Prediction of Acute Kidney Injury Development in Critically Ill Septic Patients Based on NGAL Determination

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Summary

Acute kidney injury (AKI) is a consequence of several diseases that can severely damage kidney function. It is a frequent complication of hospitalization and very common in critically ill patients because of other serious illnesses, such as septic conditions. New diagnostic options, including renal biomarkers, may help in early diagnosis. Our study included 46 patients, 31 with AKI and 15 without AKI on admission. Blood samples were collected on the first, fourth, and seventh days of treatment, and in addition to basic biochemical parameters, neutrophil gelatinase-associated lipocalin (NGAL) levels in serum were also examined. Data from the first and seventh day were used for statistical analysis. On the first follow-up day, NGAL values were categorized according to an optimal cut-off value of 290 μg/l. We demonstrated that if NGAL levels were higher, the risk of renal injury increased approximately 16 times. Other results showed that NGAL levels were moderately correlated with serum creatinine ($r = 0.613$, $p < 0.0001$), procalcitonin (PCT) ($r = 0.627$, $p < 0.0001$), and AKI stage ($r = 0.589$, $p < 0.0001$). There was also a significant positive correlation with SOFA (Sequential Organ Failure Assessment) score ($r = 0.395$, $p = 0.007$). Early diagnosis and treatment are crucial in managing AKI and preventing further kidney damage. NGAL levels can increase within a few hours after injury, making it a useful tool for early AKI detection and diagnosis.

Key words

Acute kidney injury • Biomarker • NGAL • Sepsis

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Introduction

Acute kidney injury is a sudden and rapid decline in kidney function, which can occur from a few hours to a few days. It is a common and severe condition associated with increased morbidity and mortality, especially in critically ill patients with sepsis. The previously reported incidence of acute kidney injury (AKI) is up to 15% in hospitalized patients and more than 50% in intensive care units [1]. Increased AKI severity is associated with increased mortality, and many patients with AKI have residual renal dysfunction when discharged from hospital.[2].

Septic AKI is common during the first 24 hours after intensive care unit (ICU) admission, and is independently associated with a higher risk of death and longer hospital stays. Approximately 40% of critically ill hospitalized patients in ICUs with acute kidney injury have sepsis or septic shock. This is due to the greater severity of illness in patients with septic AKI compared to patients with non-septic AKI [3]. Many factors can contribute to AKI and its progression to renal failure, including cardiovascular and hepatic disorders, malignancies, hypovolemia, intoxication, drug nephrotoxicity, anemia, surgical and vascular interventions, and the administration of nephrotoxic iodine contrast agents for radiological examinations. Consequently, AKI is often both a cause and a consequence of multiple factors. Early recognition of AKI is difficult, but is essential in clinical practice, especially in the ICU.

AKI classification is based on two markers of kidney dysfunction: increased serum creatinine (S_{crea}) and decreased urine output, as defined by the Kidney Disease Improving Global Outcome (KDIGO) association in 2012 [4]. The greatest limitations of this determination are the early diagnosis of AKI, differential diagnosis, and prognostic evaluation with adequate medical management, particularly in critically ill patients with sepsis. The most promising markers for AKI development include Cystatin C, Interleukin 18 (IL-18), Kidney Injury Molecule-1 (KIM-1), urinary tissue inhibitor of metalloproteinase 2 (TIMP-2), urinary insulin-like growth factor-binding protein-7 (IGFBP-7), calprotectin, urine angiotensinogen, and neutrophil gelatinase-associated lipocalin (NGAL) [5]. According to a meta-analysis of 63 studies involving 15,928 critically ill patients, the best evidence for the prediction of renal replacement therapy in acute kidney injury was from blood NGAL and Cystatin C, followed by urinary TIMP-2 and IGFBP-7 [6].

