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ORIGINAL ARTICLE

The Prevalence of Low Plasma Neutrophil Gelatinase-Associated Lipocalin Level in Systemic Inflammation and its Relationship with Proinflammatory Cytokines, Procalcitonin, Nutritional Status, and Leukocyte Profiles

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SUMMARY

Background: **The significance of low plasma neutrophil gelatinase-associated lipocalin (NGAL) level in systemic inflammation has not been investigated. The aim of this study was to investigate low plasma NGAL level in systemic inflammation and its relationship with proinflammatory cytokines, procalcitonin (PCT), leukocyte profiles, nutritional status, and kidney function.**

Methods: **Patients with systemic inflammation were evaluated by measuring NGAL, PCT, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), serum creatinine (sCr), and clinical scores.**

Results: **Of 191 patients, 30 (15.7%) had low NGAL levels (< 68 ng/mL), and 102 (53.4%) had elevated NGAL levels (> 150 ng/mL). Among the 30 patients with low NGAL levels, 26 (86.7%) had normal kidney function and 24 (80.0%) had low-grade inflammation. In comparison with healthy individuals, patients with low NGAL levels had higher levels of TNF-α, IL-6, PCT, and hsCRP but not absolute neutrophil count (ANC). Neutropenia was more often observed in subjects with low NGAL levels than in those with elevated NGAL levels (16.7% versus 1.9%, $p < 0.001$). In the low NGAL group, plasma NGAL was significantly associated with ANC ($r = 0.312$, $p < 0.001$) but not cytokines, sCr, nutritional parameters, and clinical scores. Receiver operating characteristic (ROC) curve analysis demonstrated that the diagnostic ability of the ANC for identifying low NGAL levels was superior to that of PCT and TNF-α [0.82 (95% CI, 0.75 - 0.89) versus 0.67 (95% CI, 0.56 - 0.79) and 0.66 (95% CI, 0.54 - 0.78), respectively, $p < 0.001$].**

Conclusions: **Low plasma NGAL level in systemic inflammation was more closely linked to the non-increment of the ANC than proinflammatory cytokines, PCT, and nutritional status, particularly in patients with low-grade inflammation who had preserved kidney function.**

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INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL) is a small glycoprotein expressed in neutrophilic leukocytes

and renal tubular epithelial cells [1]. The renal expression of NGAL is upregulated in kidney damage due to a variety of causes [2,3]. As NGAL level increases within 2 hours of an insult, particularly before a significant change in serum creatinine (sCr), NGAL has been used as an early and sensitive marker of renal dysfunction [4, 5]. NGAL is a promising indicator of acute kidney injury; however, there have been inconsistencies in its predictive value [6]. For instance, plasma NGAL level can increase in the absence of tubular damage [7]. Moreover, NGAL production is enhanced in response to different inflammatory conditions [8,9].

NGAL is an acute phase inflammatory protein produced by various cell types [10]. Plasma NGAL level can increase in response to oxidative or thermal stresses apart from being an indicator of renal dysfunction. NGAL expression has been reported to increase in mouse kidneys exposed to cold or heat stress, indicating that NGAL behaves like a stress protein [11]. A group of researchers reported that NGAL is more specific and sensitive than high-sensitivity C-reactive protein (hsCRP) in the discrimination between bacterial and viral infection [12]. NGAL is closely associated with inflammatory biomarkers in a variety of inflammatory conditions. It is upregulated via the stimulation of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [13]. TNF-α is mechanistically associated with NGAL because TNF-α specifically upregulates NGAL via the activation of TNF receptor-1, and the increased NGAL inactivates TNF receptor-2-mediated pathways [14].

Previous studies have largely focused on the clinical potential of elevated NGAL level as an indicator of acute kidney injury and as a biomarker of inflammation. However, limited studies have closely examined the significance of low plasma NGAL level in systemic inflammation. The present study investigated the prevalence of low plasma NGAL concentration in systemic inflammation and determined which parameter (TNF-α, IL-6, procalcitonin [PCT], nutrition status, sCr, and leukocyte profiles) more critically contributes to low NGAL level in patients with inflammatory diseases.

MATERIALS AND METHODS

Patient population

A total of 191 patients under clinical investigation for systemic inflammation were assessed. The patients ranged in age from 39 to 78 years (median age, 65 years), and 98 patients were male (51.3%). Age-matched and sex-matched healthy individuals (n = 53), who had no evidence of inflammation and renal dysfunction, were enrolled as the control group. Clinical and demographic data were collected from medical records. None of the subjects were pregnant or had a history of acute blood loss or proteinuria. Patients with cardiovascular diseases (n = 4), stroke (n = 3), drug administration (n = 3), or a recent operation (n = 1) were excluded because these conditions may affect plasma NGAL levels. Subjects who had incomplete data for physical examinations, laboratory tests, and anthropometric measures were also excluded from the analysis (n = 5). This study was approved by the institutional review board, and written informed consent was obtained from all subjects.

