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Urinary kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin as early biomarkers for predicting vancomycin-associated acute kidney injury: a prospective study

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Abstract. – OBJECTIVE: Previous studies have demonstrated that urinary kidney injury molecule-1 (uKIM-1) and neutrophil gelatinase-associated lipocalin (uNGAL) were superior to serum creatinine (Scr) in detecting acute kidney injury (AKI), but their ability to predict clinical vancomycin-associated AKI has not been investigated. This study aimed to investigate the abilities of uKIM-1 and uNGAL individually and in combination to predict vancomycin-associated AKI.

PATIENTS AND METHODS: Scr, uKIM-1, and uNGAL were measured on the day before and days 1, 2, and 3 of vancomycin therapy in a generalized adult population. Levels of these biomarkers between AKI and non-AKI groups were comparatively analyzed. Predictive performances were evaluated by receiver operating characteristic curve (ROC) analysis.

RESULTS: A total of 87 patients were enrolled, and among them, 11 (12.6%) patients developed AKI. Urinary KIM-1 and NGAL levels in the AKI group were higher than in the non-AKI group at all time points ($p < 0.05$), and the areas under the receiver operating characteristic curves (AUC) were 0.849 (95% confidence interval [CI] 0.750-0.948) for uKIM-1 and 0.824 (95% CI 0.726-0.922) for uNGAL, with cut-off values of 1.72 ng/mL and 9.07 ng/mL respectively. The AUC of uKIM-1 and uNGAL combined was 0.852 (95% CI 0.754-0.949), and the sensitivity and specificity were 90.9% and 75.0%, respectively.

CONCLUSIONS: Urinary KIM-1 and NGAL could efficiently discriminate patients with or without vancomycin-associated AKI earlier than Scr, and the combined urinary biomarkers showed fair discrimination compared with the individual biomarkers.

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

Introduction

Vancomycin is a glycopeptide antibiotic that remains the "gold standard" for treating methicillin-resistant *Staphylococcus aureus* (MRSA) infections, but the adverse side effects include nephrotoxicity and ototoxicity[1,2]. The occurrence rate of vancomycin-induced nephrotoxicity is approximately 10-40% in different populations[3]. A trough concentration of approximately ≥ 15 mg/L significantly increased the risk of nephrotoxicity, especially in critical patients or the elderly, leading to poor prognosis and even mortality[3,4]. However, a higher trough level (15 mg/L-20 mg/L) has been recommended by the Infectious Diseases Society of America Clinical Practice Guideline for treating serous MRSA infections since 2009[5]. Therefore, it is crucial to promptly diagnose and intervene in vancomycin-associated acute kidney injury (AKI). However, in clinical practice, the diagnosis of kidney injury relies almost entirely on serum creatinine (Scr), which is acknowledged as a lagging indicator and is influenced by many confounding factors[6,7]. This has hampered clinicians in early detection of AKI and timely intervention measures.

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Currently, noninvasive sensitive biomarkers, such as urinary kidney injury molecule-1 (uKIM-1) and neutrophil gelatinase-associated lipocalin (uNGAL), have been widely reported to outperform Scr in predicting AKI in various clinical settings[8,9]. In addition, the combination of these biomarkers may show enhanced efficacy[10,11]. However, there were also some controversies regarding the use of these markers[12-14]. KIM-1, known as a type 1 transmembrane protein, is strongly expressed by proximal tubular epithelial cells in response to injury and is released into the urine[15]. NGAL is a 25 kDa small molecular protein that belongs to the lipocalin superfamily[16], which is specifically induced in damaged nephrons and can be rapidly measured in blood and urine[17,18]. Thus far, the predictive performance of uKIM-1 has been explored among patients with ischemia[19] and those who received chemotherapy[20], cardiac surgery[21], nephrotoxicant[13], and contrast agents[22]. Urinary NGAL was assessed as an early biomarker to predict AKI in critically ill patients[23], those who received cardiac surgery[24], contrast infusion[25], amphotericin[26], and chemotherapy[27], and others. In addition, it was also shown to be an indicator for chronic renal failure[28]. Nevertheless, the usefulness of uKIM-1 and uNGAL in detecting clinical vancomycin-associated AKI has yet to be studied.

Herein, we designed a prospective study to evaluate the performance of uKIM-1 and uNGAL individually and in combination for predicting vancomycin-associated AKI in adult patients.