In our clinical study, we focused on NGAL, an acute-phase protein with a molecular weight of 25 kDa, involved in antibacterial immunity, the main source of which is neutrophils [7]. Recent evidence suggests that NGAL levels are closely associated with AKI. It is expressed in the tubular epithelial cells. When these cells are acutely injured, NGAL levels increase several times [8]. Some clinical and experimental studies have shown that the expression of urine and serum NGAL is considerably elevated in AKI, and that NGAL levels can be detected earlier than other AKI markers, meaning that it may be a promising new early AKI biochemical marker[9]. In a study by Chang et al., it was also a relatively reliable predictor of 28-day mortality and possibly outperformed creatinine in predicting the need for continuous renal replacement therapy [10]. Serum NGAL levels can also help in the assessment of sepsis severity, and appears to be an independent risk factor for 28-day mortality. Levels of this biomarker in critically ill patients with acute kidney injury in sepsis were significantly higher ($p<0.05$) than in septic patients without AKI [11]. It is clear from the text above that NGAL is a promising biomarker for the early detection and risk stratification of AKI, offering potential benefits over traditional markers like serum creatinine. However, the clinical implementation of NGAL is limited by the absence of standardized assays and reference ranges, as well as by inconsistencies in cut-off values across studies.

The first aim of our study was to determine whether NGAL is an appropriate predictor of AKI development with establishment an optimal cut-off value in critically ill patients with sepsis. The second aim was to determine the possible association between NGAL and inflammatory markers (PCT, CRP, and IL-6) and $S_{crea,}$ albumin level, SOFA (Sequential Organ Failure Assessment) score, and AKI stage.

Materials and Methods

Data source and subjects collection

For our prospective open clinical study, we used the data of critically ill adult patients with sepsis hospitalized in intermediate care or general intensive care units at University Hospitals of Ostrava and Olomouc, Czech Republic in 2019 and 2020. Of the 53 patients initially included in the study, we used data from 46 patients with complete values for statistical analysis. All patients with sepsis (defined below) were treated with wide-spectrum antimicrobial therapy and divided into two groups according to AKI presence (defined below). Patients enrolled in the chronic dialysis program, those with stage 4 or 5 chronic kidney disease, or those concurrently receiving other potentially nephrotoxic medications (such as cisplatin or colistin) or a combination of vancomycin and gentamicin were excluded. The initiation of acute intermittent hemodialysis or continuous renal replacement therapy was not an indication for exclusion. For this study, we obtained official ethical approval and written informed consent from all patients involved in the study.

Definition of acute kidney injury

Acute kidney injury is generally defined as a sudden deterioration of renal function associated with a decrease in glomerular filtration rate and is characterized by the accumulation of end products of nitrogen metabolism, decreased urine output, or both. AKI was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) 2012 classification as any of the following: Increase in S_{crea} by ≥ 0.3 mg/dl (≥ 26.5 μmol/l) within 48 hours; increase in S_{crea} to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; urine volume < 0.5 ml/kg/h for 6 hours. Based on severity, we classified AKI into three grades. Stage 1 AKI is defined as an increase in S_{crea} 1.5–1.9 times baseline or ≥0.3 mg/ dl (≥26.5 mmol/l) and/or a decrease in urine output <0.5ml/kg/hour for 6–12h. Stage 2 is defined as an increase in S_{crea} 2.0–2.9 times baseline and/or a decrease in urine output <0.5ml/kg/hour for ≥12h. Stage 3 AKI has been defined as an increase in S_{crea} of 3.0 times baseline or≥ 4 mg/dl (≥353.6μmol/l) or initiation of RRT and/or a decrease in urine output <0.3 ml/kg/hour for ≥24 h or anuria for ≥12 h [4].

Definition of sepsis

Sepsis is a syndrome of physiological, pathological, and biochemical abnormalities induced by infection, which can lead to shock, organ failure and death. It is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection [12]. The severity of organ damage was assessed using the Sequential Organ Failure Assessment (SOFA) scoring system, with a score ≥2 representing organ dysfunction. QuickSOFA (qSOFA) was used as a simplified tool to rapidly identify patients with suspected infection who are at greater risk for a poor outcome. It is derived from the more comprehensive SOFA score but is designed to be quicker and easier to use. The clinical findings included the presence of infection (SIRS), systolic blood pressure ≤ 100 mmHg, respiratory rate >22/min, altered mental status. Additional data were available for a more detailed assessment (PaO_2/FiO_2, platelet count, serum bilirubin level, mean arterial blood pressure or need for vasopressors, Glasgow Coma Scale Score, serum creatinine level, and diuresis).

Sample collection (Determination of NGAL, IL-6, PCT and Screa)

Whole blood samples were taken on the first, fourth, and seventh days of the sepsis diagnosis and antimicrobial treatment. Blood samples for IL-6, PCT, and S_{crea} were collected concomitantly with other standard care biochemical parameters (Vacuette® serum-gel, 5.0ml, red, Greiner Bio-One GmbH, Germany in University Hospital Olomouc, and S-Monovette® serum-gel, 4.9 ml, brown (Sarstedt AG & Co. KG, Germany in University Hospital Ostrava) and analyzed immediately after centrifugation at both hospitals.