Laboratory measurement

Blood samples were obtained from patients at admission, immediately centrifuged, and stored in aliquots at -80°C until assay. All specimens were collected before treatment. Several parameters were measured including inflammatory markers (TNF-α, IL-6, PCT, hsCRP, and leukocyte), nutritional status (body fat mass, serum total cholesterol, and serum albumin), and renal parameters [sCr and estimated glomerular filtration rate (eGFR)]. Plasma NGAL concentrations were measured by fluorescence immunoassay using the Triage NGAL Test (Alere, Inc., San Diego, CA, USA), which analyzes plasma NGAL with a measurable range from 15 ng/mL to 1,300 ng/mL. The intra-assay CVs (n = 20) for three samples (mean NGAL, 62 - 543 ng/mL) were 4.3 - 6.5%; the inter-assay CVs calculated from duplicate results in 10 subsequent assays were 4.5 - 7.2%. A medical decision point for plasma NGAL level was determined as 150 ng/mL [15]. A low plasma NGAL level among patients was defined as < 68 ng/mL, which was a provisional cutoff limit based on the median value of plasma NGAL level in healthy individuals. Plasma concentrations of IL-6 and TNF-α were measured using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). PCT level was measured with an electrochemiluminescence method (Elecsys BRAHMS PCT; Roche Diagnostics, Mannheim, Germany) using the Cobas e 411 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Neutropenia was defined as an absolute neutrophil count (ANC) of less than 1.5 x 10^9/L [16]. To compare inflammatory parameters under the same condition, the cutoff of hsCRP was set as 0.45 mg/dL, which corresponded to the same percentile as the values of the ANC (1.5 x 10^9/L) of the 191 patients. Serum albumin, total cholesterol, sCr, and hsCRP were assayed with a chemical analyzer (Hitachi 7600; Hitachi, Tokyo, Japan). Hypoalbuminemia was defined as a serum albumin concentration of < 3.5 g/dL [17], and hypocholesterolemia was defined as a serum total cholesterol level of < 120 mg/dL [18]. Body fat mass was measured using a body composition analyzer (InBody 720 Analyzer; Biospace, Seoul, Korea). Serum hsCRP levels were measured using a particle-enhanced immunonephelometric assay (Dade Behring, Inc., Deerfield, IL, USA). The erythrocyte sedimentation rate (ESR) was determined by the Westergren sedimentation technique using StaR-Rsed Auto-Compact (Mechatronics Manufacturing BV, Zwaag, Netherlands). The corrected erythrocyte sedimentation rate (cESR) was calculated based on a normal

hematocrit of 45% from the following formula: cESR (mm/hour) = (subject's hematocrit/45) x ESR (mm/hour). An elevated level of hsCRP and cESR was defined as > 0.3 mg/dL and > 15 mm/hour, respectively. Clinical score was determined in relation to clinical signs, such as body temperature, heart rate, respiratory rate (or hyperventilation), and leukocyte counts, which was based on the diagnostic criteria of systemic inflammatory response syndrome (SIRS) [19]. Severe inflammation (SIRS) was considered to be present when subjects had more than two of the following clinical findings: body temperature, > 38°C or < 36°C; heart rate, > 90/minute; hyperventilation evidenced by a respiratory rate of > 20/minute or a $PaCO_2$ of < 32 mmHg; and a leukocyte count of > 12,000/μL or < 4,000/μL. Subjects were categorized into two groups: low clinical score (< 2.0; non-SIRS; n = 137) and high clinical score (≥ 2.0; SIRS; n = 54). Mortality associated with diseases was assessed 28 days after the enrollment, as described previously [20].

To assess the degree of renal dysfunction, the renal score of SOFA was used [21], and the patients were categorized into five groups (grade 0 to grade 4) based on the sCr level as follows: sCr < 1.20 mg/dL (grade 0), 1.20 - 1.99 mg/dL (grade 1), 2.00 - 3.49 mg/dL (grade 2), 3.50 - 4.99 mg/dL (grade 3), and > 5.00 mg/dL (grade 4). The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula: eGFR = 186 x [sCr (mg/dL)]$^{-1.154}$ x [age (years)]$^{-0.203}$ [22]. A correction factor of 0.742 was used for women. An impaired renal function was defined as an eGFR level < 60 mL/min/1.73 m^2 [23].

The severity of inflammation was determined by an inflammation index using a scoring system based on hsCRP and cESR levels [24]. The grade of inflammation was determined by an inflammation index using hsCRP and cESR levels. The inflammation index was obtained from the sum of the individual scores. The subjects were classified into two groups: low-grade (inflammation index, > 1.0 to < 2.5) and high-grade (inflammation index, ≥ 2.5 to 4.0). Low-grade inflammation was determined based on the 95% confidence interval (CI) of the inflammation index of patients with low-grade inflammatory diseases, including cardiovascular disease, type 2 diabetes mellitus, and atherosclerosis [25].

Statistical analysis

Numerical variables with a normal distribution were expressed as the mean ± standard deviation (SD). Variables with a non-normal distribution were summarized as the median and interquartile range. The normality of data distribution was confirmed by the Shapiro-Wilk test. Categorical variables were presented as the frequency and percentage. Mann-Whitney *U* test and Student's *t*-test were used to analyze the data between the two groups. Multivariate linear regression analysis was performed to determine the correlation between NGAL and nutritional and inflammatory parameters after adjusting for potential confounders, such as age, gender, body mass index (BMI), and systolic blood pressure. The odds ratio for the risk of low NGAL level was determined by multivariate logistic regression analysis. A receiver operating characteristics (ROC) curve was analyzed to determine the diagnostic ability of PCT, ANC, and TNF-α for identifying low NGAL levels in patients with systemic inflammation. Data were analyzed using SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Patients with low plasma NGAL levels

Of the 191 patients with inflammation, 30 (15.7%) had low plasma NGAL concentrations (< 68 ng/mL), and 102 (53.4%) had high plasma NGAL concentrations (> 150 ng/mL). Patients with low NGAL levels had decreased concentrations of TNF-α, IL-6, and hsCRP compared with those with high NGAL levels. However, serum albumin and total cholesterol in the low NGAL group were higher than those in the high NGAL group. Neutropenia was observed 8.7 times more in the low NGAL group than in the high NGAL group (16.7% versus 1.9%, $p < 0.001$). The levels of TNF-α, IL-6, and PCT of patients with low NGAL levels were higher than those of the healthy controls. However, there was no significant difference in the levels of NGAL, sCr, body fat mass, serum albumin, and serum total cholesterol between the groups (Table 1).