Patients and Methods

Patients

Patients were included in this study if they received intravenous infusion of vancomycin (Eli Lilly, K.K, Seishin Laboratories, Kobe, Japan) ≥ 3 days, were aged ≥ 18 years old, had achieved at least one steady-stage vancomycin trough concentration (Van Css), and had routine measurements of Scr for determining the occurrence of AKI (on days 0-3 of vancomycin therapy and at least 2 times afterwards within 7 days of vancomycin therapy). Exclusion criteria were: patients who had disseminated intravascular coagulation, cystic fibrosis or urinary tract infections; patients who received vancomycin therapy more than 3 days within 7 days before our study; patients who had an absolute neutrophil count < 1,000 cells/mm^3 or a baseline Scr ≥ 2.0 mg/dL; patients who received continuous renal replacement treatment or hemodialysis; patients who had pre-existing AKI or chronic kidney diseases or were without sufficient monitoring of Scr.

Ethical Approval

This work complied with clinical practice guidelines and the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University. All registered patients or their relatives accepted the protocol and provided written informed consent.

Study Design

This was a prospective study conducted in The First Affiliated Hospital of Guangxi Medical University from January 2015 to November 2016. Patients were divided into the AKI group and non-AKI group, and then, the levels of Scr, uKIM-1 and uNGAL between the two groups were compared.

Data Collection

The patient's demographics (e.g., sex, age, weight), laboratory data (e.g., Scr, white blood cell count, neutrophil count, aspartate aminotransferase, albumin), diagnosis of infection, and comorbidities (e.g., diabetes mellitus, and hypertension) were collected from the electronic medical records. The Van Css was determined prior to the fourth or fifth dose of vancomycin and measured by the enzyme-multiplied immunoassay technique using the Siemens Viva-E system (Siemens Viva-E® Drug Testing System, Newark, DE, USA).

Definitions

Vancomycin-associated AKI was defined by the Improving Global Outcomes (KDIGO) criterion as an increase in Scr of ≥ 0.3 mg/dL (26.5 μmol/L) within 48 h or a Scr level ≥ 1.5 times the baseline level within 7 days[26]. Definitions of the baseline index were the values determined on the day before vancomycin therapy using the medical record system.

Specimen Collection

Urine samples were obtained from each patient on the day before and on days 1, 2, and 3 of vancomycin therapy and then centrifuged

at 3000 revolutions per minute (rpm) for 20 min. The supernatant aliquots were frozen and stored at -80 °C until they were tested in the laboratory in batches. The urinary biomarkers were only monitored up to day 3 of vancomycin therapy because they were reported to substantially increase within 24 h after AKI occurrence. Many patients were given a revised dose or another therapeutic regimen after 3 days of vancomycin therapy according to the results of the monitoring trough concentration, which might affect the biomarker levels of each individual. Additionally, our research funding was limited, and thus, we did not detect urinary biomarker levels on 7 consecutive days among all patients. We may carry out a more detailed research with more sampling points in the future.

Measurement of Urinary Biomarkers and Serum Biomarkers

Urine samples were thawed at room temperature and then centrifuged at 3000 rpm for 5 min. The levels of uKIM-1 and uNGAL were finally measured in the supernatants by a commercially available Human KIM-1 ELISA kit and Human NGAL ELISA kit, following the manufacturer's instructions (Wuhan Boster Biological Technology Co., Ltd., Wuhan, China). The assay range was 31.2 pg/mL to 2000 pg/mL for uKIM-1 and 156 pg/mL to 10,000 pg/mL for uNGAL. The sensitivity was < 2 pg/mL for uKIM-1 and < 10 pg/mL for uNGAL. Scr was determined by the oxidase method using an automatic biochemical analyzer (HITACHI 7600; Hitachi Co., Ltd. Tokyo, Japan) at the clinical laboratory in the hospital. Scr was measured in the same time frame with urinary biomarkers on days 0-3 of vancomycin therapy for comparison. In addition, it was measured at least 2 times afterwards within 7 days of vancomycin therapy. Due to the different time frames, the Scr levels measured after 3 days of treatment, which were only used for judgment of AKI, were not shown.

Statistical Analysis

Data were analyzed using SPSS version 22.0 statistical software (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as the means (with standard deviation) or medians (with interquartile range), and categorical variables are presented as percentages. Nonparametric Mann-Whitney *U*-tests or independent *t*-tests were used for comparisons of continuous variables and χ^2 or Fisher's exact tests for the categorical variables. Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive performances. In addition, biomarkers at the same time points were combined by a fitted multiple logistic regression model, which was used to obtain the maximum sensitivity and specificity[14]. The Spearman correlation test was employed to evaluate correlations between variables. All *p*-values were two-tailed, and *p*-value < 0.05 was considered statistically significant.

