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The diagnostic and prognostic values of serum and urinary kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin in sepsis induced acute renal injury patients

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Abstract. – OBJECTIVE: The kidney injury molecule-1 (uKIM-1) and neutrophil gelatinase-associated lipocalin (uNGAL, sNGAL) have been demonstrated to be diagnostic biomarkers for acute kidney injury (AKI) in a variety of diseases. However, both of them were not well validated in sepsis patients with acute kidney injury.

PATIENTS AND METHODS: This was a prospective and observational study which was performed in the three intensive care units of the Beijing Chao-Yang Hospital. Over a 12-month period, 174 patients (70 sepsis patients, 69 sepsis with AKI and 35 controls) were enrolled. Blood and urinary specimens were collected at admission as soon as possible (within 24 hours) and KIM-1 and NGAL levels were tested.

RESULTS: Levels of uKIM-1, uNGAL, sNGAL were significantly higher in the sepsis patients who developed AKI compared to those sepsis with no-AKI (0.88 ng/ml (0.37, 2.14) *vs*. 1.21 ng/ml (0.67, 3.26) *p*=0.003, 63.54 ng/ml (21.66, 125.45) *vs*. 249.85 ng/ml (86.60, 585.97) *p*<0.001, and 108.08 ng/ml (67.74, 212.22) *vs*. 200.01 ng/ml (102.76, 300.77) *p*=0.001, respectively). sKIM-1 also had significant differences between the two groups (83.98 pg/ml (54.00,147.08) vs. 193.41 pg/ml (106.90, 430.60) *p*<0.001). The four biomarkers (uKIM-1, sKIM-1, uNGAL, sNGAL) all could be predictive for AKI, and the areas under the receiver operating characteristic curves (AUROC) were 0.607, 0.754, 0.768, 0.658, respectively. The uNGAL was an independent risk factor for septic AKI, and the AUROC was 0.768 (95% CI: 0.689 to 0.835). The uNGAL and sNGAL were related to the prognosis of sepsis.

CONCLUSIONS: Our results showed that NGAL was a promising biomarker of septic AKI. Like the uKIM-1, the sKIM-1 could early predict the occurrence of septic AKI too, but both of them did not have the predictive value in judging the severity of AKI and the prognosis of sepsis.

Key Words:
Kidney injury molecule-1, Neutrophil gelatinase-associated lipocalin, Acute kidney injury.

Abbreviations

KIM=kidney injury molecule-1; NGAL=neutrophil gelatinase-associated lipocalin; AKI=acute renal injury; ICU=intensive care unit; CKD=chronic kidney disease; AIDS=Acquired Immune Deficiency Syndrome; WBC=white blood cell; PCT=procalcitonin; SOFA=Sequential Organ Failure Assessment; APACHE II=Acute Physiology and Chronic Health Evaluation II; GLS=Glasgow Coma Scale; ELISA=enzyme-linked immunosorbent assay; NT-proBNP=N-terminal pronatriuretic peptide; MAP=mean arterial pressure; DIC=Disseminated intravascular coagulations; ARDS=acute respiratory distress syndrome; AUROC=area under of the receiver operating characteristic; ECMO=extracorporeal membrane oxygenation; CVVH=Continuous veno venous hemofiltration; MV=Mechanical ventilation.

Introduction

Septicemia is a very harmful organ dysfunction. The host's abnormal response to infection

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or dysfunction may cause sepsis[1]. As the most common type of organ dysfunction, acute kidney injury (AKI) generally occurred in the early stages of sepsis. Moreover, AKI is very easy to develop from patients in the Intensive Care Unit (ICU) and nearly 30-50% of AKI patients cannot be cured and die[2-4]. However, a large part of the higher mortality of AKI was due to poor understanding of its pathology and delayed diagnosis.

At present, oliguria or elevated serum creatinine levels served as the main diagnostic indicators of AKI[5]. However, glomerular filtration rate (GFR) cannot be accurately characterized by SCr levels because of renal tubular creatinine secretion and other non-renal factors such as muscle mass hepatic function and non-renal gastrointestinal exclusion may also affect the level of GFR[6]. SCr was also considered as a late marker of kidney injury[7,8]. It is extremely important to find other indicators that can be used for early diagnosis of AKI based on above reasons.

In fact, research on potential markers for early diagnosis of AKI has been conducted for more than a decade. Among these markers, kidney injury molecule-1 (KIM-1)[9,10] and neutrophil gelatinase-associated lipoprotein (NGAL)[11-14] received the most attention among all the biomarkers studied.

KIM-1 was a transmembrane glycoprotein of renal proximal tubule epithelial cells. The extracellular domain of KIM-1 contained a mucin domain and an Ig-like domain. Generally, we could not detect KIM-1 in healthy kidneys. AKI was significantly up-regulated in dedifferentiated proximal tubule cells in some special cases such as nephrotoxicity or ischemia[9,15]. The extracellular domain of KIM-1 will rapidly lyse in AKI which caused KIM-1 to be released into the small lumen, so KIM-1 can be detected in urine. Similarly, KIM-1 could also be detected in the blood when the proximal tubule of the kidney was damaged[16]. Based on the strong translatability of KIM-1 between preclinical and clinical trials, researchers believed that KIM-1 could be one of the most promising early biomarkers. Moreover, KIM-1 was considered to be an extremely reliable predictor of early AKI because it was proved to be involved in the process of kidney injury and healing[15,16-18].

NGAL was found to be a protein expressed in various epithelial cells and neutrophils. As a member of the lipocalin protein family, NGAL lipids could transport small hydrophobic molecules such as retinoids, steroids and lipids. As an indicator of early renal injury secondary to insufficient renal perfusion, NGAL was first proposed by Mishra et al[19] in 2003. NGAL has been studied in different clinical settings since then[20], and kidney surgery, heart surgery, systemic diseases, kidney damage caused by cancer, cancer chemotherapy and other diseases were included[21,22].

In previous studies, KIM-1 and NGAL have been shown to be early markers for renal injury, but most of these studies have been performed in drug-induced renal injury, shock, or animal models, and less studies have been focusing on the sepsis induced renal damage. If KIM-1 or NGAL can be early biomarkers for diagnosing AKI in patients with sepsis, that will be a major help in early diagnosis of kidney injury in sepsis and may play an important role in the outcome of sepsis. This study was designed to determine the diagnostic and predictive value of KIM-1 and NGAL for septic AKI.

Patients and Methods

Study Population

This study was conducted in the Intensive Care Unit (ICU) of the Emergency, Surgery and Respiratory Department of Beijing Chaoyang Hospital, Capital Medical University. It was approved by the Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University. All patients signed informed consent. We excluded 87 patients from 261 patients based on the inclusion and exclusion criteria. Finally, 184 inpatients from March 2016 to March 2017 were selected as the research subjects (Figure 1). At the same time, 139 patients with sepsis were divided into "AKI-free group" (n = 70) and "AKI group" (n = 69). Pathogens of sepsis include *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Staphylococcus* and the like. We followed all patients for 28 days and recorded their prognosis. Patients discharged within 3 days were also excluded.