NGAL in SIRS and non-SIRS

Among all subjects, 54 (28.3%) had high clinical scores (≥ 2.0; SIRS), 63 (32.9%) had renal impairment, and 127 (66.5%) had high-grade inflammation. The overall mortality rate (28 days) was 8.4%. The prevalence of high NGAL level was significantly different between patients with and without renal failure (92.1% versus 34.4%, $p < 0.05$) and between those with low- and high-grade inflammation (40.6% versus 59.8%, $p < 0.05$). However, no significant difference was observed between SIRS and non-SIRS (Table 2). Plasma NGAL levels according to clinical scores were illustrated in Figure 2. There was no significant difference in median plasma NGAL levels between subjects with clinical score 0 and 2. However, median plasma NGAL levels were significantly elevated in subjects with clinical score 3 (Figure 1).

Plasma NGAL levels according to hsCRP and sCr

Plasma NGAL levels in relation to the concentrations of hsCRP and sCr are shown in Figure 2. Plasma NGAL concentrations demonstrated a sharper increase with a change in sCr compared with hsCRP. There was no significant increase in plasma NGAL concentrations until hsCRP and sCr reached the levels of 4.0 mg/dL and 1.0 mg/dL, respectively. An elevated NGAL level

Table 1. Baseline characteristics of patients with low and high NGAL levels.

	Patients with systemic inflammation		Healthy controls (n = 53)	p-value
	Low NGAL group (< 68 ng/mL; n = 30)	High NGAL group (> 150 ng/mL; n = 102)		(Low NGAL versus high NGAL)
Anthropometric parameters				
Age (years)	64 (45 - 72)	65 (42 - 75)	65 (43 - 76)	0.238
Gender (male, %)	16 (53.3)	53 (51.9)	27 (50.9)	0.403
BMI (kg/m^2)	21.6 ± 3.2	22.3 ± 3.4	22.6 ± 3.5	0.561
SBP (mmHg)	129.5 ± 34.9	127.1 ± 27.1	128.3 ± 26.2	0.597
Lipocalin levels				
NGAL (ng/mL)	61.5 (35.7 - 66.2)	386.5 (225.0 - 725.7)	68.0 (52.0 - 94.0)	< 0.001
Inflammatory markers				
TNF-α (pg/mL)	6.5 (3.2 - 19.4) *	12.1 (6.0 - 32.7)	2.7 (2.1 - 6.8)	< 0.001
IL-6 (pg/mL)	4.5 (1.9 - 8.3) *	5.6 (3.4 - 14.6)	1.3 (0.7 - 3.4)	< 0.001
PCT (ng/mL)	4.7 (0.8 - 8.9) *	5.2 (0.9 - 9.8)	0.2 (0.1 - 0.4)	0.102
hsCRP (mg/dL)	1.53 (0.41 - 3.62) *	7.21 (2.19 - 13.91)	0.07 (0.04 - 0.13)	< 0.001
cESR (mm/hour)	15.9 ± 11.3 *	38.1 ± 22.1	4.51 ± 2.82	< 0.001
Leukocyte (x 10^9/L)	7.47 ± 2.89	10.88 ± 7.01	8.18 ± 3.26	0.004
ANC (x 10^9/L)	5.02 ± 2.46	8.63 ± 7.03	5.26 ± 3.24	< 0.001
ANC < 1.5 x 10^9/L (n, %)	5 (16.7) *	2 (1.9)	0 (0.0)	< 0.001
Nutritional parameters				
Serum albumin (g/dL)	3.93 ± 0.65	3.38 ± 0.73	4.12 ± 0.74	< 0.001
Total cholesterol (mg/dL)	175.9 ± 34.8	157.4 ± 61.5	192.6 ± 57.5	0.003
Body fat mass (kg)	7.2 ± 3.5	7.1 ± 3.4	7.6 ± 5.4	0.354
Renal parameters				
sCr (mg/dL)	0.91 ± 0.32	2.01 ± 1.93	0.89 ± 0.16	< 0.001
eGFR (mL/min/1.73 m^2)	95.0 ± 44.5	61.9 ± 37.4	98.3 ± 24.5	< 0.001

Data are expressed as the mean ± SD, median (interquartile range) - or frequency (proportion).
*** - Significant (p < 0.001), compared with healthy controls, NGAL - neutrophil gelatinase-associated lipocalin, BMI - body mass index, SBP - systolic blood pressure, TNF-α - tumor necrosis factor-α, IL-6 - interleukin-6, PCT - procalcitonin, hsCRP - high-sensitivity C-reactive protein, cESR - corrected erythrocyte sedimentation rate, ANC - absolute neutrophil count, sCr - serum creatinine, eGFR - estimated glomerular filtration rate.**

(> 150 ng/mL) was observed at hsCRP and sCr levels of 4.1 - 5.0 mg/dL and 1.01 - 1.20 mg/dL, respectively (Figure 2).