Results

Basic Characteristics of the Patients and Differences Between AKI and non-AKI Groups

After a strict screen of 182 patients, multiple patients were excluded due to failure to meet the criteria. As a result, only 87 patients were included in the study. The basic characteristics of the patients and differences between the AKI and non-AKI groups are shown in Table I. Among 87 patients, 11 (12.6%) patients were diagnosed with AKI. No differences were noted between the two groups with respect to demographics, diagnosis of infection or baseline Scr. The proportions of multiple organ dysfunction syndrome (MODS) (27.3% *vs.* 6.9%, $p = 0.025$) and medians of Van Css (26.3 mg/L *vs.* 9.5 mg/L, $p < 0.001$) were statistically higher in the AKI group than the non-AKI group, while the mean hemoglobin value (89.6 g/L *vs.* 104.2 g/L, $p = 0.002$) showed the opposite trend.

Biomarker Concentrations of the Patients and Differences Between the AKI and non-AKI Groups

Table II displays the concentrations of Scr, uKIM-1, uNGAL on days 0-3 of vancomycin therapy. In brief, the median (interquartile range) uKIM-1 and uNGAL levels at baseline for total patients were 0.65 (0.38-1.26) ng/mL and 9.75 (3.88-24.10) ng/mL respectively. Scr levels were statistically higher in the AKI group than in the non-AKI group until 2 days after vancomycin therapy ($p < 0.05$, Table II). However, levels of uKIM-1 and uNGAL were statistically higher in the AKI group compared to the non-AKI group at all time points ($p < 0.05$, Table II). For analysis of the AKI group alone, there were increased

Table I. Basic Characteristics of the patients and differences between AKI group and non-AKI group.

	Total (n = 87)	AKI group (n = 11)	non-AKI group (n = 76)	*p*-values
Number of patients, n (%)	87 (100.0)	11 (12.6)	76 (87.4)	
Age, years (Mean ± SD)	(50.7 ± 16.2)	(52.2 ± 19.5)	(50.5 ± 15.8)	0.745
BMI, kg/m^2 (Median, IQR)	22.2 (19.8-23.7)	21.3 (18.2-24.0)	22.4 (20.1-23.7)	0.417
Male, n (%)	53/87 (60.9)	6/11 (54.5)	47/76 (61.8)	0.745
ICU residence, n (%)	32/87 (36.8)	7/11 (63.6)	25/76 (32.9)	0.090
Diagnosis of infection				
Pulmonary infection, n (%)	55/87 (63.2)	9/11 (81.8)	46/76 (60.5)	0.315
Intracranial infection, n (%)	35/87 (40.2)	2/11 (18.2)	33/76 (43.4)	0.187
Skin or soft tissue infection, n (%)	5/87 (5.7)	1/11 (9.1)	4/76 (5.3)	0.500
Abdominal infection, n (%)	3/87 (3.4)	1/11 (9.1)	2/76 (2.6)	0.337
Endocarditis, n (%)	4/87 (4.6)	1/11 (9.1)	3/76 (3.9)	0.424
Bone and joint infections, n (%)	2/87 (2.3)	0/11 (0.0)	2/76 (2.6)	1.00
Comorbidities				
Diabetes mellitus, n (%)	10/87 (11.5)	0/11 (0.0)	10/76 (13.2)	0.349
Hypertension, n (%)	18/87 (20.7)	3/11 (27.3)	15/76 (19.7)	0.690
MODS, n (%)	6/87 (6.9)	3/11 (27.3)	3/76 (3.9)	0.025*
Sepsis, n (%)	7/87 (8.0)	2/11 (18.2)	5/76 (6.6)	0.214
Bacteremia, n (%)	2/87 (2.3)	1/11 (9.1)	1/76 (1.3)	0.238
Septic shock, n (%)	2/87 (2.3)	1/11 (9.1)	1/76 (1.3)	0.238
Laboratory data				
Van Css, mg/L, (Median, IQR)	9.5 (6.8-15.6)	26.3 (23.5-42.4)	8.8 (6.5-13.1)	< 0.001**
Scr, μmol/L, (Median, IQR)	60.0 (46.0-78.0)	50.0 (42.0-90.0)	61.0 (48.0-77.8)	0.574
WBC, × 10^9/L, (Median, IQR)	12.8 (9.1-17.5)	15.6 (9.9-22.1)	12.8 (8.8-17.4)	0.536
NU, × 10^9/L, (Median, IQR)	10.4 (6.6-15.8)	13.6 (7.5-20.2)	9.8 (6.8-15.6)	0.338
Hemoglobin, g/L, (Mean ± SD)	104.2 ± 21.0	89.6 ± 13.3	106.3 ± 21.1	0.002**
ALB, g/L, (Mean ± SD)	34.0 ± 5.7	33.1 ± 6.4	34.1 ± 5.6	0.579
ALT, g/L, (Median, IQR)	29.0 (21.0-49.0)	27.0 (23.0-47.0)	31.0 (21.0-52.8)	0.650
AST, g/L, (Median, IQR)	39.0 (21.0-59.0)	30.0 (21.0-66.0)	28.5 (21.0-57.2)	0.934

