Am J Transl Res 2024;16(4):1266-1272
www.ajtr.org /ISSN:1943-8141/AJTR0154349

Original Article

Evaluation of urinary neutrophil gelatinase associated lipocalin in the early diagnosis of acute kidney injury with sepsis

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Received November 9, 2023; Accepted March 14, 2024; Epub April 15, 2024; Published April 30, 2024

Abstract: Background: To assess the utility of urinary neutrophil gelatinase associated lipocalin (uNGAL) in the diagnosis of acute kidney injury (AKI) in the context of sepsis. Methods: In this retrospective study, a total of 142 patients with sepsis treated in the Third Hospital of Shanxi Medical University from January 2019 to January 2021 were included. Patients diagnosed with AKI complicated with sepsis were categorized into the AKI group (n=70 cases), and patients diagnosed with sepsis were classified into the non-AKI group (n=72 cases). We collected and analyzed data on serum creatinine (Scr) and uNGAL levels. The ROC (receivers operating characteristics) curve was used to evaluate the sensitivity and specificity of uNGAL in the diagnosis of AKI with sepsis. Results: The level of uNGAL in the AKI group increased over time following admission, which was not observed in the non-AKI group. Twenty-four hours after admission, the level of uNGAL in the AKI group was significantly higher than that in the non-AKI group ($P < 0.05$), but there was no significant difference in Scr level between the two groups ($P > 0.05$). At 72 hours after admission, the AUC of uNGAL in predicting AKI was 0.989 (95% CI: 1.018-1.085), and its intercept value was 961.3 ng/ml. At the same time, the correlation analysis showed that the level of uNGAL was positively correlated with the occurrence of AKI. Conclusion: uNGAL is superior to Scr for early diagnosis of AKI patients with sepsis.

Keywords: Urine neutrophil gelatinase associated lipocalin (uNGAL), acute kidney injury, sepsis

Introduction

Acute kidney injury (AKI) is the most common complication in hospitalized patients, and it is more prominent in critically ill patients and seriously affects the prognosis of patients [1]. Sepsis is one of the independent risk factors for AKI [2], with reports suggesting that up to 47% of sever cases may attribute acute renal failure to sepsis [3]. Moreover, the prognosis of sepsis patients with recurrent AKI is markedly worse, characterized by elevated mortality rates and substantially increased risk of chronic kidney disease [4]. Therefore, it is of great clinical significance to actively prevent and treat sepsis induced AKI.

Historically, it was generally believed that sepsis-induced AKI was mainly attributed to renal ischemia, cell injury and acute tubular necrosis [5]. Patients with low blood pressure or renal hypoperfusion are at higher risk of AKI. There are three major pathological signs of sepsis-induced AKI: microcirculation disturbance, inflammation, and bioenergetic adaptive response to injury [6]. Recent studies have found that endothelial dysfunction, inflammatory response, coagulation dysfunction and cell adaptive response to injury are involved in the occurrence of sepsis-induced AKI. Hypoxia and hypoperfusion may aggravate the inflammatory response and induce the adaptive response of tubular epithelial cells. Proinflammatory factors, filtered through the glomerulus into the proximal convoluted tubules, directly activate tubular epithelial cells [7]. In practice, serum creatinine is usually used for the diagnosis of AKI [8]. However, it is both a delayed and an unreliable marker since its levels can be affected by certain factors such as age, drugs, muscle amount and metabolism, hydration, and protein intake. Most AKI markers, including cre-

https://doi.org/10.62347/PMAL7041

atinine, blood urinary nitrogen, and urine output, indicate delayed consequences of damage rather than immediate cellular injury, posing challenges in timely and accurate diagnosis.