Simultaneously, the blood samples for NGAL determination were collected into 2.6 mL neutral tubes (S-Monovette® K3 EDTA, 2.6 ml, red, Sarstedt AG & Co. KG, Germany; or Vacuette® K3 EDTA 2 ml, violet, Greiner Bio-One GmbH, Germany) and centrifuged. After centrifugation, plasma was aspirated, collected into cryotubes, and frozen at −70°C until use. Serum NGAL concentrations were determined in all patients using the immunoturbidimetric method. Bioporto NGAL Kit was used. The inter-assay variation coefficient for NGAL determination was lower than 5%.

Serum concentrations of IL-6 and PCT were determined at the University Hospital Olomouc using electrochemiluminescent immunoassays (IL-6 reagent kit, Cat. No. 05109442, Cobas 8000, Roche Diagnostics; Elecsys® BRAHMS PCT reagent kit Cat. No. 08828644, Cobas 8000, Roche Diagnostics, GmbH, Sandhofer Strasse 116, D-68305 Manheim, Germany). At University Hospital Ostrava, the ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) was used with coefficients of variation of 4.7 and 4.6%, respectively.

At the University Hospital Olomouc, serum creatinine was assessed using mass absorption spectrometry (Cobas 8000, CREP2, number 05168589 190, Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Manheim). This method is based on measuring the mass-to-charge ratio (m/z) of ions. A sample is ionized, and the ions are then separated and detected based on their m/z ratios. The AU 5820 (Beckman Coulter, Inc., Brea, CA, USA) enzyme spectrophotometry method was used at the University Hospital Ostrava. It is based on measuring the activity of enzymes by detecting changes in absorbance of a specific wavelength of light, which corresponds to the concentration of a product or substrate in an enzymatic reaction.

Adequate microbiological methods were performed to determine the cause of sepsis with sample collection for examination. Each method is selected based on the clinical presentation, suspected pathogen, sample type, and the need for speed and accuracy in diagnosis. The

most common was culture-based techniques like blood, urine, and sputum cultures to grow and identify pathogens, also molecular methods such PCR was used.

Statistical analysis

The quantitative variables are presented as medians, minimum and maximum values, means, and standard deviations (SD). Shapiro-Wilk normality tests were used to verify that the quantitative variables did not have a normal distribution. Friedman's post-hoc test with Bonferroni correction was used to compare three dependent samples. For statistical analysis was also used Fisher's exact test, Mann-Whitney U test and Wilcoxon test. The correlation between the variables was verified using Spearman's correlation coefficient. Receiver operating characteristic (ROC) analysis was used to determine the optimal cut-off value. Logistic regression was used to calculate the so-called unadjusted odds ratios (ORs) - the dependent variable in the logistic regression was AKI and the independent predictor was NGAL. Qualitative data were described using absolute and relative frequencies. All tests were performed at a significance level of 0.05. IBM SPSS Statistics for Windows, Version 23.0 Armonk, NY, USA) was used for statistical processing.

Results

The basic demographic and clinical data of the study participants are summarized in Table 1. There were

