate the association between NGAL and renal function parameters in PGN, and to investigate whether baseline NGAL can predict disease course and outcomes of PGN.

Methods

Study population and design

This single-center observational study included 60 PGN patients, enrolled between January 2012 and January 2015. The study was conducted in compliance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the University Clinical Center of Vojvodina, Serbia (No. 00–209, from June 14, 2024). Informed consent was obtained from all participants. This study represents a continuation of a prospective investigation conducted as a part of a dissertation, with a follow-up of 6–25 months [13].

Inclusion criteria were as follows: age > 18 years, percutaneous renal biopsy-confirmed PGN, absence of prior disease-specific treatment, and willingness to participate in the study. Exclusion criteria included the following: secondary GN, lack of renal biopsy confirmation, pre-existing chronic kidney disease (CKD) of other etiology, malignancy, acute infection, and severe hepatic or cardiac failure.

Established diagnostic guidelines and standardized protocols were employed to diagnose PGN and to conduct a percutaneous renal biopsy. Demographic data and information on ESRD development–including the duration in months from diagnosis to the predefined endpoint–were extracted from medical records. Five patients who were lost to follow-up after 6 months were included only in baseline analyses and excluded from subsequent calculations. The remaining patients were categorized into groups according to the specified endpoint, and these groups were subsequently compared with respect to the monitored parameters.

Follow-up assessments were conducted at two time points: five years after diagnosis and at each patient's maximum follow-up, defined as the last available clinical evaluation up to March 2024. The primary endpoint was progression of renal failure to ESRD.

Patient stratification

Results of laboratory tests (serum Cr and proteinuria) were recorded at diagnosis, and on every follow-up point. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Changes in eGFR and proteinuria from baseline were assessed, along with the average annual change in eGFR.

Patients were categorized into groups based on the annual eGFR changes during follow-up. Patients who were lost to follow-up, died within the first year, or lacked a final serum Cr were excluded from this analysis. Group 1 included patients who showed an improvement in eGFR or had an annual eGFR decline of less than 1.5 mL/min/1.73m^2. Group 2 included patients with an annual eGFR decline of more than 1.5 mL/min/1.73m^2. The groups were subsequently compared.

For Kaplan-Meier survival analysis, patients were divided into four groups according to the sNGAL interquartile range (IQR) values: 1) < IQR1, 2) IQR1–IQR2, 3) IQR2–IQR3, and 4) > IQR3.

Laboratory analysis

At diagnosis, blood and urine samples were collected for measurement of sNGAL, uNGAL, and urinary Cr. Following centrifugation, samples were stored at -80 °C until analysis.

Concentrations of sNGAL and uNGAL were measured using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique. Original microplates incorporating monoclonal antibodies to NGAL (R&D Systems, Inc., Minneapolis, USA) were used. Results were read with a Multiskan MCC 340 ELISA reader. Serum samples were diluted at a ratio of 1 : 20, whereas urine samples were analyzed undiluted. Readings were performed at a wavelength of 450 nm. Concentrations were determined from a linear regression curve based on manufacturer-provided standards. For sNGAL, results were multiplied by a factor of 20. Values are expressed in ng/mL. The uNGAL/Cr ratio, expressed in ng/mg, was calculated to account for the influence of renal failure, using the following formula:

$$\frac{\text{uNGAL}}{\text{Cr}} = \frac{\text{urinary NGAL}}{\text{urinary creatinine}}$$

Statistical analysis

An Excel database was created specifically for this study. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Variables with non-normal distributions were reported as medians with IQR. The Wilcoxson rank-sum test was used to compare independent groups of continuous variables, while paired samples were analyzed using the paired Wilcoxson rank-sum test (data with non-normal distribution). Categorical variables were compared using Fisher's exact test. The correlation

Table 1

Monitored variables at baseline, 5-year follow-up, and at the final follow-up

Variables at time points	Median (IQR)
Baseline (n = 60)	
sNGAL	154.71 (115.65–216.53)
uNGAL	13.94 (11.63–15.48)
uNGAL/creatinine	21.88 (12.20–28.97)
creatinine	75.5 (61.0–109.5)
eGFR	93.5 (64.5–108.5)
proteinuria	6,237 (3,196–12,161.5)
5-year follow-up (n = 40)	
creatinine	85 (75.0–114.5)
eGFR	81 (54.5–97.5)
Δ eGFR	-12 (-33.5–3.5)
proteinuria	358.15 (132.5–1349.5)
Δ proteinuria	-4,962.5 (-10,348.5– -2271.5)
Total follow-up (n = 36)	
creatinine	102.5 (83.5–128.0)
eGFR	74 (44.5–85.0)
Δ eGFR	-23 (-35.5– -7.0)
annual Δ eGFR	-2.54 (-4.21– -0.895)
proteinuria	190 (69.0–749.0)
Δ proteinuria	-4,962.5 (-12,242.5– -1,453)