NGAL, grade of renal failure, and severity of inflammation

The grade of renal failure and severity of inflammation in patients with low NGAL levels are summarized in Table 3. Of the 30 patients with low NGAL levels, 26 (86.7%) had normal kidney function (grade 0) and 24 (80.0%) had low-grade inflammation. In contrast, among the 102 patients with high NGAL levels, 44 (43.1%) had normal kidney function and 26 (25.5%) had low-grade inflammation (p < 0.001) (Table 3).

Linear regression analysis

In the high NGAL group, plasma NGAL levels were significantly associated with TNF-α (r = 0.423), IL-6 (r = 0.409), PCT (r = 0.438), hsCRP (r = 0.362), ANC (r = 0.337), and sCr (r = 0.528) following adjustment for potential confounders. However, in the low NGAL group, there were no significant associations between plasma NGAL concentrations and the parameters, except for the ANC (r = 0.312, p < 0.001) (Table 4). The relationship of plasma NGAL levels versus clinical scores and inflammatory- and nutritional parameters was assessed. Plasma NGAL levels were significantly associated with hsCRP (r = 0.426), ANC (r = 0.346), and clinical score (r = 0.256) in high-grade inflamma-

Table 2. Plasma NGAL levels in relation to clinical score, kidney function, and severity of inflammation.

	Clinical score		Kidney function		Inflammation	
	Low (< 2.0; non-SIRS; n = 137)	High (≥ 2.0; SIRS; n = 54)	Without renal failure (n = 128)	With renal failure (n = 63)	Low-grade (n = 64)	High-grade (n = 127)
Age (year)	67 (53 - 77)	65 (51 - 74)	65 (49 - 74)	67 (59 - 77)	62 (45 - 78)	68 (57 - 75)
Clinical score	0.48 ± 0.45	2.50 ± 0.72 [a]	0.95 ± 1.02	1.27 ± 1.05 [b]	0.86 ± 1.02	1.15 ± 1.09 [c]
Inflammation index score	2.23 ± 0.79	2.50 ± 0.87	2.26 ± 0.79	2.55 ± 0.83 [b]	1.48 ± 0.08	2.80 ± 0.64 [c]
Body temperature (°C)	36.7 ± 0.7	37.4 ± 1.3 [a]	37.0 ± 0.9	36.8 ± 0.8	36.8 ± 0.9	36.9 ± 0.9
Heart rate (/minute)	82.3 ± 14.8	102.4 ± 22.0 [a]	87.8 ± 18.4	88.3 ± 21.2	86.4 ± 20.0	88.7 ± 19.1
Respiratory rate (/minute)	17.8 ± 1.6	23.3 ± 7.8 [a]	18.8 ± 3.5	20.5 ± 7.0 [b]	19.0 ± 4.1	19.6 ± 5.3
Systolic BP (mmHg)	129.9 ± 24.8	122.1 ± 31.8	131.5 ± 22.1	119.9 ± 34.1	127.5 ± 25.6	127.8 ± 28.0
Diastolic BP (mmHg)	78.7 ± 15.1	76.6 ± 20.8	80.6 ± 13.2	73.0 ± 21.9	77.9 ± 17.2	78.2 ± 16.8
Mortality (%)	4 (2.9)	12 (22.2) [a]	3 (2.3)	13 (20.6) [b]	1 (1.5)	15 (11.8) [c]
NGAL (ng/mL)	148.0 (84.0 - 316.5)	279.5 (115.7 - 843.2) [a]	127.5 (79.2 - 234.2)	475.0 (165.0 - 980.0) [b]	97.5 (65.5 - 143.0)	242.0 (125.0 - 542.0) [c]
Low NGAL (%)	23 (16.8)	7 (12.9)	26 (20.3)	4 (6.3) [b]	24 (37.5)	6 (4.7) [c]
High NGAL(%)	68 (49.6)	34 (62.9)	44 (34.4)	58 (92.1) [b]	26 (40.6)	76 (59.8) [c]
hsCRP (mg/dL)	2.92 (0.64 - 7.29)	6.23 (2.05 - 14.13) [a]	2.87 (0.67 - 7.30)	5.80 (1.43 - 13.26) [b]	0.72 (0.17 - 2.08)	7.13 (2.92 - 13.12) [c]
cESR (mm/hour)	29.8 ± 21.1	28.2 ± 23.1	27.9 ± 21.6	32.5 ± 21.5	12.1 ± 7.2	38.0 ± 21.3 [c]
ANC (x 10^9/L)	6.48 ± 3.68	11.42 ± 8.54 [a]	7.53 ± 4.21	8.60 ± 8.38	7.31 ± 4.41	8.17 ± 6.54
sCr (mg/dL)	1.42 ± 1.41	1.78 ± 1.73 [a]	0.91 ± 0.21	2.76 ± 2.14 [b]	1.29 ± 1.45	1.64 ± 1.53 [c]
eGFR (mL/minute/ 1.73 m^2)	82.5 ± 42.8	64.8 ± 33.2 [a]	98.0 ± 33.2	36.0 ± 16.9 [b]	85.3 ± 34.8	73.6 ± 43.4 [c]

Data are expressed as the mean ± SD, median (interquartile range), or frequency (proportion).
[a, b, c] - Significant (p < 0.05), compared with the corresponding groups (non-SIRS, without renal failure, and low-grade, respectively), SIRS - systemic inflammatory response syndrome, NGAL - neutrophil gelatinase-associated lipocalin, hsCRP - high-sensitivity C-reactive protein, cESR - corrected erythrocyte sedimentation rate, ANC - absolute neutrophil count, sCr - serum creatinine, eGFR - estimated glomerular filtration rate.

tion; however, no significant association was observed in low-grade inflammation. Serum albumin and total cholesterol levels were inversely correlated with plasma NGAL levels in high-grade inflammation (Table 5).