Table 1. Demographic and clinical characteristics of the patients

	AKI on admission	non-AKI on admission	P value
N (%)	31 (67.4)	15 (32.6)	
- *AKI stage 1*	10 (21.7)	-	
- *AKI stage 2*	11 (23.9)	-	
- *AKI stage 3*	10 (21.7)	-	
Male n (%)	20 (64.5)	10 (66.6)	1.000[a]
Age n (years)	65 (24-84)	63 (25-80)	0.815[b]
BMI kg/m^2	26.0 (19.8-52.0)	29.3(19.5-41.5)	0.276[b]
SOFA	8 (3-17)	5 (2-13)	0.016[b]
RRT initiation n (%)	14 (45.2)	0 (0)	0.002[a]
Use of vasopressors n (%)	23 (74.2)	9 (60)	0.495[a]
ICU hospitalized patients n (%)	27 (87.1)	12 (80)	0.667[a]
ICU length of stay (days)	12 (0-42)	12 (0-52)	0.944[b]
Intermediate care hospitalized patients n (%)	4 (12.9)	3 (20)	0.667[a]
Intermediate care length of stay (days)	8 (0-56)	12 (0-50)	0.121[b]
Total length of hospitalization (days)	20 (10-62)	31 (12-62)	0.108[b]
Antibiotic therapy			
- *Vancomycin*	3 (9.7)	4 (26.7)	0.193[a]
- *Gentamicin*	12 (38.7)	8 (53.3)	0.527[a]
- *Other ATB*	16 (51,6)	3 (20)	0.058[a]
Days of ATB treatment	9 (4-20)	12 (0-50)	0.328[b]
Diagnoses, all n (%)			
Urogenital infection	7 (22.6)	3 (20)	1.000[a]
Pneumonia	6 (19.4)	2 (13,3)	1.000[a]
Sepsis of unknown origin	2 (6.5)	2 (13.3)	0.587[a]
GIT infection	10 (32.3)	2 (13.3)	0.285[a]
ORL infection	1 (3.2)	2 (13.3)	0.244[a]
Cardiac infection	2 (6.5)	0 (0)	1.000[a]
Orthopedics infection	3 (9.7)	2 (13.3)	1.000[a]
Other infection	0 (0)	2 (13.3)	0.101[a]

Abbreviations: AKI, acute kidney injury; BMI, body mass index; ICU intensive care unit, RRT renal replacement therapy; SOFA, Sequential Organ Failure Assessment; ATB, antibiotics; GIT, gastrointestinal tract; ORL Otorhinolaryngology. Quantitative variables are presented as medians and minimum-maximum ranges. Qualitative variables were presented as counts and percentages. P-values are from [a]Fisher's exact test; [b]Mann-Whitney U test.

no statistically significant differences between the two groups in age, sex, body mass index, SOFA score, or use of vasopressors. Furthermore, there was no statistical difference in ICU length of stay, length of hospitalization, or clinical outcomes between the defined groups of patients. We detected a statistically significant difference between the groups of patients who need of renal replacement therapy. All patients were treated with wide-spectrum antimicrobial treatment, which was initiated empirically according to the predisposed infection and changed when needed after receiving microbial results. In the AKI group we began with vancomycin in 9.7% (N=3), gentamicin in 38.7% (N=12), and other antibiotics were administered in 51.6% (N=16). In the group of patients without AKI, we initiated therapy with vancomycin in 26.7% (N=4), gentamicin in 53.3% (N=8), and other antibiotics in 20% (N=3). Therapeutic drug monitoring has been used to maintain optimal serum concentrations in all patients receiving potentially nephrotoxic therapies, such as vancomycin or gentamicin. The median values for vancomycin was C_{min} = 10.1 mg/l, and gentamicin it was C_{max} 14.4 mg/l with $C_{min,}$ 0,65 mg/l. Very detailed therapeutic drug monitoring has been done, so none of our patients had a nephrotoxic range of antibiotic.

In our patients, some changes in the AKI stage were also detected between days 1 and 7 (Fig. 1). On day 7, we observed more patients without AKI; however, there was also a noticeable increase in the number of cases of the most severe kidney injury. The p values were evaluated using the Wilcoxon test. According to the results, there was no statistically significant difference in the AKI stage of acute kidney injury between days 1 and 7.

Biochemical parameters of renal injury and inflammation during the observation period are shown in Table 2. We compared patients with and without AKI. There was a statistically significant decrease in the time delay for serum creatinine, urea, NGAL, and procalcitonin.

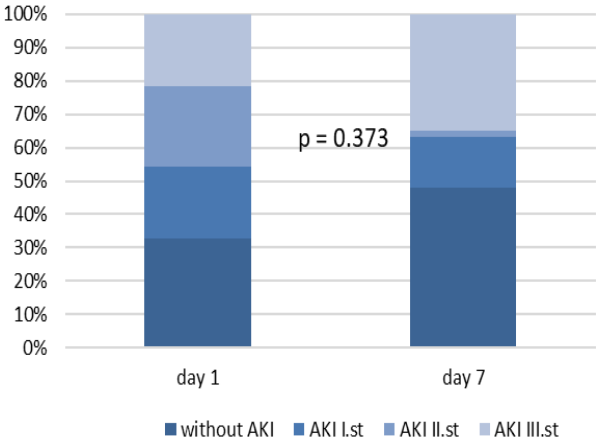

Fig. 1. Stages of AKI on the day 1 and day 7. P-value is from Wilcoxon test.
Abbreviations – AKI – acute kidney injury, st- stage