No-sepsis and no-AKI Patients

A group of 35 no-sepsis and no-AKI patients (patients who were undergoing general anesthesia) who were admitted to the ICU at the same period, were included in this study as the control group.

Inclusion and Exclusion Criteria

Patients were enrolled under the age of 18 years and with sepsis. Patients with the follow-

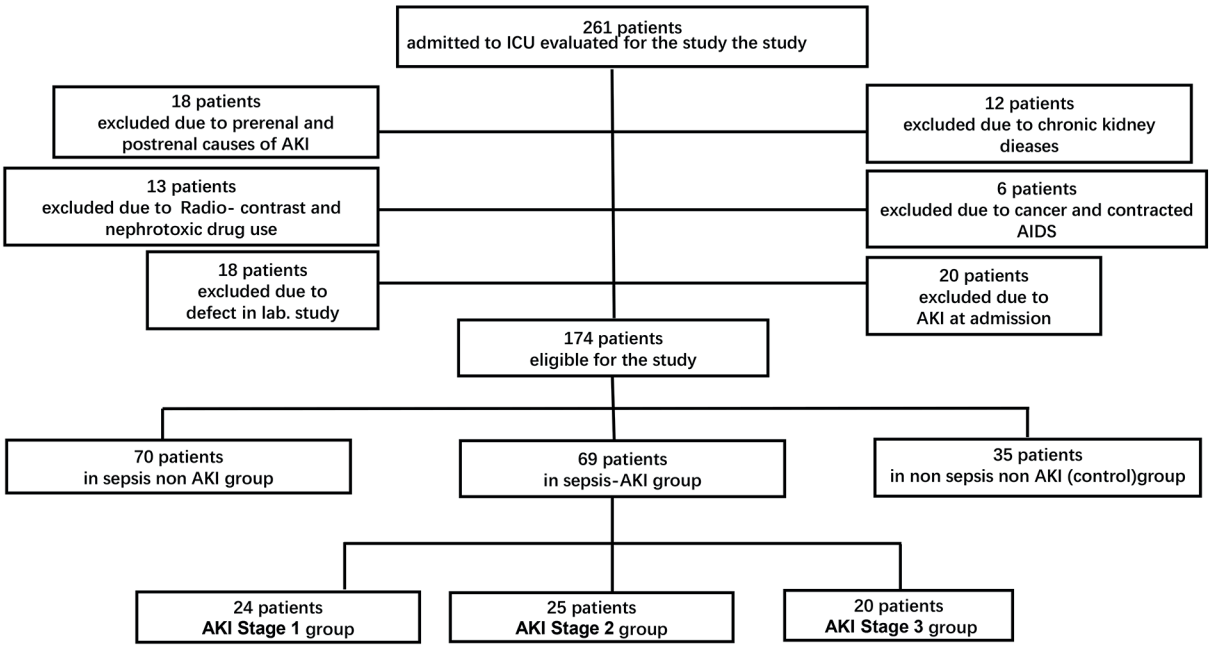

Figure 1. Selection of study patients.

ing conditions were excluded: those infected with AIDS, those receiving high-dose steroids, those receiving kidney transplants, those who have no urine and have cancer, those who have had AKI or CKD, those who refused treatment during the observation period, and were admitted exposure to nephrotoxic drugs or radiocontrast agents in the first 5 days before admission.

Definitions

Sepsis and septic shock were defined according to the Third International Consensus Definitions for Sepsis and Septic Shock[1]. We defined AKI according to Kidney Disease Improving Global Outcomes (KDIGO) criteria[5] using an increase in serum creatinine level ≥ 0.3 mg/dl (≥26.5 μmol/L) within 48 h or an increase in serum creatinine ≥ 1.5 times of baseline within 7 days. There were two baseline definitions of serum creatinine in patients with AKI. For patients without any medical records, the baseline level was the lowest serum creatinine level in the past one or two years. For patients without any medical records and without chronic kidney disease requiring dialysis, the baseline level was the patient's admission serum creatinine level. To assess whether KIM-1 and NGAL could be served as an early predictor of AKI, we included all patients with 'injury' and 'failure' in the statistical analysis. They were evaluated in 'the AKI group'.

Data Collection

We recorded the patient's basic information within 24 hours of admission, including the patient's name, age, gender, source of infection, comorbidities and medical history. It was provided a standardized treatment for all patients including basic technical support such as broad-spectrum antibiotics, drainage and fluid resuscitation. We measured SCr levels at the time of admission and every 24 hours thereafter and recorded the patient's urine volume every hour. To determine the severity of the inflammation, we tested various basic indicators including C-reactive protein (CRP), procalcitonin (PCT) levels and white blood cell (WBC) counts. At the same time, we collected other physiological and clinical information of patients by using acute physiological and chronic health assessment II (APACHE II) and sequential organ failure assessment (SOFA) scores.

Sample Processing and Measurement

Blood and urine of patients were used as samples to measure NGAL and KIM-1 levels. Firstly, we centrifuged the blood for 15 minutes (3,000 rpm) and centrifuged the urine for 5 minutes (2,000 rpm). Then, the supernatant was transferred to an Eppendorf (EP) tube and stored at -80°C. All samples were renumbered before the experiment. Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA; R&D, Minneapo-

lis, MN, USA) was used to measure the urinary KIM-1 levels with a measurable range of 0.2 to 10 ng/mL. ELISA was also used to detect the serum KIM-1 levels with a measurable range of 10.9 to 700 pg/ml. Serum and urinary neutrophil gelatinase-associated lipocalin (NGAL) were measured using an ELISA kit (Life Technologies, Carlsbad, CA, USA) with a measurable range of 7.81 to 500 pg/ml. Their concentrations were determined from standard curves. ELISA was performed in duplicate, and in strict accordance with the instructions of the manufacturers.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows version 20.0 (SPSS Inc., Armonk, NY, USA). Data for normal distribution variables were expressed as mean ± standard deviation (SD), data for non-normal distribution variables were expressed as median. At the same time, we used independent *t*-test or Mann-Whitney test to analyze the comparison of the above variables. In the parametric analysis, the methods of single factor analysis of variance (ANOVA) and post-hoc least significant difference (LSD) analysis were used. The area under the sensitivity and 1-specific receptor operating characteristic (AUROC) curve was used to characterize the diagnostic and predictive indicators of KIM-1 and NGAL for AKI. The concentration of maximum specificity and sensitivity was used to characterize the optimal cutoff. Regression curves were used to characterize the correlation between clinical variables and the occurrence of AKI. p <0.05 was considered statistically significant.