Neutropenia as a risk factor for low NGAL level

In a multivariate logistic regression analysis, neutropenia was significantly associated with low plasma NGAL level (odds ratio, 1.32; 95% CI, 1.05 - 3.14; p < 0.001). However, a serum albumin level of < 3.5 g/dL and a total cholesterol level of < 120 mg/dL were not significantly associated with low NGAL concentration (Table 6).

ROC curve analysis

The diagnostic efficacy of TNF-α, PCT, and ANC for identifying low plasma NGAL levels in patients with systemic inflammation was investigated. In ROC curve analysis, the area under the curve (AUC) of the ANC was significantly larger than that of PCT and TNF-α [0.82 (95% CI, 0.75 - 0.89) versus 0.67 (95% CI, 0.56 - 0.79) and 0.66 (95% CI, 0.54 - 0.78), respectively, p < 0.001] (Figure 3). Additionally, the diagnostic abilities of NGAL, ANC, hsCRP, and sCr for predicting high clinical scores (≥ 2.0; SIRS) were evaluated. The diagnostic value of ANC was superior to that of NGAL, hsCRP, and sCr [0.71 (95% CI, 0.61 - 0.82) versus 0.63 (95% CI, 0.54 - 0.73), 0.65 (95% CI, 0.56 - 0.74), and 0.64 (95% CI, 0.55 - 0.72), respectively, p < 0.001] (Figure 4).

Table 3. Kidney function and severity of inflammation of patients with low and high NGAL levels.

	Patients with inflammation		
	Low NGAL group (n = 30)	High NGAL group (n = 102)	p-value
Grade of renal failure (sCr; mg/dL)			
Grade 0 (< 1.20)	26 (86.7)	44 (43.1)	< 0.001
Grade 1 (1.20 - 1.99)	4 (13.3)	31 (30.4)	0.032
> Grade 2 (2.00 - 3.49)	0 (0.0)	27 (26.5)	< 0.001
Severity of inflammation (inflammation index)			
Low-grade inflammation (1.0 - < 2.5)	24 (80.0)	26 (25.5)	< 0.001
High-grade inflammation (2.5 - 4.0)	6 (20.0)	76 (74.5)	< 0.001

Data are expressed as the frequency (percentage). NGAL - neutrophil gelatinase-associated lipocalin, sCr - serum creatinine.

Table 4. Association of plasma NGAL level with inflammatory and nutritional parameters in patients with low and high NGAL levels.

	Univariate		Multivariate *	
	High NGAL group	Low NGAL group	High NGAL group	Low NGAL group
Inflammatory markers				
TNF-α (pg/mL)	0.457 (< 0.001)	0.197 (0.214)	0.423 (< 0.001)	0.168 (0.395)
IL-6 (pg/mL)	0.423 (0.005)	0.186 (0.257)	0.409 (< 0.001)	0.171 (0.343)
PCT (ng/mL)	0.469 (< 0.001)	0.175 (0.291)	0.438 (< 0.001)	0.143 (0.471)
hsCRP (mg/dL)	0.391 (< 0.001)	0.150 (0.428)	0.362 (< 0.001)	0.107 (0.615)
ANC (x 10^9/L)	0.375 (< 0.001)	0.341 (< 0.001)	0.337 (< 0.001)	0.312 (< 0.001)
Nutritional parameters				
Serum albumin (g/dL)	-0.425 (< 0.001)	-0.205 (0.261)	-0.404 (< 0.001)	-0.194 (0.278)
Total cholesterol (mg/dL)	-0.290 (< 0.001)	-0.159 (0.175)	-0.281 (0.001)	-0.146 (0.183)
Body fat mass (kg)	0.154 (0.428)	0.142 (0.492)	0.126 (0.532)	0.115 (0.602)
Renal parameters				
sCr (mg/dL)	0.591 (< 0.001)	0.134 (0.506)	0.528 (< 0.001)	0.090 (0.635)
eGFR (mL/minute/1.73 m^2)	-0.548 (< 0.001)	0.216 (0.252)	-0.532 (< 0.001)	0.186 (0.365)
Clinical score	0.327 (< 0.001)	0.192 (0.288)	0.314 (0.004)	0.038 (0.875)

Data are expressed as standardized β (p values).
*** - Adjusted for age, gender, BMI, and systolic blood pressure, NGAL - neutrophil gelatinase-associated lipocalin, TNF-α - tumor necrosis factor-α, IL-6 - interleukin-6, PCT - procalcitonin, hsCRP - high-sensitivity C-reactive protein, ANC - absolute neutrophil count, sCr - serum creatinine, eGFR - estimated glomerular filtration rate.**

DISCUSSION

In this study, patients with low plasma NGAL levels were assessed by measuring TNF-α, IL-6, PCT, ANC, nutritional parameters, and renal function. The patients were stratified into subgroups according to the degree of renal failure, severity of inflammation, and clinical score. The ANC was more closely associated with a decreased NGAL level than proinflammatory cytokines, PCT, and nutritional parameters. This study demonstrated that patients with low NGAL levels were characterized by low-grade inflammation, neutropenia, and normal or subnormal kidney function.