Table 2. Biochemical parameters of renal injury and inflammation

Biochemical parameter	AKI		non- AKI		
	Median	Range	Median	Range	p – value
S_{crea} (µmol/l) day 1	162.0	85.0-796.0	72.0	43.0-114.0	<0.0001
S_{crea} (µmol/l) day 7	143.0	58.0-547.0	58.5	30.0-85.0	<0.0001
urea (mmol/l) day 1	12.8	4.7-37.2	6.8	3.7-18.1	0.0028
urea (mmol/l) day 7	12.0	5.3-32.7	6.1	2.2-14.5	<0.0001
NGAL (µg/l) day 1	566.5	47.4-1429.7	140.2	47.4-732.3	0.0002
NGAL (µg/l) day 7	447.9	44.3-3184.0	133.0	17.9-608.4	0.0002
CRP (mg/l) day 1	245.5	8.9-513.0	176.5	31.3-413.3	0.015
CRP (mg/l) day 7	111.2	13.0-296.9	84.3	17.2-204.5	0.496
PCT (µg/l) day 1	5.92	0,60-100	0.46	0.14-12.50	<0.0001
PCT (µg/l) day 7	1.24	0.08-10.1	0.18	0.03-1.45	0.0003
IL-6 (ng/l) day 1	236.0	15.1-9592	92.0	12.2-1209.8	0.113
IL-6 ng/l) day 7	86.1	3.8-5500	26.9	10.2-869.9	0.275

P-values were derived using the Mann–Whitney U-test. Data are presented as median, minimum, and maximum values. Abbreviations: AKI, acute kidney injury; IL-6, interleukin 6; min, minimum value; max, maximum value; NGAL, neutrophil gelatinase–associated lipocalin; PCT, procalcitonin; CRP, C-reactive protein; S_{crea}, serum creatinine.

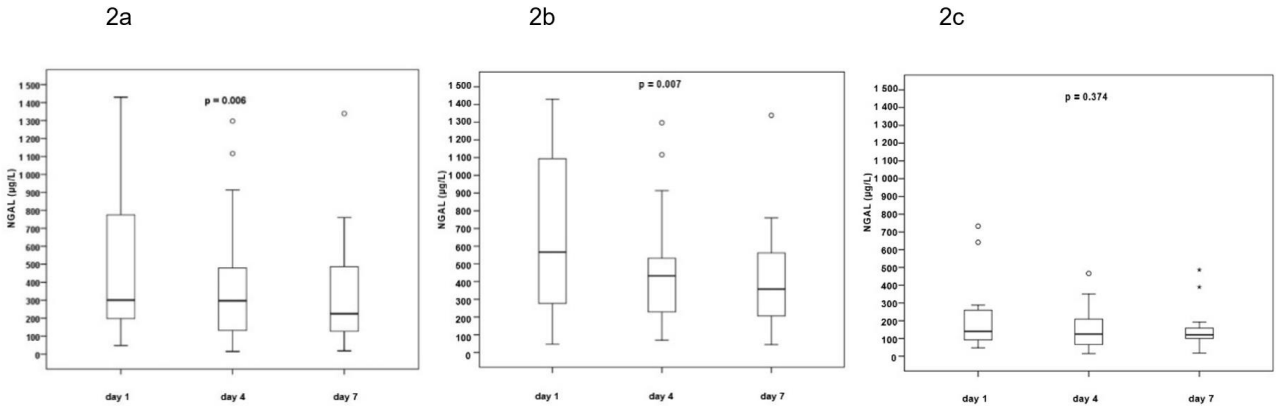

Fig. 2. Evaluation of NGAL over time. On the graphical presentation: a) serum NGAL concentrations in whole group of patients b) serum NGAL concentrations in patients with AKI on admission c) serum NGAL concentrations in patients without AKI on admission. The distribution of measured values was shown by a box plot. Data are presented as median and range. P values are from Friedman's test and post hoc analysis by Bonferroni correction

Table 3. Odds ratio for AKI prediction on day 1 according to NGAL serum concentration

NGAL (µg/l)	OR	CI	P value
> 290	15.9	3.0 – 85.1	0.001
> 240	11.5	2.7 – 48.9	0.001

Abbreviations: CI, confidence interval; NGAL, Neutrophil Gelatinase-associated Lipocalin, OR - Odds ratio; P value, logistic regression

The serum NGAL concentrations significantly changed in the entire group of patients (Fig. 2a). Friedman's test revealed statistically significant changes in NGAL levels over time (p = 0.006). Subsequently, post hoc test analysis with Bonferroni correction showed that NGAL values in AKI group were significantly higher on day 1 than on day 4 (p = 0.001) and day 7 (p = 0.017). The difference between days 4 and 7 was not statistically significant (p = 0.970). We also observed a higher NGAL levels in patients with AKI upon admission compared to patients with normal renal function (Fig 2b,c).