Urinary neutrophil gelatinase related carrier protein (uNGAL) plays a crucial role in the pathophysiological process of AKI and has emerged as one of the extensively studied biomarkers [9]. uNGAL is primarily secreted by the thick ascending limb of the renal tubular medullary loop and collecting duct in the kidney, and filtered through the glomerulus and reabsorbed in the proximal tubules. Following an AKI event, there is a significant increase in NGAL secretion from the thick ascending limb of the loop, coupled with impaired reabsorption function of the renal tubules, leading to the increase of blood and urine NGAL. No matter whether the renal injury is reversible or not, NGAL can increase within 3 hours after injury and reach the peak at 6-12 hours, which is directly proportional to the degree of injury. In case of severe injury, high level NGAL can last for nearly 5 days. Studies have shown that sustained high levels of NGAL are associated with an increased risk of death [10]. However, there is a paucity of data on its use in sepsis patients, a high-risk group to renal injury.

In our study, we investigated the utility of uNGAL in the early diagnosis of the acute kidney injury in patients with sepsis, aiming to fill the gap in current research and provide insights into early detection strategies for this high-risk population.

Materials and methods

Study design and human subject collection

In this retrospective study, a total of 142 patients with sepsis treated in the Third Hospital of Shanxi Medical University from January 2019 to January 2021 were included. This study was reviewed and approved by the ethics committee of Shanxi Bethune Hospital.

Inclusion criteria: (1) Patients diagnosed with sepsis according the Definition and Diagnostic Criteria of Sepsis 3.0 jointly issued by the American Society of critical care (SCCM) and the European Society of critical care (ESICM) in 2017 [14]; (2) The diagnostic criteria of AKI refer to the relevant definitions in the 2019 Kidney Disease: Improving Global Outcomes (KDIGO) guide [15]: an increase in the absolute Scr ≥ 26.5 μ Mol/L (0.3 mg/dl) within 48 hours, or 1.5 times higher than the basic value within 1 week, or urine volume < 0.5 ml/(kg/h) for more than 6 h.

Exclusion criteria: (1) Patients with pre-existed renal impairment or diseases that affect renal function; (2) Patients with hypertension that cannot be stabilized with medication; (3) Patients who had used drugs affecting renal function; (4) Patients with immune system diseases (including rheumatoid arthritis, allergic bronchial asthma, etc.), hypovolemic shock and thyroid diseases; (5) Patients who were discharged, had treatment interruptions, or died within 24 hours; (6) Patients with malignant tumors, hematological malignancies, connective tissue diseases or immunosuppressants; (7) Pregnant women.

Clinical data and sample collection

In this study, the basic information of patients, including name, gender, age, body temperature, heart rate and respiratory rate were collected. Urine and blood samples were collected for 3 consecutive days. Patients with non-indwelling catheterization provided about 2 ml of middle urine samples (female patients avoided their menstrual period time when giving urine samples); Patients with indwelling catheterization provided about 2 ml of a newly collected urine sample. The urine samples were placed into a pyrogen-free and endotoxin-free test tube, and centrifuge at 3000 R/min for 1 min using a bench type low-speed centrifuge (800D, produced by Jiangsu Zhengji Instrument Co., Ltd.). Subsequently, the supernatant was collected for analysis.

Detection index

uNGAL: The bedside scattering turbidimetric analyzer (norman-2, produced by Nanjing Norman Biotechnology Co., Ltd.) was used for immunoenhanced turbidimetry. The test reagents R1 and R2 were provided by Nanjing Norman Biotechnology Co., Ltd. and the reagents to be used were stored in a refrigerator at 2-8°C away from light.

