The relationship of neutrophil gelatinase-associated lipocalin (NGAL) as a predictor of events acute kidney injury (AKI) in septic patients treated in ICU

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Abstract

***Introduction*: Sepsis is defined as life-threatening organ dysfunction caused by dysregulation of the body's response to infection. Sepsis can cause multiple organ dysfunctions, including kidney dysfunction, leading to sepsis-related acute kidney injury (S-AKI). Recent studies have found that neutrophil gelatinase-associated lipocalin (NGAL) can be a biomarker responsive to tissue stress and injury to the nephron, so acute kidney damage is recognized more quickly. This biomarker also functions as a monitor of development and recovery, and predicts the final outcome.**

***Method*: This study was a prospective observational study to see the relationship between NGAL levels as a predictor of AKI incidence in septic patients in the intensive care unit (ICU). The research sample was taken according to the inclusion and exclusion criteria, and the sample size was 40.**

***Results*: NGAL levels >150 ng/ml with the occurrence of sepsis with AKI were found in 32 (94.1%) samples, and sepsis without AKI in 2 (5.9%) samples. NGAL levels <150 ng/ml with the occurrence of sepsis with AKI was 1 sample (16.7%), with sepsis without AKI in 5 (83.3%) samples. It was found that NGAL had a relationship with the occurrence of sepsis with AKI (p<0.001, specificity 96%, specificity 71%, positive predictive value [PPV] 93%, negative predictive value [NPV] 94%, and area under the curve [AUC] 86%).**

***Conclusion*: There was a relationship between NGAL and the incidence of AKI, and it had a specificity of 97%, specificity of 71%, PPV of 94%, NPV of 83%, and AUC of 86%.**

Key words: Sepsis, ICU, neutrophil gelatinase-associated lipocalin, AKI, critical patient, SOFA.

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Introduction

European Society of Intensive Care Medicine's and the Society of Critical Care Medicine's Third International Consensus Definition for Sepsis and Septic Shock in 2016 stated that sepsis is defined as life-threatening organ dysfunction caused by dysregulation of the body's response to infection. Sepsis can cause multiple organ dysfunctions, including kidney dysfunction, leading to sepsis-related acute kidney injury (S-AKI). (1,2)

AKI is a common complication in patients treated in the intensive care unit (ICU). AKI occurs due to a sudden decline in kidney function within hours to weeks, followed by kidney failure to excrete nitrogenous metabolic waste with or without fluid and electrolyte balance disturbances. Acute kidney fail-

ure, or AKI, is a serious complication that often occurs in patients with critical illnesses. (2,3)
The incidence of AKI, according to the Indonesian Renal Registry in 2020, was around 4625 people, and the incidence of patients with sepsis at Haji Adam Malik Hospital in 2019 was 111 people, while in 2020, it was 63 people and in 2021, it was 39 people. Patients who died due to sepsis in 2019 were 91 people; in 2020, it was 43 people, and in 2021, it was 23 people. The number of patients at Haji Adam Malik Hospital who experienced sepsis with AKI in 2019 was 40 people. In 2020, the number of patients experiencing sepsis with AKI was 25 people, while in 2021, patients with sepsis and AKI were 19 people. (4)
In establishing the diagnosis of S-AKI using the Kidney Disease: Improving Global Outcomes (KDIGO) criteria, serum creatinine (sCr) and urine output are assessed, which are still problematic due to lack of sensitivity and specificity. In septic patients, aggressive fluid therapy often causes dilution of sCr so that S-AKI is not diagnosed. A recent study found that neutrophil gelatinase-associated lipocalin (NGAL) can be a biomarker responsive to tissue stress and injury to the nephron, so acute kidney damage is detected more quickly. (5,6)
In critically ill conditions, there will be impaired perfusion in the kidneys, which can damage the renal tubular epithelial cells, so in AKI, NGAL levels will increase more significantly. This condition occurs due to impaired NGAL reabsorption by proximal tubular epithelial cells and increased release of NGAL from neutrophil secondary granules. (7)
The purpose of this study was to determine the relationship between NGAL and AKI in septic patients treated in the ICU and assess the sensitivity, specificity, and positive and negative predictive values of NGAL and the area under the curve (AUC) as predictors of AKI in septic patients treated in the ICU.