Results

Patient Demographics

Between March 2016 and March 2017, 139 patients with sepsis were finally included in the study (Figure 1). Among them, 70 patients included in the no-AKI group; 69 patients included in the AKI group, as those patients reached the standard of diagnosing AKI within 72 hours after enrollment, and 35 patients who require general anesthesia for non-infectious diseases with no-AKI included as control group. Of the 69 patients who developed AKI within 3 days, 24 [34.8% (23.2-46.4%)] developed stage 1 AKI, 25 [36.2% (24.6-47.8%)] developed stage 2 AKI, 20 [29.0% (18.8-46.6%)] developed stage 3 AKI and 13 [18.8% (10.1-27.5%)] received renal replacement therapy.

Patients ranged in age from 18 to 96 years (66.61±16.87), and 105 patients were male (60.3%). The three groups of patients had no significant differences in gender, combined underlying disease and baseline creatinine levels (**Additional Chart 1**). However, in the APACHE II score and GCS score, the 'no-AKI group' and 'AKI group' were significantly higher than the control group. Compared with the no-AKI and AKI group, the control group had more stable vital signs and fewer abnormalities, required a shorter ICU stay, and the mortality rate was lower than the two sepsis groups (2.86%, 11.43%, 50.72%, respectively, p <0.001) (Table I).

Compared to the no-AKI patients, AKI patients were older and had greater illness severity on presentation and more comorbidities, the AKI group had more patients with septic shock and coagulation dysfunction, and used more antihypertensive drugs, and there were significant differences in cardiac biomarkers (NT-proBNP) and MAP in the two groups. The AKI group had higher APACHE II scores and SOFA scores, and the mortality of AKI group was significantly higher than the no-AKI group. By 28 days after ICU admission, 35 patients (50.7%) in the AKI group died compared to 8 (11.4 %) of the 70 non-AKI patients (p <0.001) (Table I).

Biomarker Characteristics

The urinary and serum KIM-1 and NGAL levels were compared between the 3 groups, and significant differences were found on both urinary and serum KIM-1 and NGAL levels (p = 0.009, <0.001, <0.001, 0.003, respectively) (**Additional Table I, Additional Table II**). In addition to sKIM-1, uKIM-1, uNGAL, sNGAL all showed no significant difference between the control group and the no-AKI group (p=0.242, 0.644, 0.366, respectively) (Figure 2). We found that both urinary and serum KIM-1 and NGAL in the AKI group were significantly higher than the no-AKI group (p-values were 0.030, <0.001, <0.001, 0.001, respectively) (Figure 2). In the meantime, we evaluated the value of KIM-1 and NGAL in AKI severity grading (based on the KDIGO grading scale), we divided the 69 sepsis patients with AKI into AKI Stage 1, 2 and 3, and found that both uNGAL and sNGAL were significantly different between severe AKI (AKI Stage 2 and 3) and mild AKI (AKI Stage 1). The uNGAL and sNGAL in severe AKI were significantly higher (p = 0.002, 0.039, respectively). But the uKIM-1 and sKIM-1 did not show the differences between severe AKI and mild AKI in this study (Figure 2).

Table I. The positive expression rates of CRKL in laryngeal squamous cell carcinoma (LSCC) and normal laryngeal mucosa (control).

		Sepsis (n=139)		
	Control (n=35)	no-AKI (n=70)	AKI (n=69)	*p*-value
Male sex (n)	18 (51.43%)	47 (67.14%)	40 (57.91%)	0.262
Age (years)	68.71±13.82[a]	60.33±18.78[a,b]	66.88±15.41[b]	0.019
Basal sCr (μmol/L)	65.04±16.34	65.93±18.58	70.20±13.42	0.189
Admission sCr (μmol/l)	66.75±15.65[c]	66.88±16.59[b]	88.75±23.81[b,c]	<0.001
APACHE II score	8.31±3.65[a,c]	13.63±5.60[a,b]	21.67±6.65[a,b,c]	<0.001
GCS score	14±2.16[a,c]	11.91±3.32[a]	11.19±3.47[c]	<0.001
SOFA score	2.43±2.15[a,c]	5.36±2.85[a,b]	9.55±4.17[c,b]	<0.001
Diabetes mellitus	6 (17.14%)	14 (20.20%)	22 (31.88%)	0.146
Hypertension	17 (48.57%)	23 (32.86%)	31 (44.93%)	0.203
Ischemic heart disease	4 (11.43%)	11 (15.71%)	18 (26.09%)	0.132
COPD/asthma	0	6 (8.57%)	4 (5.80%)	0.205
Use of vasopressor drugs	——	15 (21.43%)	35 (50.72%)	<0.001
MAP (mmHg)	92.92±12.54[a,c]	80.40±18.73[a,b]	72.36±16.59[b,c]	<0.001
Heart rate (bpm)	86.77±14.22	108.39±23.92[b]	121.61±30.34[b]	0.005
RR (bpm)	20 (16,22)[c]	25.5 (20.75,32)[b]	29 (20,35.5)[b,c]	<0.001
FIO2	0.4 (0.29,0.50)[c]	0.385 (0.30,0.50)[b]	0.5 (0.33,0.70)[b,c]	0.011
Oxigenation Index	396.67 (292.00,447.62)[a,c]	233.15 (148.50,334.00)[a,b]	192.68 (106.75,281.29)[b,c]	<0.001
PCO2 (mmHg)	37.00 (32.90,42.00)	39.50 (35.00,46.00)[b]	36.00 (32.00,43.00)[b]	0.052
PH	7.4 3 (7.40,7.47)[a]	7.45 (7.42,7.48)[a,b]	7.42 (7.38,7.46)[b]	0.001
HCO3 (mmol/L)	22.95±7.07[a]	28.52±7.53[a,b]	24.48±6.73[b]	<0.001
BE (mmol/L)	0.47±3.15[a]	4.69±6.60[a,b]	1.03±6.98[b]	0.001
WBC (x10^9/L)	9.41 (6.55,12.24)	10.16 (7.03,14.24)	11.14 (6.71,16.46)	0.235
NE (%)	85.20 (76.9,90.4)	85.25 (73.375,90.6)	85.30 (77.05,91.75)	0.263
RBC (x10^12/L)	3.68±0.57	3.59±0.94	3.44±0.96	0.388
HCT	32.63±5.01	32.19±7.42	30.90±7.71	0.315
HGB (g/L)	110.83±17.40	107.90±25.90	103.09±25.48	0.261
PLT (x10^9/L)	186.94±68.78	198.73±100.64[b]	152.06±95.98[b]	0.017
CRP (mg/dl)	——	7.52±7.37	12.80±8.26	0.003
PCT (ng/ml)	——	0.61 (0.13,2.67)	5.71 (1.43,17.78)	0.001
D-Dimer (mg/L)	3.96 (2.19,9.44)	2.72 (1.22,7.37)	4.35 (2.63,10.41)	0.493
PT (s)	12.13±1.32[c]	12.89±2.22[b]	15.98±11.54[b,c]	0.017
PA (%)	85.63±9.14[c]	81.31±12.50[b]	72.50±17.23[b,c]	<0.001
FBG (mg/dl)	345.07±148.77	401±163.49[b]	342.06±177.25[b]	0.087
APTT (s)	34.55±5.28[c]	36.93±10.20[b]	49.29±30.63[b,c]	<0.001
TBIL (umol/L)	14.90 (9.07,20.305)	13.05 (8.50,13.05)[b]	15.2 (10.80,38.90)[b]	0.085
NT-proBNP (pg/mL)	227.70 (68.56,684.10)	451.95 (176.83,1916.00)[b]	2234.00 (704.20,9763.00)[b]	<0.001
Mechanical ventilation (MV)	23 (65.71%)	50 (71.43%)	53 (76.81%)	0.469
CVVH	——	0	13 (18.84%)	<0.001
ECMO	——	4 (5.71%)	6 (8.70%)	0.496
Coagulation dysfunction	——	10 (14.29%)	37 (53.62%)	<0.001
ARDS	——	20 (28.57%)	30 (43.48%)	0.067
Septic shock	——	21 (30.30%)	46 (66.67%)	<0.001
MV (hours)	15.17±2.66[a,c]	179.71±42.57[a]	221.97±34.37[c]	0.442
ICU LOS (days)	3.89±1.87[a,c]	13.6±1.98[a]	15.39±1.67[c]	0.492
Death within 28 days	1 (2.86%)[a,c]	8 (11.43%)[a,b]	35 (50.72%)[b,c]	<0.001