Plasma NGAL concentration is elevated under certain

Table 5. Relationship of plasma NGAL level versus inflammatory and nutritional parameters in relation to kidney function and severity of inflammation.

	Kidney function *		Inflammation *	
	Without renal failure (n = 128)	With renal failure (n = 63)	Low-grade (n = 64)	High-grade (n = 127)
hsCRP (mg/dL)	0.558 (< 0.001)	0.432 (0.002)	0.035 (0.825)	0.426 (< 0.001)
cESR (mm/h)	0.553 (< 0.001)	0.322 (0.028)	0.175 (0.341)	0.351 (0.001)
ANC (x 10^9/L)	0.354 (< 0.001)	0.293 (0.032)	0.016 (0.920)	0.346 (< 0.001)
Serum albumin (g/dL)	-0.554 (< 0.001)	-0.389 (0.005)	-0.318 (0.075)	-0.359 (0.001)
Total cholesterol (mg/dL)	-0.245 (0.023)	-0.345 (0.021)	-0.275 (0.084)	-0.285 (0.007)
sCr (mg/dL)	0.269 (0.014)	0.610 (< 0.001)	0.878 (< 0.001)	0.573 (< 0.001)
eGFR (mL/minute/1.73 m^2)	-0.211 (0.075)	-0.607 (< 0.001)	-0.737 (< 0.001)	-0.527 (< 0.001)
Clinical score	0.438 (< 0.001)	0.060 (0.680)	0.167 (0.281)	0.256 (0.008)

*** - Data are expressed as standardized β (p values) after adjusting for age, gender, BMI, and systolic blood pressure.**
hsCRP - high-sensitivity C-reactive protein, cESR - corrected erythrocyte sedimentation rate, ANC - absolute neutrophil count, sCr - serum creatinine, eGFR - estimated glomerular filtration rate.

Table 6. Multivariate logistic regression analysis of the association of low NGAL level with inflammatory and nutritional parameters.

	Odds ratio (95% CI)			
Low NGAL level (< 68 ng/mL)	Unadjusted	p-value	Adjusted *	p-value
hsCRP < 0.45 mg/dL	2.15 (1.01 - 6.14)	< 0.001	1.01 (0.89 - 2.45)	0.105
ANC < 1.5 x 10^9/L	2.61 (1.12 - 5.06)	< 0.001	1.32 (1.05 - 3.14)	< 0.001
Serum albumin < 3.5 g/dL	0.86 (0.76 - 1.29)	0.128	0.93 (0.71 - 1.35)	0.157
Total cholesterol < 120 mg/dL	0.94 (0.74 - 1.32)	0.149	0.98 (0.85 - 1.42)	0.161

*** - Adjusted for age, gender, BMI, and systolic blood pressure.**
NGAL - neutrophil gelatinase-associated lipocalin, hsCRP - high-sensitivity C-reactive protein, ANC - absolute neutrophil count, CI - confidence interval.

inflammatory conditions. Some studies reported that NGAL is a novel biomarker for inflammatory diseases because NGAL is secreted by activated neutrophils during inflammation [26]. However, in our study, approximately 46.6% of inflamed patients did not have elevated plasma NGAL levels. Furthermore, in comparison with healthy individuals, 15.7% of patients with systemic inflammation had lower plasma NGAL levels. Interestingly, a well-known inflammatory marker, hsCRP, was 21.8 times higher in patients with low NGAL levels than in healthy subjects. These results suggest that plasma NGAL concentration may not be elevated in parallel with the severity of inflammation in a substantial number of inflamed patients.

Imamaki et al. [27] reported that a decreased NGAL level could be associated with current nutritional status and could play a role as a marker of malnutrition in patients under hemodialysis, which was based on lower levels of nutritional markers including serum albumin and serum total cholesterol. However, in our study, there was no evidence of a significant decrease in nutritional parameters among patients with low NGAL levels. On the other hand, in comparison with patients with high NGAL levels, patients with low NGAL levels had elevated serum albumin and total cholesterol levels. Both serum albumin and total cholesterol levels were inversely correlated with plasma NGAL concentrations. A possible reason can explain the difference in the association between NGAL and nutritional parameters. For instance, a downward shift of nutritional status can occur in proportion to aggravation of clinical signs, particularly in critically ill patients. Additionally, these discrepancies may reflect a difference in the subject population, extent of inflammation, and impaired renal function among studies. Imamaki et al. [27] measured serum NGAL level in patients with chronic renal failure,

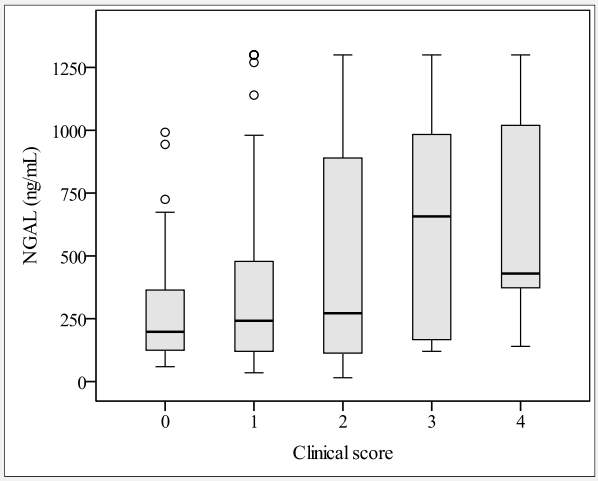

Figure 1. Plasma NGAL levels according to clinical scores in patients with systemic inflammation.