IQR – interquartile range; n – number of patients included in analysis; sNGAL – serum neutrophil gelatinase-associated lipocalin (NGAL); uNGAL – urinary NGAL; eGFR – estimated glomerular filtration rate; Δ eGFR – change in eGFR from baseline; Δ proteinuria – change in proteinuria from baseline.
***Note*: Units of measurement are given as ng/mL for sNGAL and uNGAL; ng/mg for uNGAL/creatinine; μmol/L for creatinine; mL/min/1.73 m^2 for eGFR and Δ eGFR; mg/day for proteinuria and Δ proteinuria.**

between NGAL levels and monitored parameters was assessed using Spearman's correlation coefficient. The Kaplan-Meier curve was used to visualize time-to-ESRD, stratified by sNGAL levels. Additionally, the odds ratio (OR) for ESRD development was calculated for these four groups. The ability of NGAL to discriminate between ESRD developers and those who remained dialysis-independent was evaluated using logistic regression. Results were presented using receiver operating characteristic (ROC) curves. Binomial logistic regression analyses were used to assess the discriminative ability of multivariate models in predicting renal survivor. All statistical tests were two-tailed, with a significance threshold set at $p < 0.05$. Statistical analysis was performed using RStudio (version 2023.03.1 + 446 "Cherry Blossom" Release) and Jamovi (version 2.6.26).

Results

The baseline median age of the 60 included patients was 53 years (IQR 18–77). Data regarding baseline characteristics, 5-year, and the final follow-up are presented in Table 1. Patients who died or became dialysis-dependent during the monitoring period were excluded from analyses at both follow-up time points.

A total of 34 (56.67%) patients had proliferative PGN, and 26 (43.33%) had non-proliferative PGN. Representation of different PGN subtypes is presented in Figure 1. There was no difference between proliferative and non-proliferative GN in sNGAL ($p = 0.445$, U statistic = 390, Effect size = -0.118), uNGAL ($p = 0.331$, U statistic = 376, Effect size = -0.149), or uNGAL/Cr ($p = 0.662$, U statistic = 412, Effect size = - 0.068).

When comparing different PGN subtypes using multiple comparisons analysis, no significant difference in uNGAL ($p = 0.652$, $F = 0.698$) or sNGAL ($p = 0.087$, $F = 1.97$) was observed.

However, rapidly progressive GN patients had significantly higher sNGAL compared to mesangioproliferative GN patients [$p = 0.039$, mean difference (MD) = -134, 95% confidence interval (CI): 40.25–227.75], and membranous nephropathy patients ($p = 0.025$, MD = 153.7, 95% CI: 53.25–254.15). The groups differed in uNGAL/Cr ($p = 0.021$, $F = 2.74$). Membranoproliferative GN patients had higher uNGAL/Cr compared to mesangioproliferative GN patients ($p = 0.044$, MD = 17.3, 95% CI: 3.8–30.8).

Five (8.33%) patients were lost to follow-up 6 months after diagnosis and were excluded from further analyses. The remaining patients were followed for a median of 112 months (IQR 67–124).

Table 2 summarizes the correlations between NGALs and the monitored variables at baseline and at two follow-up time points. At baseline, sNGAL and uNGAL correlated directly with serum Cr levels and negatively with eGFR. Proteinuria correlated positively with both NGAL measures; however, the correlation with sNGAL showed only a trend toward significance ($p < 0.1$) and did not reach statistical significance ($p < 0.05$). Over the entire follow-up, uNGAL/Cr correlated directly with both absolute and annual changes in eGFR, while uNGAL demonstrated a near-significant positive correlation with eGFR change. Change in proteinuria correlated negatively with both NGALs at the 5-year checkpoint and across the entire observation period.