Scr: The determination of serum creatinine (Scr) was conducted using the sarcosine oxi-

Table 1. Clinical characteristics of the two groups

	AKI group (n=70)	Non-AKI group (n=72)	t/X^2	*P*
Age (years)	72.1±7.47	69.75±11.23	9.65	0.47
Sex			4.18	0.32
Male (n%)	42 (60%)	44 (61.1%)		
Female (n%)	28 (40%)	28 (38.9%)		
BMI	19.15±0.85	19.7±1.23	5.19	0.12
Original infection site			22.83	0.34
Severe pneumonia	15 (21.4%)	17 (23.6%)		
Blood stream infection	20 (28.6%)	19 (26.4%)		
Gastrointestinal and abdominal cavity infection	10 (14.3%)	8 (11.1%)		
Central nervous system infection	9 (12.9%)	11 (15.3%)		
Urinary system infection	6 (8.6%)	7 (9.7%)		
Osteomyelitis	4 (5.7%)	5 (6.9%)		
Other infections	6 (8.6%)	5 (6.9%)		
Complication			16.73	0.52
ARDS	11 (15.7%)	13 (18.1%)		
Gastrointestinal dysfunction	16 (22.9%)	19 (26.4%)		
Cardiac dysfunction	15 (21.4%)	14 (19.4%)		

Note: BMI: body mass index; AKI: acute kidney injury; ARDS: acute respiratory distress syndrome.

dase method utilizing a fully automatic biochemical analyzer (AU5800, Beckman Company in the United States). The Scr quantification assay kits were purchased from Beckman and carried out in strict accordance with the operating instructions.

Statistical analyses

SPSS 16.0 statistical software was used for data analysis. The measurement data were expressed as mean ± standard deviation. The Shapiro Wilk was used to assess the normality of data distribution. For data following a normal distribution, differences between the two groups were evaluated using the independent samples t-test. For data not adhering to a normal distribution, the Mann-Whitney test was used for comparisons between the two groups. Chi square test was used for the comparison of count data. Receiver Operating Characteristic (ROC) curve analysis was performed, and the Area Under the Curve (AUC) was calculated. The difference between AUC and 0.5 was compared by a 95% confidence interval (CI). If 0.5 fell outside the 95% CI, it was considered that the difference was statistically significant. Statistical significance was established at $P < 0.05$.

Results

Characteristics of subjects

Table 1 shows the characteristics of the participants. A total of 142 patients were included in this study, involving 70 patients in the AKI group, with a mean age of (52.1±7.47), and 72 patients in the Non-AKI group, with a mean age of (59.75±11.23). There were no significant differences between two groups in terms of BMI, organ infections, ratio of severe pneumonia and complications (all $P > 0.05$).

Comparison of uNGAL and Scr levels between the two groups

The level of uNGAL in the AKI group increased with the extension of admission time, while the level of uNGAL in the non-AKI group did not increase significantly. There was no significant difference in the level of uNGAL between the two groups at admission ($P > 0.05$), but the level of uNGAL in AKI group was higher than that in non-AKI group at 24, 48 and 72 hours after admission (all $P < 0.05$). There was no significant difference in Scr level between the two groups 24 hours after admission ($P > 0.05$), as shown in **Table 2**.

Table 2. Comparison of uNGAL and Scr levels between the two groups

	Group	Number of cases	T0	T1	T2	T3
Scr	AKI group	70	78.13±10.24	90.22±14.11	378.44±13.27	223.57±14.27
	Non-AKI group	72	75.87±10.02	84.14±13.25	79.49±14.56	91.53±13.95
	t	-	3.737	2.245	9.275	8.536
	P	-	0.142	0.053	0.001	0.003
uNGAL	AKI group	70	461.45±111.23	737.21±114.15	908.32±209.51	986.14±340.19
	Non-AKI group	72	441.57±122.19	420.20±128.03	613.36±119.43	525.16±196.29
	t	-	1.237	3.245	13.125	12.289
	P	-	0.312	0.009	0.012	0.023

Note: T0: On admission; T1: 24 hours after admission; T2: 48 hours after admission; T3: 72 hours after admission. AKI: acute kidney injury.

Table 3. ROC analysis of the performance of uNGAL at different time point in predicting sepsis-related AKI

Time	AUC	Cut-off	OR (95% CI)		*P*
T0	0.609	441.3	1.038	1.123	0.030
T1	0.901	736.5	0.911	1.083	0.006
T2	0.973	889.4	0.999	1.037	0.003
T3	0.989	961.3	1.018	1.085	0.002

Note: T0: On admission; T1: 24 hours after admission; T2: 48 hours after admission; T3: 72 hours after admission.