Values in table are reported as the frequency [number] with the percentage in parenthesis for categorical variables, mean ± standard deviation or median and interquartile range. p-value of statistical significance. ***a $p < 0.05$ between non-sepsis non AKI group (control group) and sepsis with non-AKI group (no-AKI group); ***b $p < 0.05$ between sepsis with non-group (no-AKI group) and sepsis with AKI group (AKI group); ***c $p < 0.05$ between Control group and AKI group. AKI acute kidney injury, APACHE II acute physiology and chronic health evaluation II, ICU intensive care unit, LOS length of stay, SOFA sequential organ failure assessment score, CVVH Continuous veno venous hemofiltration, ECMO extracorporeal membrane oxygenation.

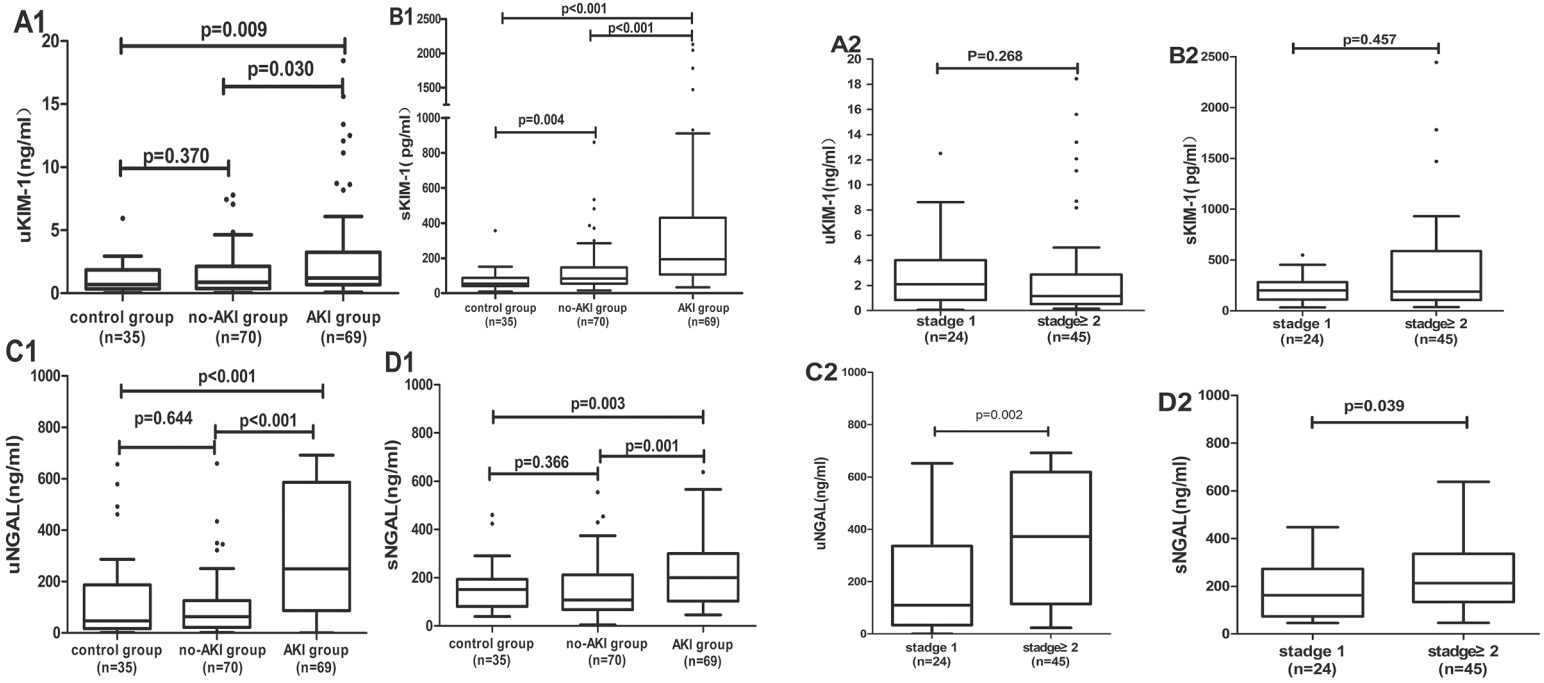

Figure 2. Comparison of the urinary and serum levels of KIM-1 and NGAL in different groups. **A1, B1, C1, D1** show the different concentration levels of uKIM-1, sKIM-1, uNGAL and sNGAL in the non-sepsis non-AKI group (the control group), the sepsis with no-AKI group (the no-AKI group) and the sepsis with AKI group (the AKI group). **A2, B2, C2, D2** delegate the different concentration levels of uKIM-1, sKIM-1, uNGAL and sNGAL in mild AKI (AKI Stage 1) and severe AKI (AKI Stage 2 or 3). Lines denote median values, boxes represent 25th to 75th percentiles and whiskers indicate the range. Numbers of samples are indicated in parentheses.

Table II. Univariate and multivariable analysis of different variables for prediction of AKI.