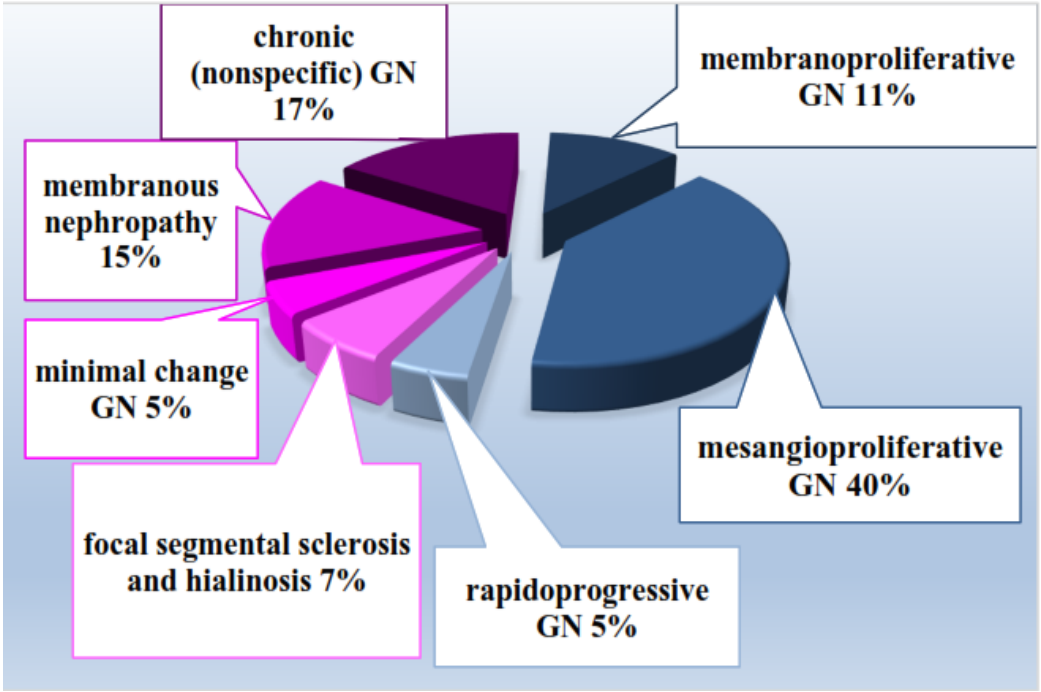

Fig. 1 – Representation of different primary glomerulonephritis (GN) subtypes.

Table 2

Correlations between NGAL and monitored variables

Variables at time points	sNGAL		uNGAL		uNGAL/Creatinine	
	p-value	*r*	*p*-value	*r*	*p*-value	*r*
Baseline						
age	0.759	-0.040	0.686	-0.053	0.381	-0.115
creatinine	< 0.001	0.510	0.047	0.258	0.270	0.145
eGFR	< 0.001	-0.431	0.046	-0.258	0.149	-0.189
proteinuria	0.069	0.237	0.018	0.306	0.701	0.050
5-year follow-up						
creatinine	0.041	0.325	0.660	0.072	0.698	0.063
eGFR	0.082	-0.279	0.840	-0.033	0.614	-0.082
Δ eGFR	0.817	0.038	0.207	0.204	0.065	0.295
proteinuria	0.548	-0.098	0.805	0.040	0.621	-0.081
Δ proteinuria	0.002	-0.470	0.006	-0.424	0.480	-0.115
Total follow-up						
creatinine	0.178	0.229	0.730	0.060	0.670	0.074
eGFR	0.480	-0.122	0.921	-0.017	0.651	-0.078
Δ eGFR	0.240	0.201	0.052	0.322	0.048	0.327
annual Δ eGFR	0.385	0.149	0.164	0.237	0.023	0.376
proteinuria	0.883	-0.025	0.838	0.035	0.518	0.111
Δ proteinuria	< 0.001	-0.541	0.005	-0.459	0.428	-0.136
time to ESRD	0.528	-0.203	0.914	-0.035	0.683	-0.133

NGAL – neutrophil gelatinase-associated lipocalin; sNGAL – serum NGAL; uNGAL – urinary NGAL; eGFR – estimated glomerular filtration rate; Δ eGFR – change in eGFR from baseline; annual Δ eGFR – average annual change in eGFR; Δ proteinuria – change in proteinuria from baseline; ESRD – end-stage renal disease.