ROC analysis

The areas under the ROC curve (AUCs) of uNGAL at admission, 24-, 48-, and 72-hour after admission for predicting AKI were 0.609, 0.901, 0.973 and 0.989, respectively. The intercepted value of 961.3 ng/ml was more sensitive and specific for the diagnosis of AKI (**Table 3** and **Figure 1**).

Associations between uNGAL and AKI

In this study, as shown in **Figure 2**, there was a significant correlation between the increase in uNGAL levels and the occurrence of AKI among patients (Correlation coefficient: 0.333, and *p*-value: < 0.001).

Discussion

Sepsis poses a significant threat to the lives of critically ill patients, with acute kidney injury (AKI) emerging as one of the most common and serious complications. AKI is closely correlated to the mortality of patients and is an independent risk factor for the death of patients [11-13]. At present, serum Scr is often used as an index for the diagnosis of AKI in the clinic, but it can be affected by many factors, such as age, gender, drugs, volume load, nutritional status, muscle metabolism, protein intake, and gastrointestinal bleeding. Therefore, the sensitivity of Scr in the diagnosis of AKI is not ideal [14-16]. A significant decrease in glomerular filtration rate, about 50%, is required before Scr levels rise to a point where AKI can be diagnosed. At this time, renal function may have been seriously damaged. There is a lag in the diagnosis of AKI by Scr [17, 18]. It is urgent to screen sensitive and reliable biomarkers for early diagnosis of AKI and its early treatment, so as to improve the prognosis and survival rate of patients with sepsis [19].

Neutrophil gelatinase-associated lipocalin (NGAL) is a kind of apolipoprotein that was originally found in activated neutrophils and is a small molecule secretory protein. NGAL not only binds and transports hydrophobic small molecules, but also participates in the inflammatory response, immune response, chemotaxis, signal transduction, as well as the occurrence and development of tumors [20]. A study [21] found that in the early stage of renal ischemia (within 2 h), the level of uNGAL in blood and urine increased significantly; Injection of recombinant uNGAL into ischemia-reperfusion modeled mice could reduce the occurrence of azotemia and renal injury; uNGAL promoted the differentiation of renal progenitor cells into early renal tubular epithelial cells, repaired neurocadherin, upregulated heme oxygenase and inhibited cell death. Therefore, it can be inferred that NGAL can be used as one of the effective biomarkers for the diagnosis of AKI. A study