	Univariate		Multivariable	
Variables	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Age	1.023 (1.002,1.044)	0.029	——	——
MAP	0.974 (0.955,0.994)	0.010	——	——
PH	0.001 (0.000,0.205	0.010	——	——
PLT	0.995 (0.992,0.999)	0.012	——	——
PCT	1.013 (1.001,1.025)	0.041	——	——
HCO3	0.917 (0.867,0.970)	0.003	——	——
TBIL	1.014 (1.000,1.027)	0.047	——	——
NT-proBNP	1.000 (1.000,1.000)	0.001	——	——
Coagulation dysfunction	6.937 (3.056,15.748)	<0.001	3.66 (1.337,10.048)	0.012
Use of vasopressor drugs	3.775 (1.800,7.917)	<0.001	——	——
Septic shock	5.000 (2.432,10.280)	<0.001	4.989 (1.287,19.337)	0.020
APACHE II	1.241 (1.149,1.341)	<0.001	——	——
SOFA at ICU admission	1.391 (1.230,1.574)	<0.001	1.551 (1.065,2.259)	0.022
uKIM-1	1.198 (1.037,1.384)	0.014	——	——
sKIM-1	1.002 (1.001,1.004)	0.011	——	——
uNGAL	1.006 (1.004,1.009)	<0.001	1.004 (1.001,1.007)	0.022
sNGAL	1.004 (1.002,1.007)	0.002	——	——

In addition to the analysis of the value of urinary and serum KIM-1 and NGAL in predicting the occurrence of AKI and the severity of AKI, an assessment was made in order to find the values of the four markers in prediction or diagnostic of the sepsis with the mortality, shock, DIC, and ARDS. We found that all the four biomarkers had no difference in sepsis with or without ARDS. As to uKIM-1 and sKIM-1, there were significant differences between sepsis with or without shock (p=0.028, 0.039, respectively) and DIC (p=0.05, 0.018, respectively). However, both uKIM-1 and sKIM-1 could not be used to predict the mortality of sepsis (p=0.848, 0.199, respectively). Urinary and serum NGAL had good performance in prediction of the septic AKI and the severity of AKI; besides that, in the sepsis with or without shock, and with or without DIC group, there were significantly differences (p=0.001, 0.012, <0.001, 0.022, respectively), and in the sepsis death and survival group, significantly differences were also found (p= 0.031, 0.011, respectively). The uNGAL and sNGAL were potential biomarkers for judging sepsis complications and prognosis according to this study (Figure 3).

Value of KIM-1 and NGAL for Predicting the Development of AKI and the Severity of AKI

AUROC for the diagnosis of AKI was 0.607 for uKIM-1, 0.754 for sKIM-1, 0.768 for uNGAL and 0.658 for sNGAL. The four biomarkers had fair value for the early prediction of AKI caused by sepsis. For KIM-1, the AUROC of sKIM-1 to predict AKI development was as good as the uKIM-1 (p-values were <0.001, 0.0254, respectively) (Figure 4, **Additional Table III**).

In predicting the severity of AKI, AUROC for the diagnosis of sever AKI was 0.727 for uNGAL, 0.652 for sNGAL, and when the cut-off of uNGAL was 493.45 ng/ml, the specificity to predict the sever AKI (KIDGO grade ≥ 2) could reach 91.67%. The value of sNGAL for predicting the severity of AKI was slightly lower than that of uNGAL. When the cut-off was set at 195.59 ng/ml, the sensitivity and specificity of severe AKI prediction could reach more than 60% (Figure 4). The urinary and serum NGAL could both predict the septic AKI and severity of AKI, but as to the urinary and serum KIM-1, there were no significant values in predicting the severity of renal injury.

We further combined of urinary and serum KIM-1 and NGAL in different groups, to predict the occurrence of septic AKI and found that uNGAL combined uKIM-1 and sKIM-1 together could increase the prediction of septic AKI to 0.806 in AUROC, and the combination detection of the four biomarkers to predict the septic AKI's AUROC could also reach 0.806.

Univariate and Multivariable Analysis of Different Variables for Prediction of AKI

We first conducted a one-way regression analysis of various factors that may affect AKI in sepsis patients and we found the significant differences of 17 indicators (including relevant tests on