Method

This type of research was a prospective observational study to see the relationship between NGAL levels as a predictor of AKI incidence in septic patients in the ICU. This study was conducted in the ICU of Prof. Dr. Chairuddin P. Lubis Hospital, Medan, and Dr. Pirngadi Regional General Hospital with a sample size of 40 people who met the research criteria. The inclusion criteria were patient families who agreed to be research samples, patients treated in the ICU, and patients diagnosed with sepsis using Sequential Organ Failure Assessment (SOFA). Meanwhile, the exclusion criteria were patients who previously had renal insufficiency, patients who used nephrotoxic drugs before the study, patients who had chronic kidney disease, patients who had urinary tract infections or kidney inflammation, patients undergoing hemodialysis, and kidney transplant recipients. The drop-out criterion was patients who died before 24 hours of treatment in the ICU.
Univariate data consisted of gender, age, diagnosis, and S-AKI displayed with distribution, frequency, and percentage of each variable, and they were presented in the table. Meanwhile, heart rate, temperature, respiration rate, leukocytes, urine output, and sCr were presented with mean and standard deviation. Bivariate data to assess the relationship between NGAL and S-AKI used a 2x2 table to determine sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and AUC using IBM SPSS Statistics Version 25 software.

Result

Demographic data characteristics of septic patients with AKI and without AKI
This study consisted of 40 septic patients divided into 2 groups, i.e., sepsis with AKI and sepsis without AKI. The characteristics of septic patients with AKI and without AKI consisted of age and gender. The age of septic patients with AKI was 55.12±13.81 years, consisting of 20 males (60.6%) and 13 females (39.4%). In septic patients without AKI, the age was 59.42±12.88 years, consisting of 2 males (28.6%) and 5 females (71.4%) (**Table 1**).

Clinical data characteristics of septic patients with AKI and without AKI
Their characteristics were systolic and diastolic blood pressure, mean arterial pressure (MAP), sCr, and 24-hour urine. In septic patients with AKI, the systolic blood pressure was 113.21±14.42 mmHg, the diastolic blood pressure was 68.63±12.82 mmHg, MAP was 90.69±11.75 mmHg, sCr was 1.97±0.98 mg/dl, and 24-hour urine of 1256±326 ml. In septic patients without AKI, the systolic blood pressure was 119.14±15.44 mmHg, the diastolic blood pressure was 68.28±5.7 mmHg, MAP was 93.57±9.12 mmHg, sCr was 0.73±0.12 mg/dl, and 24-hour urine of 921.42±107.45 ml (**Table 2**). In septic patients with AKI, urine examination could not be used as a reference in establishing AKI because the patients received furosemide therapy, making 24-hour urine more than sepsis without AKI.

Characteristics of NGAL levels in septic patients with AKI and without AKI
NGAL samples were collected in the emergency

room (ER) or inpatient rooms, and then patients were treated in the ICU. NGAL was re-examined 24 hours after being in the ICU. Septic patients with AKI had an NGAL level of 179.06±28.46 ng/ml and a 24-hour NGAL of 203.51±42.08 ng/ml, while in septic patients without AKI, the NGAL level was 126.71±21.62 ng/ml and a 24-hour NGAL of 151±27.54 ng/ml (**Table 3**). There was a difference in the figures between NGAL in septic patients with AKI and without AKI, so we conducted a dependent t-test to see the relationship between NGAL as a predictor of AKI incidence in septic patients, as in **Table 4**.

Relationship of NGAL levels in septic patients with AKI and without AKI

The dependent test was to see if there was a difference in NGAL levels in sepsis with AKI and without AKI. NGAL level in sepsis with AKI was 179.06±28.46 ng/ml, and in sepsis without AKI was 126.71±21.62 ng/ml. It was found that NGAL levels had a relationship to the incidence of sepsis with AKI with $p<0.001$ (**Table 4**).