Table 3

NGAL levels and ESRD development at two follow-up periods

Variables at time points	n	sNGAL	uNGAL	uNGAL/Creatinine
5-Year follow-up				
no ESRD	44	144.3 (100.2)	14 .0 (4.0)	19.4 (14.6)
ESRD	11	217.9 (127.7)	14.1 (2.5)	23.0 (28.8)
p		0.068	0.860	0.411
U statistic		155	233	202
effect size *r*		0.360	-0.037	0.165
Final follow-up				
no ESRD	43	138.2 (98.7)	14.1 (4.1)	21.5 (14.8)
ESRD	12	222.5 (117.1)	13.6 (2.3)	22.6 (30.3)
p		0.027	0.695	0.802
U statistic		150	238	245
effect size *r*		0.419	-0.077	0.050

NGAL – neutrophil gelatinase-associated lipocalin; ESRD – end-stage renal disease; sNGAL – serum NGAL; uNGAL – urinary NGAL.

Values are given as median (interquartile range).

***Note*: For units of measurement performed, see Table 1.**

Twelve (20%) patients developed ESRD, with the median time from diagnosis to ESRD of 38 months (IQR 19.5–47.5).

Patients were stratified according to renal survival at two follow-up time points. Baseline levels of sNGAL, uNGAL, and uNGAL/Cr were compared between groups (Table 3). Patients who progressed to ESRD had significantly higher baseline sNGAL than those who remained dialysis-independent. This difference was evident at the 5-year follow-up time point and reached statistical significance after completion of the follow-up period.

Four groups were created based on sNGAL IQR values: 1) sNGAL < 115.65 ng/mL, 2) sNGAL 115.66–154.71 ng/mL, 3) sNGAL 154.72–216.53 ng/mL, and 4) sNGAL > 216.53 ng/mL. They were compared in terms of renal survival over the full follow-up period. Kaplan-Meier analysis demonstrated a significant difference in time-to-ESRD between the groups (Tarone-Ware test, $\chi^2 = 61.4$, $p < 0.001$). The median time to ESRD for Group 4 was 92 months, while it was not estimable for the other groups due to the small number of events. Five-year kidney survival was 84.8% (95% CI: 67.4–100%) for Group 1; 90.9% (95% CI: 75.4–100%) for Group 2; 85.7% (95% CI: 69.2–100%) for Group 3; and 57.1% (95% CI: 36.3–89.9%) for Group 4. Compared with Group 1, OR for ESRD development in Group 4 was 6.50 (95% CI: 1.19–52.38, $p = 0.044$), while no significant differences were found for Group 2 (OR = 0.59, $p = 0.684$) or Group 3 (OR = 1.08, $p = 0.941$). The Kaplan-Meier curve and OR plot are shown in Figure 2 A, B.

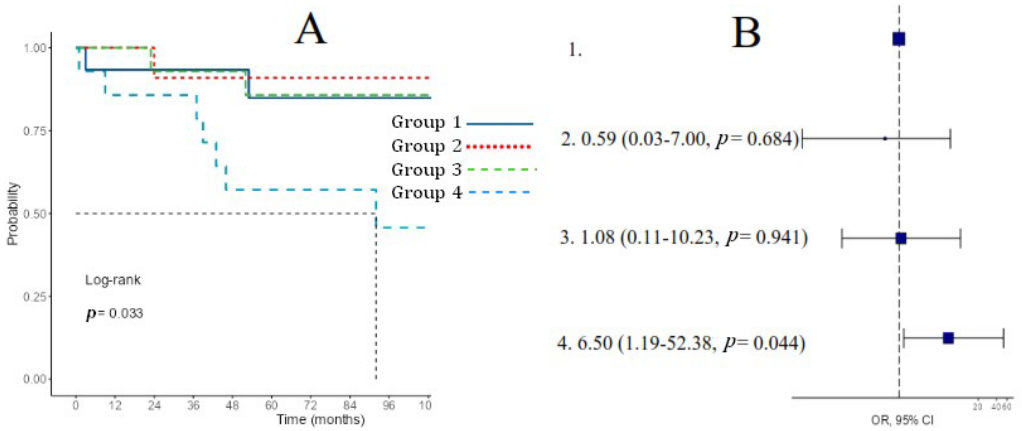

Fig. 2 – Serum neutrophil gelatinase-associated lipocalin (sNGAL) and development of end-stage renal disease: A) Kaplan-Meier curve (Tarone-Ware $p < 0.001$, Group 4 median – 92 months); B) OR plot. OR – odds ratio; CI – confidence interval.