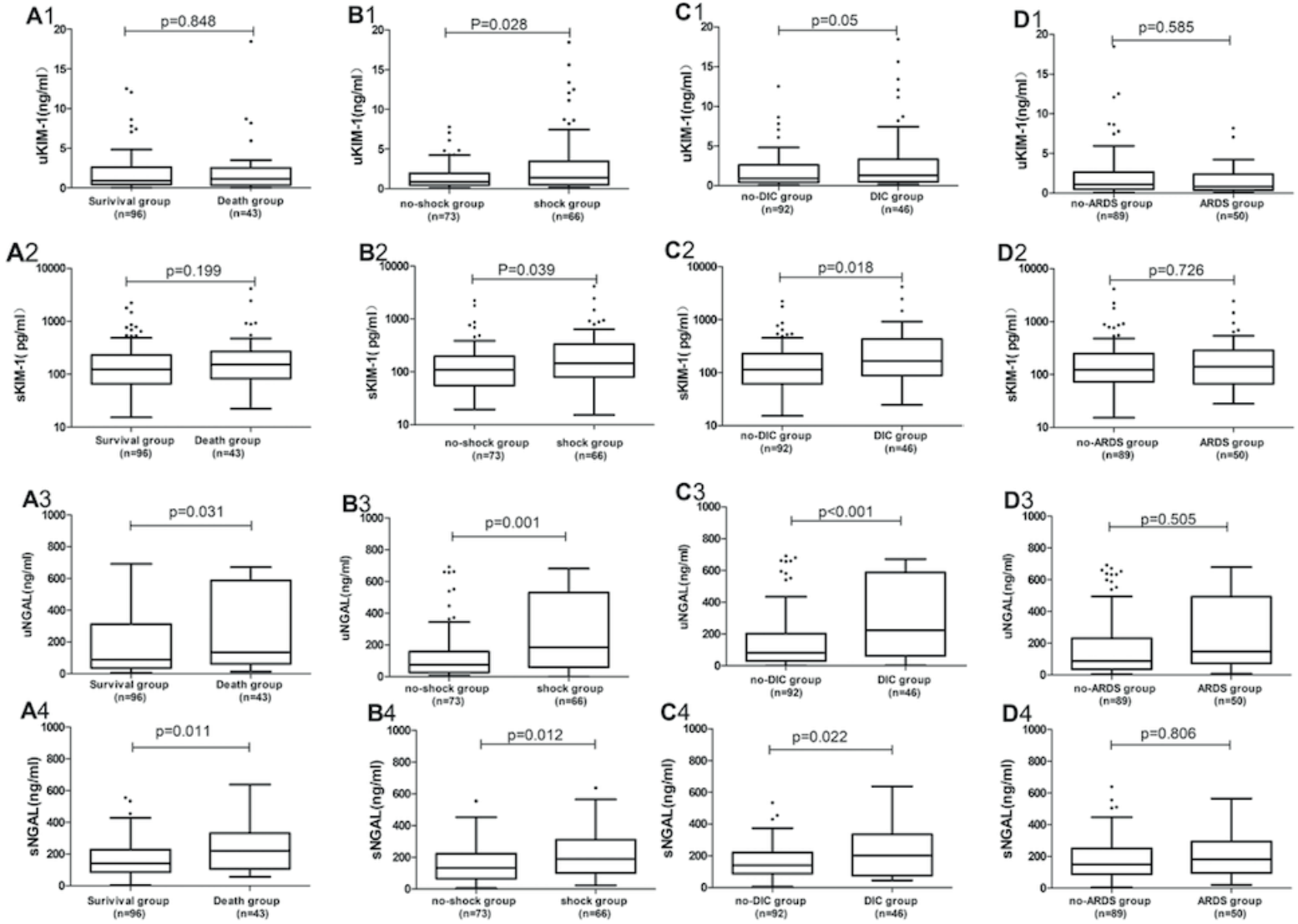

Figure 3. Comparison of the levels of KIM-1 and NGAL in sepsis with different condition. **A1, A2, A3, A4** shows the differences between the survival and the death group of the sepsis patients in the uKIM-1,sKIM-1,uNGAL,sNGAL; **B1, B2, B3, B4** shows the differences between the sepsis patients with or without shock in the uKIM-1, sKIM-1, uNGAL and sNGAL; **C1, C2, C3, C4** show the differences between the sepsis patients with or without DIC in the uKIM-1, sKIM-1, uNGAL and sNGAL; **D1, D2, D3, D4** show the differences between the sepsis patients with or without ARDS in the uKIM-1, sKIM-1, uNGAL and sNGAL.

admission such as arterial blood gas, coagulation index, liver function, heart function status, presence or absence of shock, etc.) in the AKI and no-AKI group. Multivariate regression analysis was then performed on the 17 univariate variables, and we finally found that sepsis shock at enrollment, coagulation dysfunction (DIC), SOFA scores at admission, and uNGAL were four independent risk factors for sepsis patients developed AKI (Table II). The four indexes predicted the AUROC of septic AKI were 0.690 (OR=4.989), 0.697 (OR=0.3666), 0.788 (OR=1.551) and 0.768 (OR=1.004), respectively. The four indexes were able to predict AUROC of septic AKI as high as 0.857 (95% CI, 0.787-0.910) (Figure 5, **Additional Table IV- Additional Table V**).

The Role of Urinary and Serum KIM-1 and NGAL In Predicting the Prognosis of Sepsis

In this study, we analyzed the role of urinary and serum KIM-1 and NGAL in predicting the prognosis of sepsis patients. We found that both the uNGAL and sNGAL could predict the survival of sepsis. The uNGAL AUROC for death risk of sepsis was 0.619. If we set the cut-off value as 85.33 ng/ml, the sensitivity was 69.23% and specificity was 50%. The sNGAL had an AUROC of 0.640 for predicting the risk of death, and a cut-off value of 185.28 ng/ml had a sensitivity and specificity of more than 60%. However, in our study, both uKIM-1 and sKIM-1 had no value in predicting sepsis mortality (Figure 6, **Additional Table VI**).

Discussion

We conducted a prospective, exploratory study assessing the diagnostic and prognostic values of two novel potential AKI biomarkers (KIM-1 and NGAL) in septic AKI patients. In this study, we found that both urinary and serum KIM-1 and NGAL levels were higher in patients who developed AKI than in those who did not within 72 hours after confirmed as sepsis in ICUs. This study revealed the values of using urinary and serum KIM-1 and NGAL as early prediction of biomarkers of septic AKI. Besides, we found that the urinary and serum NGAL had fair value in predicting the severity of AKI, and only the uNGAL at admission besides SOFA score, coagulation dysfunction and septic shock were independently risk factors for predict septic AKI.

Acute kidney injury is frequently associated with sepsis, particularly when presence of shock[4,23]. In this study, of the 139 sepsis patients, 66 (49.6%) patients with septic shock, and 69 patients (49.6%) developed AKI. AKI incidence rate and severity correlate with the severity of the underlying sepsis[24]. AKI is an important cause of mortality and morbidity in ICUs. Septic AKI is a hallmark of sepsis and septic shock and is associated with worse outcomes including prolonged hospital length of stay, fewer ventilator-free days and increased mortality when compared to patients with non-septic AKI[4,25]. Our study remarkably pointed out as the rate of mortality was significantly higher in 'sepsis with AKI' patients when compared with 'sepsis with no-AKI' patients (11.43% *vs.* 50.72% $p<0.001$). We found that the chances of occurrence of AKI in sepsis shock group were significantly higher than that of sepsis without shock group (28.67% *vs.* 66.67% $p<0.001$). In the meantime, patients with septic AKI were generally sicker, with a higher burden of illness, and have greater abnormalities in acute physiology compared with patients with non-septic AKI. There was also a relatively bigger difference between the two groups in demanding of respiratory support (FIO_2 0.385 *vs.* 0.5 $p=0.042$), and the incorporate of ARDS (28.57% *vs.* 43.48 $p= 0.067$), but in the ratio of ECMO support, there was no significant difference between the two groups (0.06% *vs.* 0.09% $p= 0.496$).

Several factors have been implicated in the pathogenesis of septic AKI. Hemodynamic changes in the macro circulation (i.e., vasodilatation and increased cardiac output), and systemic and renal microcirculation contribute to renal hyperemia coupled with inefficient cellular oxygen. Sepsis is also associated with systemic inflammation and endothelial dysfunction, which also have been shown to contribute to renal injury and enhance microcirculation perturbations[26,27]. In this clinical study, CRP and PCT, which were associated with the severity of response to inflammation, were significantly higher in the sepsis with AKI group than in the sepsis with no AKI group ($p=0.003$, $p=0.001$, respectively). We also found that the incidence of AKI in patients with coagulopathy was significantly higher than those without coagulopathy (14.29% *vs.* 53.62%, $p<0.001$), and all of those were consistent with the pathophysiological mechanism of sepsis-induced AKI.