There was no significant difference in median plasma NGAL levels between subjects with clinical score 0 and 2. However, median plasma NGAL levels were significantly elevated in subjects with clinical score 3.

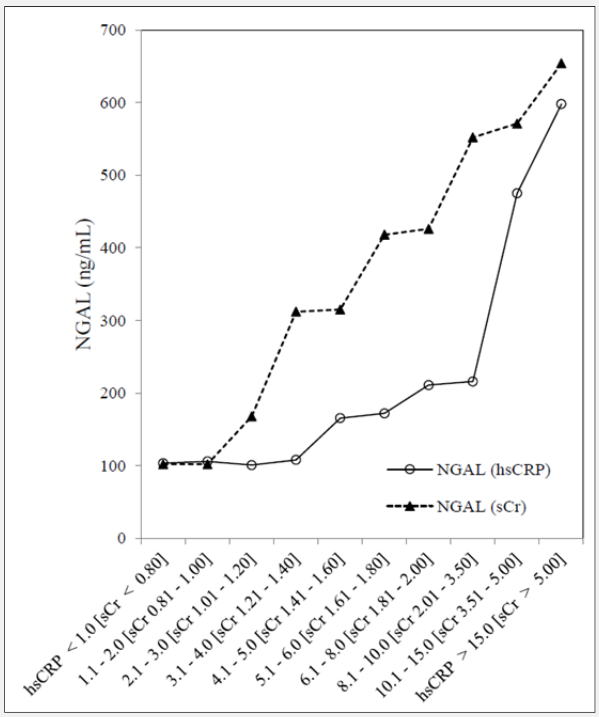

Figure 2. Plasma NGAL levels in relation to the concentrations of hsCRP and sCr in patients with inflammation.

No significant increase in plasma NGAL levels was observed until hsCRP was 4.0 mg/dL. An elevated NGAL level (> 150 ng/mL) was observed at hsCRP and sCr levels of 4.1 - 5.0 mg/dL and 1.01 - 1.20 mg/dL, respectively.

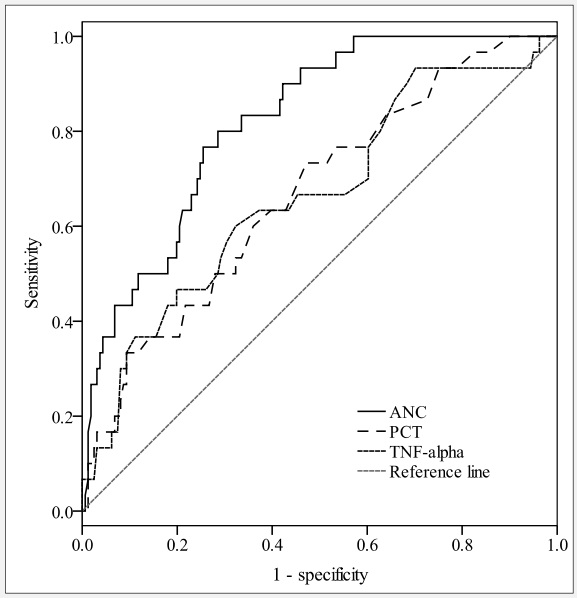

Figure 3. ROC curve analysis of ANC, PCT, and TNF-α for identifying low plasma NGAL levels (< 68 ng/mL) in patients with systemic inflammation.

The AUC of ANC was significantly larger than that of PCT and TNF-α [0.82 (95% CI, 0.75 - 0.89) versus 0.67 (95% CI, 0.56 - 0.79) and 0.66 (95% CI, 0.54 - 0.78), respectively, p < 0.001].

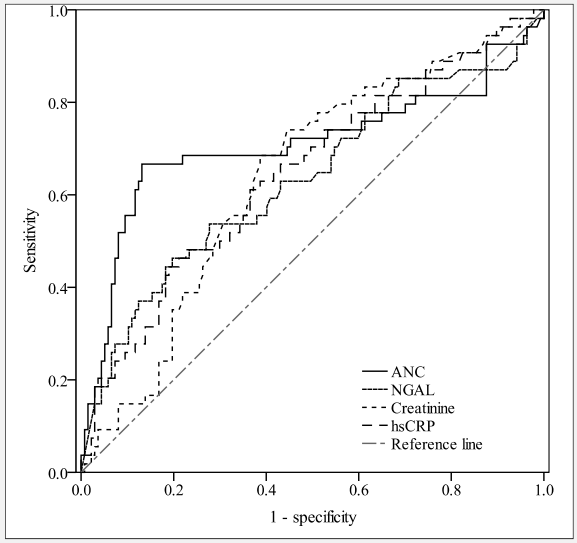

Figure 4. The diagnostic abilities of NGAL, ANC, hsCRP, and sCr for predicting high clinical scores (≥ 2.0; SIRS) among subject populations.

The diagnostic efficacy of ANC was superior to that of NGAL, hsCRP, and sCr [0.71 (95% CI, 0.61 - 0.82) versus 0.63 (95% CI, 0.54 - 0.73), 0.65 (95% CI, 0.56 - 0.74), and 0.64 (95% CI, 0.55 - 0.72), respectively, p < 0.001].

whereas the present study measured serum NGAL level in patients with systemic inflammation.