Characteristics of NGAL levels against SOFA scores

NGAL levels of 193±32.46 ng/ml were highest at SOFA score 11 (n=12 [30%]). At SOFA score 10 (n=10 [25%]), NGAL was 174.2±25.37 ng/ml; at SOFA score 8 (n=6 [15%]), NGAL was 174±31.15 ng/ml; at SOFA score 9 (n=3 [7.5%]), NGAL was 168±18.33 ng/ml; at SOFA score 6 (n=4 [10%]), NGAL was 134.75±32.20 ng/ml; and at SOFA score 7 (n=5 [12.5%]), NGAL was 134.60±20.61 ng/ml (**Table 5**).

Characteristics of NGAL levels against AKI incidence

NGAL levels >150 ng/ml in sepsis with AKI were obtained from 32 (94.1%) samples and without AKI from 2 (5.9%) samples, while NGAL levels <150 ng/ml in sepsis with AKI were obtained from 1 (16.7%) sample and without AKI from 5 (83.3%) samples (**Table 6**).

NGAL diagnostic test

NGAL diagnostic test results showed a sensitivity of 97%, a specificity of 71%, a PPV of 94%, and an NPV of 83% (**Table 7**); meanwhile, the area under the receiver operating characteristic (ROC) was 86% (**Figure 1**).

Discussion

Sepsis causes changes in regional microcirculatory flow characterized by increased heterogeneity of blood flow, decreased proportion of capillaries carrying occluded blood flow, and decreased proportion of capillaries carrying sluggish blood flow. This pattern of microcirculatory dysfunction occurs in humans who experience septicemia. Endothelial injury, autonomic dysfunction, glycocalyx release, and activation of the coagulation cascade result in increased rolling and adhesion of leukocytes and platelets, decreased blood flow velocity, and microthrombi formation, which ultimately disrupt the microvascular flow. (8,9)

Inflammatory responses, named pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), are inflammatory mediators derived from bacteria and host immune cells, respectively. These inflammatory mediators bind to pattern recognition receptors (PRRs) expressed on the surface of innate immune cells, endothelial cells, and renal tubular epithelial cells, initiating downstream signaling cascades. This cascade increases the synthesis of proinflammatory cytokines, reactive oxygen species (ROS), oxidative stress, and endothelial activation by upregulating nitric oxide and nitric oxide synthase (iNOS). (10)

These cytokines, produced in very large amounts during the early phase of sepsis, activate leukocytes, endothelial cells, and epithelial cells, leading to leukocyte and platelet activation, microvascular dysfunction, hypoxia, and tissue damage. Loss of glomerular filtration rate (GFR) in sepsis is due to impaired microcirculatory hemodynamics at the glomerular level. This study, conducted with a total sample of 40 people to find the relationship between neutrophil gelatinase-associated lipocalin (NGAL) to predict AKI in septic patients, had a specificity of 96%, a specificity of 71%, a PPV of 93%, an NPV of 94%, and an AUC of 86%. This was the same as the study conducted by Meili Andriani et al., which assessed the sensitivity and specificity of NGAL as an early marker of AKI in ICU and high-care unit patients with a sensitivity of 88%, specificity of 71%, PPV of 88%, NPV of 81%, and accuracy of 85%. A study conducted by Khaled Abdelwahad and colleagues compared blood NGAL with urine NGAL in AKI. The findings revealed that blood NGAL had a sensitivity of 82.4% and a specificity of 73.9%, with an AUC of 0.82. In contrast, urine NGAL also showed a sensitivity of 82.4% and a higher specificity of 78.3%, with the same AUC of 0.82. (11,12)

In the study conducted by Gomes et al., AKI was detected in septic patients by examining blood NGAL and urine NGAL obtained from critically ill patients with urinary ROC 0.881. In a study by Karina Soto et al., which examined NGAL to predict