Recent diagnostic and staging criteria for AKI included an absolute increase of serum creatinine or a urinary output. Severity of septic AKI may be

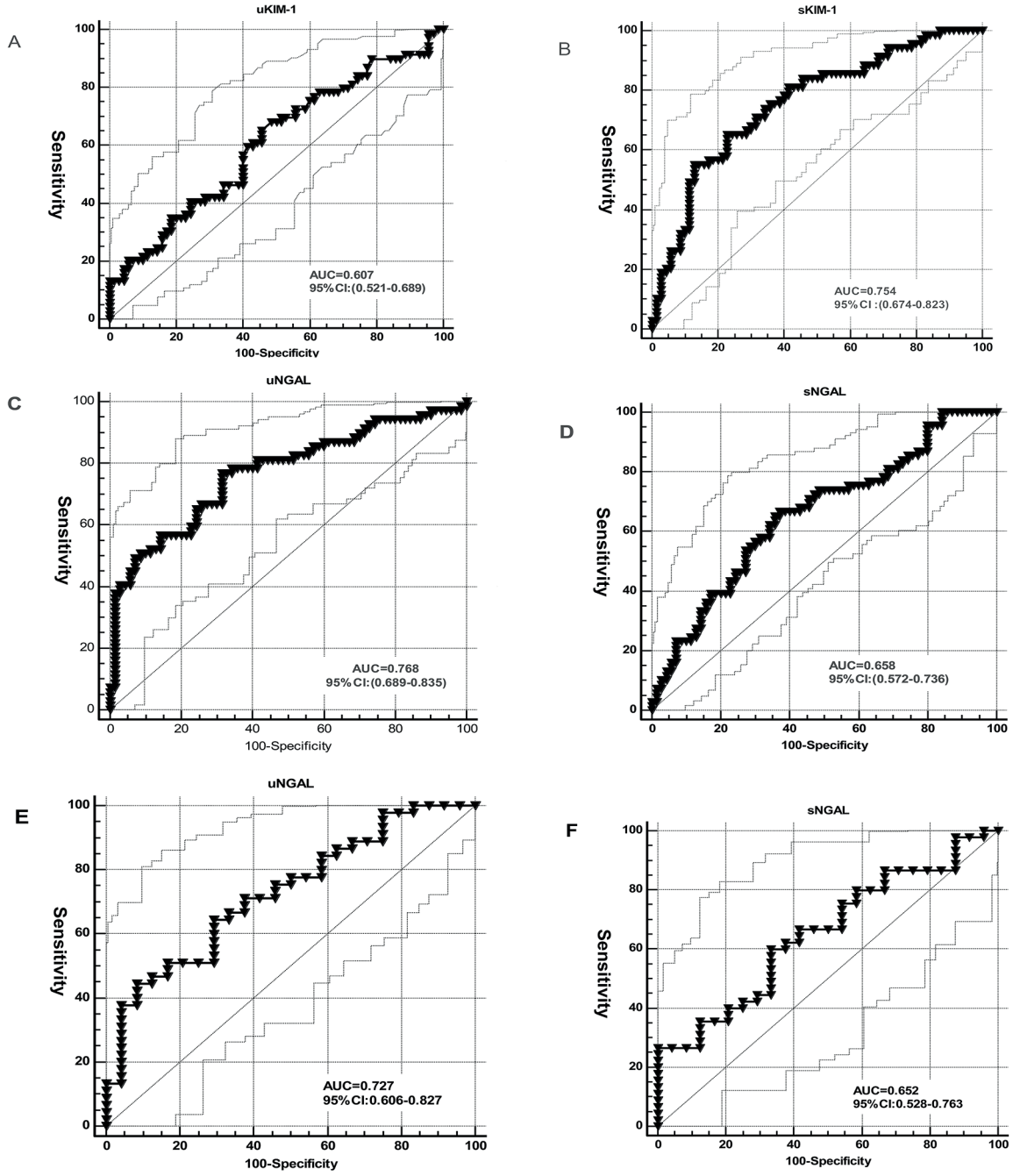

Figure 4. Receiver operating characteristic curves of KIM-1 and NGAL for diagnosis of septic AKI. AUROC curves A, B, C, D demonstrating the predictive ability of urine and serum KIM-1 and NGAL to predict septic AKI. AUROC curves E and F demonstrating the predictive ability of Serum of NGAL in Predict severity of AKI.

classified using the well documented consensus KDIGO criteria for AKI staging, and outcomes appear to be correlated with the presence and severity of AKI as defined by this classification system[24,28]. Several pitfalls are associated with the use of serum creatinine and urinary output for the diagnosis of septic AKI. As both these are late consequences of injury and are not markers of the injury itself[29]. But an early identification of AKI in septic patients is crucial, so newer biomarkers are increasingly being studied for rapid AKI diagnosis.

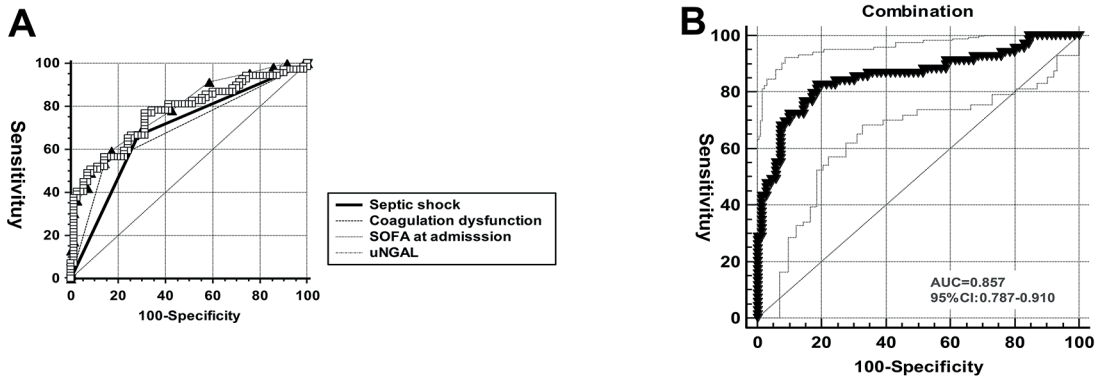

Figure 5. Receiver operating characteristic (ROC) curves for septic AKI of different risk factors. **A**, demonstrating the predictive ability of septic shock, coagulation dysfunction, SOFA scores at admission and uNGAL for predicting AKI in septic patients. **B**, demonstrating the predictive ability of the four risk factors together for predicting septic AKI.

KIM-1 and NGAL were studied more in recent years, both in the temporary and animal experiments. However, the conclusions were not consistent, especially the sNGAL. As to KIM-1, most studies were focused on urinary KIM-1, and only little attention had been paid to the KIM-1 in the blood[16]. The two markers (KIM-1 and NGAL) were less frequently studied in sepsis patients jointly. The purpose of this study was to investigate the diagnostic utility of urinary and serum KIM-1 and NGAL in prediction of AKI in sepsis patients. To the best of our knowledge, there have been no studies that investigated both urinary and serum KIM-1 and NGAL for septic AKI so far.