NGAL was originally identified as a novel protein isolated from the secondary granules of human neutrophils [28]. NGAL is constitutively synthesized during a narrow window of granulocyte maturation in the bone marrow, almost exclusively in myelocytes and metamyelocytes [29]. Landrø et al. [30] reported the strong correlation of plasma NGAL levels with neutrophil counts in infected patients. In our study, neutropenia was observed 8.7 times more in patients with low NGAL levels than in those with elevated NGAL levels. To assess the potential role of a decreased neutrophil count as a risk factor for low plasma NGAL level, multivariate logistic regression analysis was performed. Based on the odds ratio, the presence of neutropenia resulted in a 1.32 times increase in the risk for low NGAL level. Therefore, neutropenia could play a crucial role in decreased plasma NGAL concentration in patients with inflammation. In our study, low plasma NGAL level was linked to non-increment of ANC, suggesting that low NGAL level may be more attributable to bone marrow failure than kidney or nutritional status. Synthesis of NGAL from granulocytic precursors in bone marrow seems to be disturbed in some patients with systemic inflammation, which is responsible for low NGAL level. To determine which parameter (TNF-α, IL-6, PCT, hsCRP, ANC, and nutritional status) is more closely linked to plasma NGAL level, the patients were categorized into low and high NGAL groups. In the high NGAL group, a significant association was observed between plasma NGAL concentration and the levels of proinflammatory cytokines, PCT, hsCRP, and sCr. However, in the low NGAL group, no significant association was observed between the parameters. Only the ANC was significantly associated with plasma NGAL concentration in the low NGAL group. Additionally, the diagnostic ability of TNF-α, PCT, and ANC for identifying low NGAL levels was investigated by ROC curve analysis. ANC had better performance for detecting low plasma NGAL levels than did PCT and TNF-α. This is presumably because activated neutrophils are directly associated with secretion of NGAL as a main production site. It seems more likely that PCT and TNF-α act as a potentiator to increase NGAL with other proinflammatory cytokines.

Inflammation is a known risk factor for decreased kidney function [31]. Systemic inflammation contributes to the development of acute kidney injury via leukocytic infiltration, thrombosis, hypoperfusion, and apoptosis [32,33]. On the other hand, inflammation commonly occurs in patients with renal failure because uremia can contribute to the inflammatory process by stimulating proinflammatory cytokines [34]. In this study, the levels of proinflammatory cytokines and PCT were higher in patients with low NGAL levels than in healthy individuals. However, there were no significant differences in kidney function and neutrophil counts between the groups. In particular, normal kidney function was observed in 86.7% of patients with low NGAL levels compared with 43.1% of patients with high NGAL levels. Additionally, 80% of patients with low NGAL levels had low-grade inflammation. These findings suggest the development of an unexpectedly low plasma NGAL level when systemic inflammation is accompanied by neutropenia, particularly in patients with preserved renal function and low-grade inflammation.

PCT is secreted by a variety of tissues in response to bacterial endotoxin in the bloodstream. PCT is detected in the blood within 2 - 4 hours of infection, and reaches a peak 8 - 24 hours after infection, whereas hsCRP takes 12 - 24 hours to increase and remains elevated for up to 3 - 7 days [35]. In our study, elevated serum PCT levels in the low NGAL group were similar to those in the high NGAL group. However, hsCRP levels in the low NGAL group were significantly lower than those in the high NGAL group. A possible explanation for the results of PCT and hsCRP in the low NGAL group is that PCT may increase earlier than hsCRP. Furthermore, in our study, no significant increase in plasma NGAL concentration was observed until hsCRP was 4.0 mg/dL. The results suggest that active NGAL production occurs only when inflammation is moderately advanced. It is possible that patients with low plasma NGAL levels are in the early phase of inflammation prior to active NGAL production.

In the current study, NGAL did not accurately reflect clinical scores and inflammation status, particularly in low-grade inflammation and in patients with renal failure, although it was a potential indicator in high-grade inflammation and in subjects with normal kidney function. A ROC curve analysis revealed that NGAL does not surpass the diagnostic accuracy of eGFR, ANC, and inflammation index score for predicting SIRS. Thus, the clinical relevance of NGAL as an inflammatory marker appears to be limited according to the intensity of inflammation or kidney function. We stress that NGAL should be used with caution as an indicator for screening patients with low-grade inflammation or patients with leukopenia, which is not infrequently encountered in the diverse clinical settings of severe sepsis or septic shock.

This study has several limitations. We did not measure plasma NGAL level in serial samples to assess the changes in NGAL in association with the progression of clinical status. The cross-sectional analysis of our study limited the demonstration of a cause-and-effect relationship. As in any observational study, there may be unmeasured confounders for which we did not adjust during statistical analysis. Despite these limitations, this is the first study to investigate the significance of low plasma NGAL level in systemic inflammation. However, the present findings obtained in a single-center uncontrolled study may need to be validated in larger randomized prospective trials.

CONCLUSION

This study demonstrated that low plasma NGAL level in systemic inflammation was linked to a decrease in neutrophil counts. The ANC exhibited better diagnostic performance than PCT and TNF-α for identifying low plasma NGAL levels. These results suggest that an unexpectedly low plasma NGAL level in systemic inflammation may be attributed to the non-increment of circulating neutrophils in the early phase of inflammation, particularly in patients with low-grade inflammation who had preserved kidney function.

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Declaration of Interest:
The authors declare that they have no conflicts of interest.

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