Relationship between AKI and NGAL

We performed ROC analysis to predict acute renal injury based on NGAL serum concentrations on days 1 and 7 of observation (Fig. 3a, b).

The optimal cut-off value according to AUC (area under the curve) = 0.842, 95% CI (0.722-0.962) for NGAL on day 1 was 290 µg/l with sensitivity 0.710 and specificity 0.867. The positive predictive value is 0,9167, negative predictive value is 0,5909. If we would like to increase sensitivity, then the optimal cut off value was 240 µg/l, when the sum of sensitivity and specificity is second highest (sensitivity 0.806, specificity 0.773). According to the determined cutoff values of NGAL, we performed logistic regression to calculate the odds ratio (OR) (Table 3).

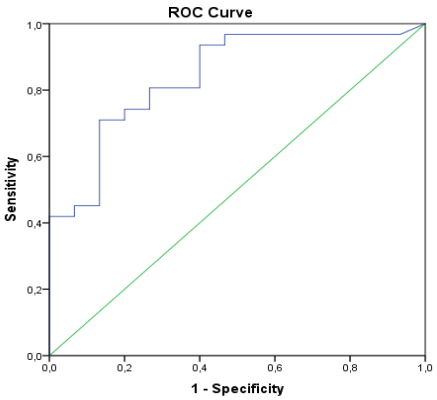

Fig. 3a. Relationship of AKI and NGAL on day 1 in all study patients

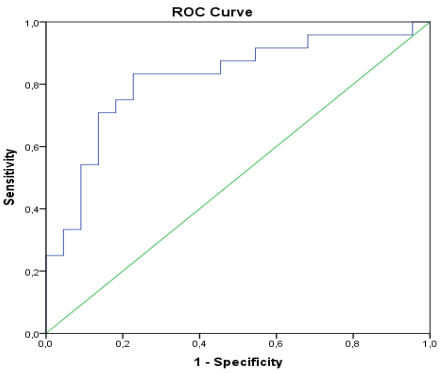

Fig. 3b. Relationship of AKI and NGAL on day 7 in all study patients

If a patient has an NGAL level > 290 μg/l on day 1, the chance of renal impairment increases by approximately 16 times. NGAL values were categorized also according to cut-off value of 240 μg/l. Logistic regression was performed to calculate the odds ratios (ORs). If the patient has an NGAL level > 240 μg/l on day 1, the risk of renal impairment increases 11.5 times. However, the cut-off values of NGAL on day 1 used for AKI prediction on day 7 were not significant, with AUC=0.671, 95% CI (0.513 - 0.830) and p = 0,047.The optimal cut-off value according to AUC = 0.818, 95% CI (0.692-0.945), for NGAL on day 7 was 210 μg/l, with sensitivity 0.833 and specificity 0.773. If the patient had an NGAL level > 210 μg/l on day 7, the chance of renal impairment increased 17 times (Table 4).

Table 4. Odds ratio for AKI prediction according to NGAL serum concentration

NGAL (μg/l)	OR	CI	P value
> 210	17.0	3.9-73.6	0.0002

Abbreviations: CI, confidence interval; NGAL, Neutrophil Gelatinase-associated Lipocalin, OR - Odds ratio; P value, logistic regression

Table 5. Relationship between NGAL, selected biochemical and inflammatory markers, AKI stage and SOFA score