The previous studies of KIM-1 mostly focused on uKIM-1, and the uKIM-1 is a sensitive and specific urinary biomarker of kidney injury in both rodent models and humans[15,30,31]. The US Food and Drug Administration and the European Medicines Agency qualified KIM-1 as a urinary biomarker in the context of drug-induced nephrotoxicity[32]. Patients with severe kidney damage may soon experience anuria, and urine specimens are not readily available, so it is necessary to investigate the characteristic of the blood KIM-1. In this study, we not only examined the uKIM-1 but also the sKIM-1 in the sepsis patients, and we found that the concentration of KIM-1 was higher in the sepsis with AKI group than the sepsis with no-AKI group in both urinary and serum. There was a significant increasing of sKIM-1 between the non-infected group and the sepsis group, as also reported by Angiari et al[33]. KIM-1 might be served as a potential inflammatory marker. Our study found that both serum and urinary KIM-1 could predict the septic AKI within 72 hours. However, neither serum or urinary of KIM-1 showed any significant values in predicting the severity of AKI, which is consistent with the secretory mechanism of KIM-1, as KIM-1 not only reflects the injury, but also increases at the time of repair and the regeneration process. Many mild AKI begins to repair at the time of injury, and KIM-1 may play an important role in this process[10], so the elevation of KIM-1 does not distinguish well whether the kidney injury is in the process of 'injury' or 'repair'. That is why KIM-1 could not be used as a predictive marker for the severity of AKI.

A previous research found that, after ischemic or nephrotoxic kidney injury, intrarenal NGAL is dramatically upregulated, elevated NGAL protein can detect in the urinary as early as 3 hours after injury, and the peak is about 6 hours after injury; there is some evidence of sustained elevation in its concentration as long as 5 days post injury[11,34]. Serum NGAL also increases after AKI as a result of increased hepatic production[35]. Thus, both urinary and serum NGAL can potentially exert an effect on the intrarenal molecular and cellular events that occur during AKI, and both have been used to predict the onset and course of AKI. Consistent with previous studies, we indicated that the NGAL was elevated in the urinary and serum of the sepsis patients with AKI, and there was significant difference between the mild AKI and the sever AKI. The AUROCs of uNGAL and sNGAL all showed fair value in the prediction of the occurrence of AKI, the severity of AKI and the prognosis of sepsis. Besides that, the different concentration levels of urinary and serum NGAL in the sepsis patients with or without shock, DIC, ARDS and mortality also displayed that NGAL could reflect the severity of sepsis.

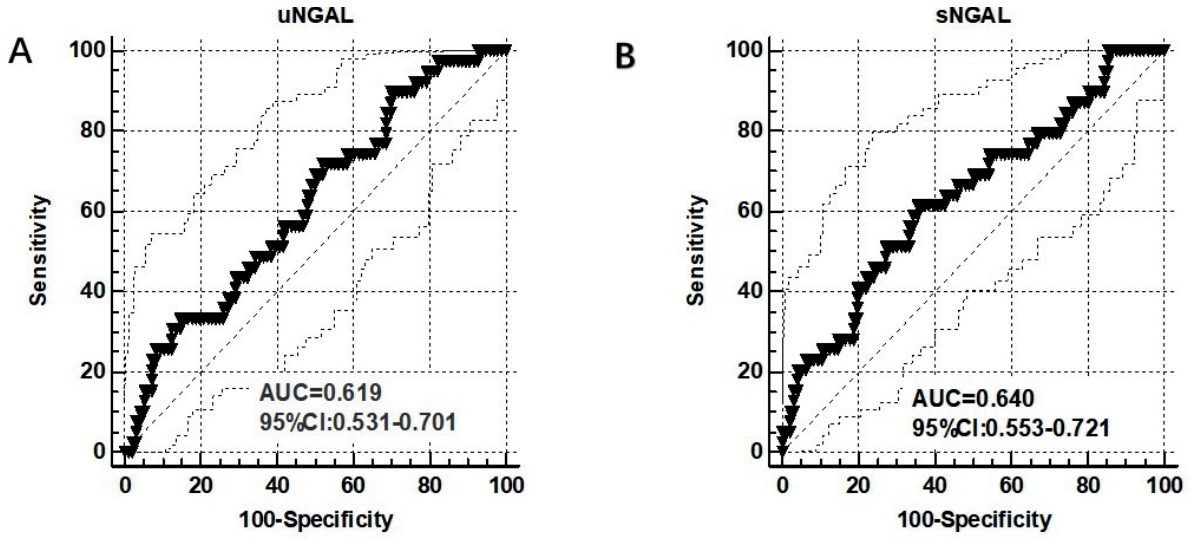

Figure 6. Receiver operating characteristic (ROC) curves of urine and serum NGAL in predicting the death of sepsis. **A**, demonstrating the predictive ability of uNGAL for predicting 28-day mortality in sepsis patients. **B**, demonstrating the predictive ability of the sNGAL for predicting 28-day mortality of sepsis patients.

In this study, we only tested one time the urinary and serum of KIM-1 and NGAL within 24 hours; after the patients who were diagnosed as sepsis, were included in our study, and the AUROCs of the four biomarkers also showed fair value in prediction of septic AKI. KIM-1 and NGAL once again proved to be promising early biomarkers of AKI, even in the sepsis setting. Meanwhile, we found that the AUROC of the combination of KIM-1 and NGAL was higher than that of either biomarker alone, and that was consistent with previous reports that a combination of two biomarkers may have better diagnostic performance than a single biomarker[36].

In our univariate logistic regression analysis, we found a number of factors associated with the development of sepsis AKI; however, when conducting the multivariate logistic regression analysis, we found that the uNGAL, SOFA score at admission, coagulation dysfunction and septic shock were independently risk factors for septic AKI, and their combined predicted AKI AUROC could be as high as 0.857 with both the sensitivity and the specificity were over 80%. This is consistent with the conclusions of the current relevant studies[37,38].

Conclusions

This was the first study that explored both urinary and serum KIM-1 and NGAL for the diagnosis of AKI in sepsis. We found that sKIM-1, like the uKIM-1 and serum and urine NGAL, also had the ability to predict septic AKI. Besides that, both urinary and serum NGAL had the value of diagnosis the severity of the AKI and could predict the prognosis of sepsis, and elevated urinary NGAL levels besides SOFA score, coagulation dysfunction and septic shock were independently risk factors in the prediction of septic AKI. The urinary and serum KIM-1 could predict the occurrence of septic AKI, but they could not be used to judge the severity of renal injury's stage and predict the prognosis of sepsis.

Declarations

Ethics approval and consent to participate

The protocols were approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University (2016-R-115).

Consent for publication

Patients or their family members were fully informed of the study details and signed the informed consent forms of their own accord.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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Authors' contributions

HJW, CFZ, and ZHT conceived of the study. CZ and HQY were involved with retaining specimens. CFZ, YSW and RRG conducted data collection. CFZ and HJW were responsible for statistical analysis. CFZ, HJW and ZHT drafted the manuscript. HJW and HZS were responsible for verifying data and modifying articles. All authors were involved in reviewing the manuscript.

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