***Note*: sNGAL concentrations (ng/mL) in Groups 1–4 were < 115.65, 115.66–154.71, 154.72–216.53, and > 216.53, respectively.**

Table 4

NGAL in relation to eGFR change during follow-up

Variables	Group 1	Group 2	*U* stat.	*p*-value	Effect size
n	12	37			
sNGAL	172.9 (69.2)	160.4 (102.2)	203	0.671	-0.086
uNGAL	15.2 (2.3)	13.3 (3.4)	141	0.061	-0.365
uNGAL/creatinine	22.9 (26.1)	17.0 (14.4)	137	0.049	-0.383
Age, years	39 (22.8)	53 (13.0)	157	0.133	0.293
eGFR	68 (30.8)	101 (34.0)	118	0.016	0.471
Total follow-up, months	110 (38.3)	113 (46.0)	200	0.609	0.101

NGAL – neutrophil gelatinase-associated lipocalin; eGFR – estimated glomerular filtration rate; *U* stat – *U* statistic; n – number of patients in the group; sNGAL – serum NGAL; uNGAL – urinary NGAL. Values are given as median (IQR). For units of measurement, see Table 1.

***Note*: Group 1 – an average annual eGFR increase or decline of < 1.5 mL/min/1.73m2; Group 2 – an average annual eGFR decline of > 1.5 mL/min/1.73m2.**

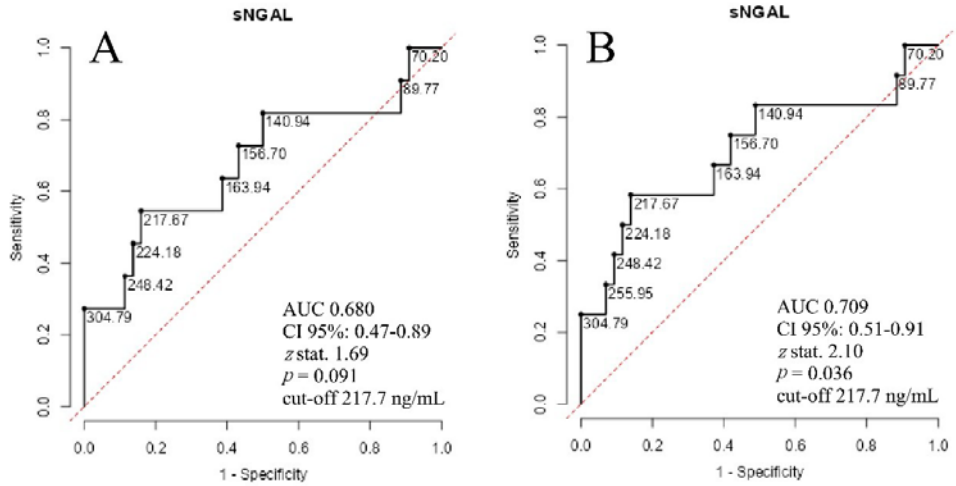

Fig. 3 – ROC curves for serum neutrophil gelatinase-associated lipocalin (sNGAL) in predicting renal survival: A) sNGAL for predicting end-stage renal disease (ESRD) development in a 5-year period; B) sNGAL for predicting ESRD development in the total follow-up period.
ROC – receiver operating characteristic; AUC – area under the curve; CI – confidence interval.

According to eGFR changes (average annual) during follow-up, 49 patients were divided into groups. Patients who were lost to follow-up, who died within the first year, or who lacked a final serum Cr were excluded from this analysis. Comparisons between the groups are presented in Table 4. Group 2 had a significantly lower baseline uNGAL/Cr ratio.

Logistic regression was used to assess the discriminatory ability of NGAL in predicting renal survivor. The ROC curves with *p*-values < 0.1 are shown in Figure 3A, B. The