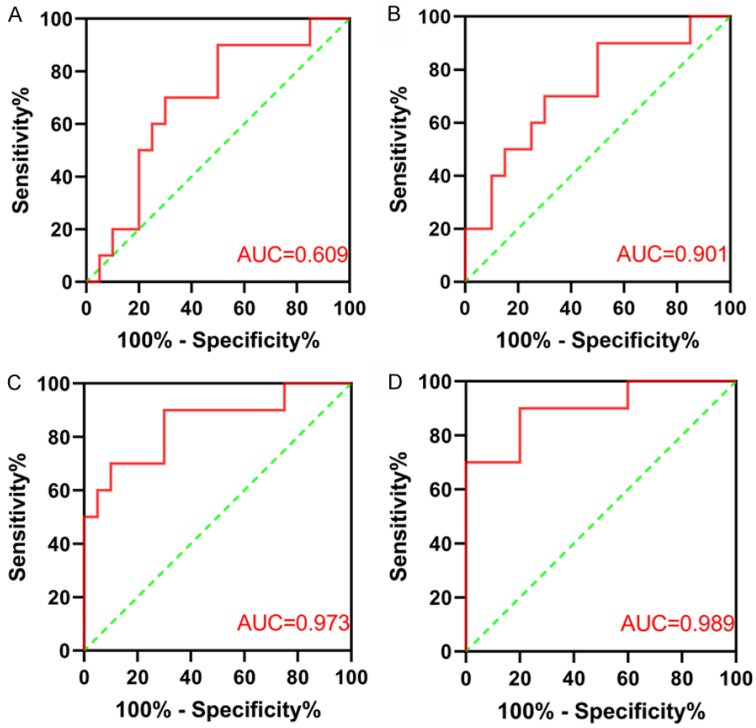

Figure 1. ROC analysis of uNGAL at different time points in predicting sepsis-related AKI. A: at admission; B: 24 hours after admission; C: 48 hours after admission; D: 72 hours after admission. Note: AKI: acute kidney injury; uNGAL: urinary neutrophil gelatinase associated lipocalin.

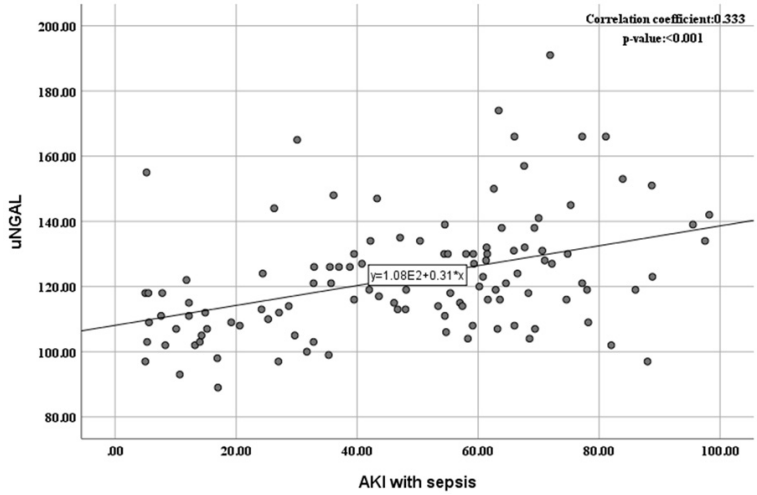

Figure 2. Pearson analysis of uNGAL and sepsis-related AKI. AKI: acute kidney injury; uNGAL: urinary neutrophil gelatinase associated lipocalin.

has important clinical significance. In patients with sepsis, an elevated release of uNGAL into the bloodstream is observed. Under normal circumstances, uNGAL can be reabsorbed by the renal tubules, implying that NGAL should not be present in the urine of sepsis patients without AKI. Therefore, considering the accuracy of the test results, the urine samples of sepsis patients were taken as the test specimen in this study. Clinically, relying on a single marker often yields limited effect on the early diagnosis of AKI. Therefore, it is more effective to study the combination of two or more indicators to diagnose whether patients with sepsis are complicated with AKI.

The results of this study show that at the time of admission, there was no significant difference in the levels of uNGAL between patients with sepsis who developed AKI and those didn't. However, a significant increase in uNGAL levels was observed in the AKI group at the 24th hour after admission and further increased with the progress of time. In contrast, the level of uNGAL in the non-AKI group did not increase significantly. There was no significant difference in Scr level between the two groups at the 24th hour after admission. ROC curve analysis showed that uNGAL had a high sensitivity and specificity in the diagnosis of sepsis-related AKI at the 72th hour after admission.

[22] showed that in the early stages of sepsis-related AKI (within 6 hours), the levels of NGAL in blood and urine increased to varying degrees, outperforming Scr in terms of sensitivity and specificity. Taking uNGAL as an early biomarker for the diagnosis of sepsis-related AKI

The present study is subject to a few limitations, including its retrospective design and the fact that it was conducted with a relatively small patient cohort at a single institution. Despite these constraints, the findings underscore the potential utility of uNGAL as a supe-

rior biomarker over serum creatinine for the early diagnosis of acute kidney injury (AKI) in sepsis patients.

Disclosure of conflict of interest

None.

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