Parameter		**NGAL (ug/l) day 1**	**Parameter**		**NGAL (ug/l) day 7**
serum creatinine (μmol/l) day 1	r_s	0.613	serum creatinine (μmol/l) day 7	r_s	0.719
	p	<0.0001		*p*	<0.0001
albumin (g/l)	r_s	-0.229	albumin (g/l)	r_s	-0.175
	p	0.134		*p*	0.256
CRP (mg/l) day 1	r_s	0.363	CRP (mg/l) day 7	r_s	0.27
	p	0.013		*p*	0.073
PCT (μg/l) day 1	r_s	0.627	PCT (μg/l) day 7	r_s	0.635
	p	<0.0001		*p*	<0.0001
SOFA score	r_s	0.239	SOFA score	r_s	0.395
	p	0.114		*p*	0.007
AKI day 1	r_s	0.589	AKI day 7	r_s	0.620
	p	<0.0001		*p*	<0.0001
IL-6 day 1	r_s	0.484	IL-6 day 7	r_s	0.475
	p	0.0007		*p*	0.0011

Relationship between NGAL and the other biochemical parameters

The association of NGAL with creatinine, albumin, CRP, procalcitonin, and SOFA stage was verified using Spearman's correlation coefficient (r_s) and the corresponding p-value (p). The results showed that NGAL on day 1 correlated moderately strongly with creatinine (r_s = 0.613, p < 0.0001), PCT (r_s = 0.627, p < 0.0001), and AKI stage (r_s = 0.589, p < 0.0001), while a weak correlation was observed with CRP (r_s = 0.363, p = 0.013). Furthermore, NGAL values measured on day 7 were moderately strongly correlated with creatinine (r_s = 0.7193, p < 0.0001), PCT (r_s = 0.635, p < 0.0001), and AKI stage (r = 0.620, p < 0.0001) and significantly positively correlated with SOFA score (r_s = 0.395, p = 0.007). An overview of the results is presented in Table 5.

Discussion

In this prospective clinical study, we confirmed that plasma NGAL is a good biomarker for the early detection of acute kidney injury in critically ill patients with sepsis. This conclusion is supported by some recently published studies. But according to our current knowledge,

this is an original study with the determination of optimal cut-off values of plasma NGAL concentrations in predicting the development of AKI in time delay of 7 days treatment in septic critically ill patients. For example, a systematic review and meta-analysis of 12 studies showed that plasma NGAL levels were significantly higher in adult septic patients with AKI than in those without AKI. Plasma NGAL level has high sensitivity and a high negative predictive value for the detection of AKI in adult patients with sepsis. However, its low specificity and positive predictive value may limit its clinical use [13]. These observations are also supported by other recent studies that confirm that plasma NGAL is a candidate biomarker of AKI with very good sensitivity and specificity (AUC 0.80) that can predict AKI before elevated serum creatinine level [14]. Reference ranges for serum creatinine are 49- 90 μmol/l for women and 64-104 μmol/l for men in our study. Other systematic reviews and meta-analyses that tried to quantitatively summarize published data to evaluate the value of urinary and plasma NGAL for kidney risk prediction proved that both urinary and plasma NGAL may identify patients at high risk for AKI in clinical research and practice. This systematic review included 52 observational studies with 13,040 heterogenous intensive care patients. Cut-off concentrations at 95% specificity for urinary NGAL were >580 ng/ml with 27% sensitivity for severe AKI and >589 ng/ml with 24% sensitivity for AKI-D (AKI requiring dialysis). Corresponding cut-offs for plasma NGAL were >364 ng/ml with 44% sensitivity and >546 ng/ml with 26% sensitivity, respectively [15]. In our study, we focused on the determination of plasma NGAL concentration and found that the optimal cut-off value for plasma NGAL at the onset of infection was 290 μg/l, with a sensitivity of 0.710 and specificity of 0.867 and 240 μg/l, respectively, with a sensitivity of 0.806 and specificity of 0.773. In our septic patient cohort, the optimal NGAL value was lower than in the previously mentioned study, but if we take cut-off values similar to the above (364 ng/ml), the sensitivity is 0.645 and specificity 0.867. The variations in results can be explained by differences in study parameters, furthermore our study was quite small with potential for bias.

The aim of our study was also to determine possible associations with other biochemical parameters and inflammatory markers. Our results were very similar to other research papers. Numerous studies have reported NGAL as a reliable marker for AKI and its strong correlation with serum creatinine. For instance, Haase et al. indicated that NGAL is a sensitive marker for AKI and its levels often precede increases in serum creatinine by 24-48 hours, however in heterogenous groups of patients including contrast induced nephropathy, postsurgical AKI, and critically ill patients.[16]. We also achieved similar results as other study authors when comparing NGAL and inflammatory markers.

Serum NGAL is a highly sensitive indicator for detecting acute kidney injury in critically ill patients with septic shock, but it lacks specificity [17].