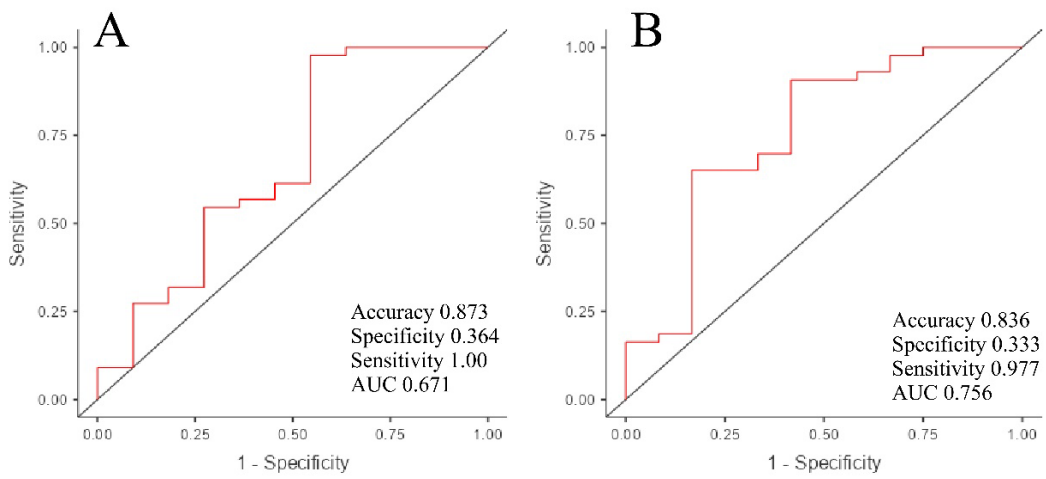

Fig. 4 – ROC curves for multivariate models in predicting renal survival: A) model for predicting end-stage renal disease (ESRD) after 5 years; B) model for predicting ESRD at the end of follow-up. ROC – receiver operating characteristic; AUC – area under the curve.

ROC curve for sNGAL indicated fair performance in identifying patients who would develop ESRD by the end of follow-up [area under the curve (AUC) = 0.709, 95% CI: 0.51–0.91, p = 0.036, cut-off = 217.7 ng/mL] (Figure 3B). Although the number of outcome events was limited, the analyses included a single predictor, which reduced the risk of overfitting.

Multivariate models with the discriminative ability of p-values < 0.1 for predicting renal survival, based on binomial logistic regression, are shown in Figure 4A, B. The model for predicting 5-year renal survival (Figure 4A), including baseline sNGAL, uNGAL, Cr, eGFR, diagnosis of proliferative vs. non-proliferative GN, and age, showed high sensitivity (1.0), but low specificity (0.364) and moderate discrimination (AUC = 0.671, p = 0.099). Supplementary Table 1 additionally shows the model for predicting ESRD development after 5 years.

The model for overall renal survival (Figure 4B), including baseline sNGAL, uNGAL, uNGAL/Cr, Cr, and eGFR, demonstrated good discriminative ability (AUC = 0.756), with high sensitivity (0.977), but low specificity (0.333) (p = 0.035). Supplementary Table 2 additionally shows the model for predicting overall ESRD development.

The results of both multivariate models should be interpreted with caution owing to the small number of patients who reached the study endpoint.

Discussion

Reference values for NGAL in healthy individuals are not clearly defined; the reported range for sNGAL is 7.8–109 ng/mL [10, 14, 15]. Previous studies have shown elevated levels of sNGAL and uNGAL in GN [4–6, 8, 11, 12, 16]. In our cohort, the median sNGAL (154.7 ng/mL) was consistent with these findings. In contrast, the median uNGAL (13.9 ng/mL) fell within the reported range for healthy individuals (0.34–20.41 ng/mL) [17, 18] and was lower than the 26.1 ng/mL reported in patients with PGN and lupus nephritis [12]. Our median uNGAL/Cr ratio (21.9 ng/mg) exceeded the value reported for the general population (4.2 ng/mg) [15].

No significant differences in uNGAL or sNGAL were observed among PGN subtypes in our study, consistent with Coppolino et al. [7] results. Values of uNGAL/Cr differed significantly between PGN subtypes in our cohort.

Both sNGAL and uNGAL correlated directly with baseline Cr and indirectly with eGFR, in line with previously reported data [5, 11]. However, some studies of glomerular diseases and diabetic nephropathy have not reported such correlations [7, 12, 16, 19]. Patients were followed for a median of 112 months. After 5 years, 18.33% of patients progressed to ESRD, increasing to 20% over the entire follow-up period, aligning with previously reported PGN rates (12.9–19.4% over 5 years; up to 60% over 10 years) [1]. The median time to ESRD was 38 months. Patients who progressed to ESRD had higher baseline sNGAL, compared to those who remained dialysis-independent, whereas Coppolino et al. [7] identified uNGAL as the stronger predictor. Kaplan-Meier analysis of renal survival demonstrated the lowest renal survival probability in patients with the highest sNGAL, with 5-year renal survival of 57.1% (vs. 84.8–90.9% in other groups). A fair discriminatory ability for predicting progression to ESRD was observed for sNGAL, with an optimal cut-off of 217.7 ng/mL. Elevated sNGAL in patients with CKD progression may reflect extra-tubular NGAL sources during active PGN. Renal mesangial cells produce NGAL in response to inflammation [8]. The cytokine environment in active PGN likely increases NGAL release into the circulation. NGAL influences cell motility and invasiveness in malignant cells [9], and functions as a chemoattractant [20]. Similarly, it may promote neutrophil recruitment to the kidney and modulate other inflammatory cells, thereby amplifying its own production. CKD progression in patients with elevated sNGAL may be related to its pro-fibrotic effects, which accelerate renal deterioration. Supporting this, Bonnard et al. [9] reported that a chemical NGAL inhibitor attenuated its pro-fibrotic and pro-inflammatory effects in renal and cardiac tissues.