AKI in patients in the Emergency Department, the sensitivity was 71% and the specificity 70%. (13,14)

Kidney damage should be detected by measuring certain markers or biomarkers, indicating the presence of disease. Ideally, the detection process using biomarkers should be a non-invasive procedure that can easily detect the disease, provide rapid results, and clearly see specific damage to cells. In general, acute renal failure can be seen from the results of sCr and diuresis, though kidney damage is known to cause increased levels of various biomarkers, including NGAL. Traditionally, sCr and urine output play an important role in assessing kidney injury, but today, many clinical trials have been conducted using other biomarkers that are also sensitive in determining kidney function and predicting kidney damage. Serum creatinine levels have limitations because they cannot differentiate structural damage to the kidney and damage due to a decreased renal hemodynamic function that causes a decrease in the GFR. Furthermore, sCr levels increase only after renal function has been impaired by almost 50%. Therefore, it usually takes several days to see an increase in sCr concentration. The time required to obtain sCr test results can cause a delay in diagnosis, which affects subsequent treatment management. (15,16)

NGAL, also known as human neutrophil lipocalin, lipocalin-2, siderocalin, and 24p3, is a neutrophil that binds to neutrophil granulocyte particles and undergoes gelatinization. It is a protein from the lipocalin protein family with a small molecular weight of only 25 kD. Lipocalin comprises 6 β-strands from a β-barrel composed of calyx that bind and are transported by small molecules in the body. NGAL is secreted from secondary granules of activated neutrophils. Therefore, its plasma levels increase during inflammation or infection. NGAL can be identified in neutrophils, but is also expressed by renal epithelial cells, so it can indicate pathological responses in the body, for example, in the occurrence of inflammation, infection, ischemia, poisoning, acute renal failure, and neoplastic transformation. (17)

One limitation of this study was that urine output could not be used as a reference in establishing AKI because all patients diagnosed with AKI used furosemide. Other limitations included the lack of recording of antibiotic use before establishing sepsis and the inability to compare NGAL in patients who had been diagnosed with AKI to those who had not been diagnosed with AKI using the same number of samples.

Conclusion

The demographic characteristics of the study showed the age of 55.87±13.59 years, consisting of 22 (55%) males and 18 (45%) females. Demographic clinical hemodynamic characteristics showed mean systolic blood pressure of 114.25±14.42 mmHg, mean diastolic blood pressure of 68.57±11.82 mmHg, and mean MAP of 91.20±11.28 mmHg. In the meantime, the mean creatinine was 1.76±1.01 mg/dl, with mean 24-hour urine of 1197±325 ml. The mean NGAL value was 169.29±33.79 ng/ml; meanwhile, the mean 24-hour NGAL was 194.35±44.45 ng/ml. NGAL had a specificity of 97%, a specificity of 71%, a PPV of 94%, an NPV of 83%, and an AUC of 86%. There was a relationship between NGAL and the incidence of AKI.

Table 1. Demographic data characteristics of septic patients with AKI and without AKI

Characteristics	Sepsis with AKI (n=33)	Sepsis without AKI (n=7)
Age (years), mean±SD	55.12±13.81	59.42±12.88
Gender, n (%) - Male - Female	 20 (60.6%) 13 (39.4%)	 2 (28.6%) 5 (71.4%)

Legend: AKI=acute kidney injury; SD=standar deviation.

Table 2. Clinical data characteristics of septic patients with AKI and without AKI

Characteristics	Sepsis with AKI (n=33)	Sepsis without AKI (n=7)
Systolic blood pressure (mmHg)	113.21±14.42	119.14±15.44
Diastolic blood pressure (mmHg)	68.63±12.82	68.28±5.7
MAP (mmHg)	90.69±11.75	93.57±9.12
sCr (mg/dl)	1.97±0.98	0.73±0.12
24-hour urine (ml)	1256±326	921.42±107.45

Legend: AKI=acute kidney injury; MAP=mean arterial pressure; sCr=serum creatinine; SD=standar deviation. All data are in mean±SD.