In the study by Wu Y et al., serum NGAL could play a role in evaluating the severity of sepsis. Both serum NGAL and lactate might serve as independent risk factors for predicting 28-day mortality in sepsis patients. Additionally, serum NGAL has the potential to predict the occurrence of septic-AKI [11]. The results suggest that we should use a combination of biomarkers, such as a multimarker approach, to improve the diagnosis of critically ill patients with sepsis, which is also supported by some studies [18,19].

We also focused on the use of NGAL levels as a predictor of acute kidney injury. If we use the NGAL value on day 1 to predict acute kidney injury on day 7, it does not look so promising (AUC = 0.671; 95% CI (0.513-0.830), p = 0.047); however according to some studies, for example human study by Jahaj et al., NGAL was shown to be more accurate in predicting AKI development than creatinine in initially non septic patients [20]. In a prospective human study by Kümpers et al., plasma NGAL levels independently predicted AKI severity in critically ill patients with severe systemic inflammation and 28-day survival. There was a significant difference between serum NGAL levels in healthy humans (39.0 [37.5–42.75] ng/ml), critically ill patients with systemic inflammatory response syndrome (297 [184–490] ng/ml), and critically ill patients with sepsis (708 [365–1301] ng/ml) [21]. Another prospective cohort study by Casas-Aparicio et al. on critically ill COVID-19 patients recently showed that patients with NGAL values > 45 ng/ml develop acute kidney injury faster than the other [22].

The severity of sepsis does influence NGAL values. Higher NGAL levels are generally associated with more severe sepsis, greater inflammation, and a higher likelihood of renal impairment. There are also some limitations to the use of NGAL as a biomarker for AKI. Sepsis enhances plasma NGAL production in critically ill adult patients irrespective of the presence of AKI. However, the diagnostic test accuracy for AKI is unaffected by sepsis, although the optimal cutoff values

are elevated [23]. In addition, it seems that the levels of this marker are significantly higher in patients with acute kidney injury following several cardiac surgeries, especially percutaneous coronary intervention (PCI), coronary angiography, or angioplasty, and the prognostic and diagnostic potential of urinary and serum NGAL in determining kidney damage in children. This biomarker and its predictive value have been proven in children with urolithiasis, metabolic disorders, renal transplantation, diabetic nephropathy, and diarrhea-associated hemolytic uremic syndrome [24]. The role of NGAL in determining acute kidney injury in critically ill patients with severe trauma, surgical interventions, and sepsis has been well established in several other studies [25-27]. Therefore, it is essential to interpret NGAL levels in the context of clinical history. The results of another human study by Bolignano et al. showed increased levels of NGAL in a small cohort of 46 patients with heart failure compared to age-matched controls. Notably, the highest levels were found in patients with the most severe class IV congestive heart failure. Moreover, the values also successfully predicted mortality in these patients with levels above baseline NGAL > 783 ng/ml in a two-year follow-up [28].

Finally, different assays for NGAL measurement are available for commercial use, leading to variations in serum/plasma value determination. Therefore, standardization of the assays is necessary to ensure accurate and reliable results. Limited utility could be a disadvantage in certain populations, such as patients with chronic kidney disease or those on dialysis.

Conclusion

Our study was relatively small, with potential for bias. We have confirmed that NGAL is a promising biomarker for the early detection of AKI in critically ill patients with sepsis, and its use in clinical practice has the potential to improve patient outcomes. However, in critically ill patients with initial sepsis, NGAL values may not be an optimal predictor of AKI development over the next seven days. The septic state itself, where NGAL reflects more than just renal function, is likely to play a role. Its interpretation should be performed in the context of clinical history and other laboratory findings. The optimal cut-off levels for NGAL in the diagnosis of AKI are still debated and may vary depending on the clinical context. However, further studies are needed to determine its optimal clinical use and to evaluate its performance in different patient populations and clinical settings.

Ethical approval.

This study was approved by the institutional ethics review boards of the University Hospital Olomouc (Reference number 13/19) and University Hospital Ostrava (Reference number 55/2019, protocol code number III), Czech Republic. The study adhered to the Declaration of Helsinki, 2013 and Good Clinical Practice. Written informed consent was obtained from all participants involved in the study.

Conflict of Interest

There is no conflict of interest.

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Informed consent was obtained from all individual participants included in the study.

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