In dialysis-independent patients, the uNGAL/Cr ratio was directly correlated with both absolute and annual eGFR change during the follow-up period, while uNGAL showed a borderline significant direct correlation with eGFR change. When comparing groups with different eGFR changes during follow-up, those with improved and/or stable eGFR had higher baseline uNGAL/Cr than those with eGFR decline. They also had higher uNGAL, but the difference did not reach significance. Data on the association between NGAL and longitudinal changes in renal function in PGN are limited. As mentioned, Copolino et al. [7] linked higher baseline uNGAL with CKD progression. Data on type 2 diabetes are inconsistent. Chou et al. [21] observed no association, whereas Żyłka et al. [19] found higher NGAL in patients with declining eGFR. Kielar et al. [22] linked higher uNGAL with eGFR decline after kidney transplantation. Our patients with improved and/or stable eGFR during follow-up had lower baseline eGFR compared with those with eGFR decline. When initiating treatment in PGN, therapy primarily aims to limit existing renal injury and to restore impaired kidney function, whereas in diabetes or after successful kidney transplantation, therapeutic strategies are largely aimed at preventing the onset and progression of renal damage. This difference limits the direct comparability of results across studies.

We observed a positive correlation between uNGAL and baseline proteinuria, consistent with findings from studies on glomerular diseases and lupus nephritis [4, 5, 11, 12, 16], but not previously reported in PGN [7]. This correlation can be explained by the increase in uNGAL during proteinuria. Filtration of sNGAL through the damaged GBM is increased [12]. Increased protein concentration in the tubules causes the cubilin-megalin transport mechanism overload, with reduced NGAL reabsorption [12, 16]. Tubular release of NGAL is increased due to the toxic effects of high cellular concentration of reabsorbed proteins, and decreased oncotic pressure and renal perfusion due to massive proteinuria [12]. Reduction in proteinuria during follow-up correlated negatively with both sNGAL and uNGAL. Those with greater reductions had higher baseline sNGAL and uNGAL. A positive correlation between the change in proteinuria and uNGAL change over time was reported before, but no comparison was made with baseline uNGAL [12].

Based on our findings, we can assume that abundant proteinuria leads to increased NGAL release in the kidneys, which may activate different mechanisms that contribute to proteinuria reduction. Other authors have arrived at a similar conclusion [5]. The association of high baseline uNGAL normalized by urine Cr with eGFR improvement may also suggest NGAL's protective role during active PGN. T-cell immunity plays an important role in PGN pathogenesis [23]. NGAL downregulates the Th17 cell effects, thereby preventing the development of severe forms of GN [6]. PGN is an inflammatory disease with intense tissue oxidative processes associated with GBM changes and podocyte injury leading to CKD progression [24–26]. NGAL can mitigate oxidative stress (OS), which is linked to CKD progression and mortality in CKD [14, 24, 25]. OS-induced NGAL release is believed to be a compensatory mechanism aimed at reducing the toxic OS effects [14, 25]. By binding to chemotactic proteins, NGAL clears inflamed tissue and regulates inflammation [14]. NGAL reduces apoptosis of endotoxemia-damaged tubular cells and protects against ischemia-reperfusion injury [27]. In addition to mitigating OS and inflammation and modulating T-cell-mediated immunity, NGAL may be involved in other mechanisms that prevent pathological changes in the kidneys, influencing a better PGN course.

It remains unclear which mechanisms would be triggered by NGAL in individual patients and which factors influence the occurrence or predominance of specific effects. Distinct biological roles of NGAL in serum and urine are possible, as in our study, sNGAL was more strongly associated with CKD progression, whereas uNGAL was associated with improvement in renal function. Several questions regarding the role of NGAL, particularly in PGN, remain unresolved, underscoring the need for further research to clarify the diagnostic and prognostic significance of NGAL in PGN.