Table 3. Characteristics of NGAL levels in septic patients with AKI and without AKI

Characteristics	Sepsis with AKI (n=33)	Sepsis without AKI (n=7)
NGAL (ng/ml)	179.06±28.46	126.71±21.62
24-hour NGAL (ng/ml)	203.51±42.08	151±27.54

Legend: NGAL=neutrophil gelatinase-associated lipocalin; AKI=acute kidney injury; SD=standar deviation. All data are in mean±SD.

Table 4. Relationship of NGAL levels in septic patients with AKI and without AKI

Characteristics	Sepsis AKI (n=33)	Sepsis (n=7)	p
NGAL (ng/ml)	179.06±28.46	126.71±21.62	0.0001

Legend: NGAL=neutrophil gelatinase-associated lipocalin; AKI=acute kidney injury; SD=standar deviation. All data are in mean±SD.

Table 5. Characteristics of NGAL levels against SOFA scores

Characteristics	Samples (n=40), n (%)	NGAL (ng/ml)
SOFA score 6	4 (10%)	134.75±32.20
SOFA score 7	5 (12.5%)	134.60±20.61
SOFA score 8	6 (15%)	174±31.15
SOFA score 9	3 (7.5%)	168±18.33
SOFA score 10	10 (25%)	174.2±25.37
SOFA score 11	12 (30%)	193±32.46

Legend: NGAL=neutrophil gelatinase-associated lipocalin; SOFA=Sequential Organ Failure Assessment.

Table 6. Characteristics of NGAL levels against AKI incidence

Characteristics	Sepsis with AKI	Sepsis without AKI
NGAL>150 ng/ml	32 (94.1%)	2 (5.9%)
NGAL<150 ng/ml	1 (16.7%)	5 (83.3%)

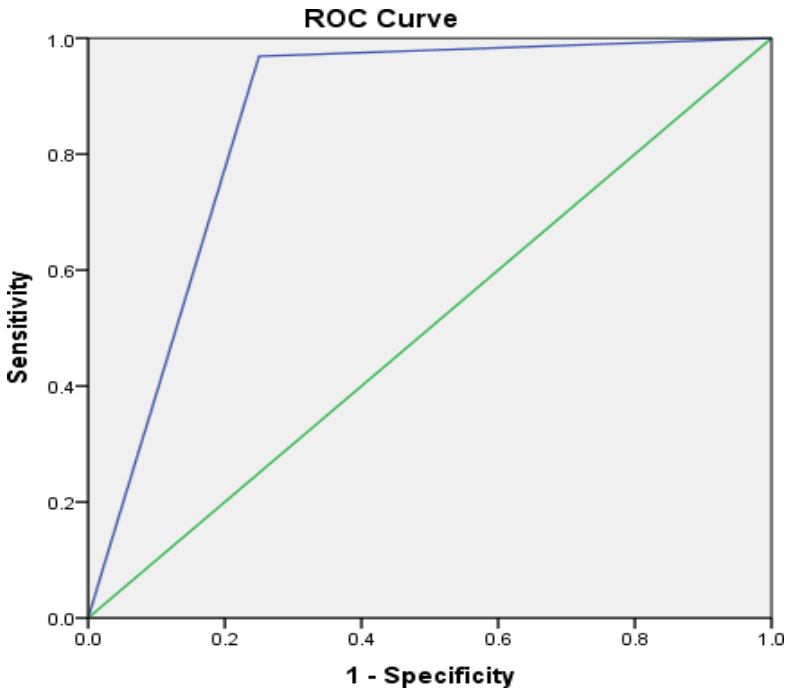
Legend: NGAL=neutrophil gelatinase-associated lipocalin; AKI=acute kidney injury.

Table 7. NGAL diagnostic test

Characteristics	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
NGAL	97	71	94	83

Legend: NGAL=neutrophil gelatinase-associated lipocalin; PPV=positive predictive value; NPV=negative predictive value.

Figure 1. ROC curve



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