Limitation of the study

This study has several limitations and strengths. It was conducted at a single center and included a relatively small number of patients. Because all PGN subtypes were analyzed collectively, the findings cannot be attributed to individual subtypes. The absence of a control group limited comparisons of baseline findings. The small number of patients reaching the predefined endpoint may have reduced statistical power. Furthermore, not all clinical, biochemical, and histological biomarkers known to influence renal survival and mortality in PGN were included in the analyses. Despite these limitations, the study has notable strengths. All patients had biopsy-confirmed PGN, were naïve to disease-specific treatment at enrollment, and were followed over a prolonged period. CKD due to causes other than PGN was an exclusion criterion.

Conclusion

Higher sNGAL was associated with the development of end-stage renal disease, possibly reflecting its pro-fibrotic effects. In contrast, elevated uNGAL/Cr was associated with eGFR improvement. Both NGALs correlated inversely with changes in proteinuria during follow-up. The observed correlations between NGAL levels and reductions in proteinuria, together with the relationship between the uNGAL/Cr ratio and improved or stable renal function, suggest that NGAL may activate adaptive processes associated with favorable outcomes. Measurement of both sNGAL and uNGAL at diagnosis may help identify high-risk patients with primary glomerulonephritis, who could benefit from intensified treatment. However, the small sample size, the limited number of patients reaching study endpoints, and the incomplete understanding of cellular mechanisms of NGAL underscore the need for further research to validate NGAL as a biomarker in primary glomerulonephritis.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Table 1

Model for predicting ESRD development after 5 years

Predictor	Estimate (95% CI: lower–upper)	SE	*Z*	*p*-value	OR (95% CI: lower–upper)
Intercept	6.031 (-6.945–19.006)	6.620	0.911	0.362	416.032 (9.64×10^{-4}–1.80×10^{8})
sNGAL	-0.003 (-0.017–0.009)	0.006	-0.580	0.562	0.996 (0.983–1.01)
uNGAL	0.095 (-0.128–0.317)	0.114	0.836	0.403	1.100 (0.880–1.37)
Age	-0.021 (-0.089–0.047)	0.035	-0.595	0.552	0.980 (0.915–1.05)
Creatinine baseline	-0.023 (-0.065–0.020)	0.022	-1.034	0.301	0.978 (0.937–1.02)
eGFR baseline	-0.023 (-0.096–0.050)	0.037	-0.612	0.540	0.977 (0.908–1.05)
Pr/nonpr GN	0.193 (-1.536–1.923)	0.882	0.219	0.827	1.213 (0.215–6.84)

ESRD – end-stage renal disease; CI – confidence interval; SE – standard error; OR – odds ratio; sNGAL – serum neutrophil gelatinase-associated lipocalin (NGAL); uNGAL – urinary NGAL; eGFR – estimated glomerular filtration rate; Pr/nonpr GN – proliferative or non-proliferative glomerulonephritis; AIC – Akaike information criterion; McF – McFadden's.
***Note*: This model corresponds to the results shown in Figure 4A. Model fit measures: deviance = 44.4, AIC = 58.4, R^2McF = 0.194; Overall model test: $\chi^2 = 10.7$, $df = 6$, p = 0.099. Model estimated using a sample size of 55 patients.**

Supplementary Table 2

Model for predicting overall ESRD development

Predictor	Estimate (95% CI: lower–upper)	SE	*Z*	*p*-value	OR (95% CI: lower–upper)
Intercept	6.085 (-2.603–14.772)	4.432	1.373	0.170	439.053 (0.074–2.60×10^{6})
sNGAL	-0.008 (-0.021–0.005)	0.007	-1.177	0.239	0.992 (0.980–1.01)
uNGAL	0.138 (-0.098–0.375)	0.121	1.147	0.251	1.149 (0.907–1.46)
uNGAL/creatinine	-0.024 (-0.082–0.033)	0.029	-0.839	0.402	0.976 (0.922–1.03)
Creatinine baseline	-0.022 (-0.056–0.012)	0.017	-1.284	0.199	0.978 (0.945–1.01)
eGFR baseline	-0.030 (-0.088–0.029)	0.030	-0.974	0.330	0.971 (0.916–1.03)

ESRD – end-stage renal disease; CI – confidence interval; SE – standard error; OR – odds ratio; sNGAL – serum neutrophil gelatinase-associated lipocalin (NGAL); uNGAL – urinary NGAL; eGFR – estimated glomerular filtration rate; AIC – Akaike information criterion; McF – McFadden's.
***Note*: This model corresponds to the results shown in Figure 4B. Model fit measures: deviance = 45.8, AIC = 57.8, R^2McF = 0.207; Overall model test: $\chi^2 = 12.0$, $df = 5$, p = 0.035. Model estimated using a sample size of 55 patients.**