*: $p < 0.05$; **: $p < 0.005$; IQR: inter-quartile range; SD: standard deviation; BMI: body mass index; MODS: multiple organ dysfunction syndrome; Van Css: Vancomycin steady state trough concentration; Scr: serum creatinine; WBC: white blood cell; NU: neutrophil; ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase

trends in concentrations of uKIM-1 and uNGAL from day 0, but they were not statistically significant ($p > 0.05$), and they remained stable after day 2. In contrast, the levels of Scr significantly increased on day 2 and day 3 compared with day 0 ($p < 0.05$).

Table II. Differences of biomarker concentrations (Median, IQR) between AKI and non-AKI groups.

	Total (n = 87)	AKI group (n = 11)	non-AKI group (n = 76)	*p*-values
Scr on day 0, μmol/L	60.0 (46.0-78.0)	50.0 (42.0-90.0)	61.0 (48.0-77.8)	0.574
Scr on day 1, μmol/L	53.0 (47.0-76.0)	88.0 (50.0-122.0)$^{\Delta}$	53.0 (46.2-70.5)	0.059
Scr on day 2, μmol/L	53.0 (44.0-74.0)	109.0 (60.0-139.0)$^{\Delta\Delta}$	50.0 (44.0-69.2)	0.003**
Scr on day 3, μmol/L	56.0 (44.0-75.0)	119.0 (61.0-160.0)$^{\Delta\Delta}$	53.0 (43.0-68.8)	0.001**
uKIM-1 on day 0, ng/mL	0.65 (0.38-1.26)	1.18 (0.85-3.55)	0.56 (0.35-1.16)	0.004**
uKIM-1 on day 1, ng/mL	0.90 (0.59-1.79)	1.79 (1.10-2.92)$^{\Delta}$	0.88 (0.59-1.49)	0.017*
uKIM-1 on day 2, ng/mL	0.86 (0.44-1.67)	2.43 (1.76-2.81)$^{\Delta}$	0.78 (0.37-1.29)	< 0.001**
uKIM-1 on day 3, ng/mL	0.99 (0.52-2.04)	2.30 (1.64-3.20)$^{\Delta}$	0.83 (0.42-1.87)	0.003**
uNGAL on day 0, ng/mL	9.75 (3.88-24.10)	16.51 (9.97-42.80)	8.87 (3.73-22.85)	0.003**
uNGAL on day 1, ng/mL	10.75 (5.10-24.40)	30.40 (13.51-67.45)$^{\Delta}$	9.57 (4.73-23.00)	0.013*
uNGAL on day 2, ng/mL	8.41 (3.60-21.97)	31.73 (12.12-42.32)$^{\Delta}$	6.68 (3.01-19.06)	< 0.001**
uNGAL on day 3, ng/mL	9.50 (4.62-18.10)	31.59 (13.83-40.14)$^{\Delta}$	8.70 (3.47-16.24)	0.001**

IQR: inter-quartile range; Scr: serum creatinine; uKIM-1: urinary kidney molecule-1; uNGAL: urinary neutrophil gelatinase-associated lipocalin; **: $p < 0.005$ compared with AKI group; *: $p < 0.05$ compared with AKI group; $^{\Delta\Delta}$: $p < 0.05$ compared with day 0; $^{\Delta}$: $p > 0.05$ compared with day 0.

ROC Analysis of Biomarkers and Their Predictive Performance in Vancomycin-associated AKI

We performed ROC analyses of Scr, uKIM-1 and uNGAL, and they are described in Figure 1 (a), (b), and (c) respectively. The AUC values, optimal biomarker cut-off values and corresponding sensitivities and specificities are displayed in Table III. Both biomarkers significantly discriminated the development of AKI from the baseline (AUC: uKIM-1 0.769, uNGAL 0.703; all $p < 0.05$). The highest AUC values for uKIM-1 (0.849 [95% CI 0.750-0.948] and uNGAL (0.824 [95% CI 0.726-0.922]) were obtained on day 2 of vancomycin therapy, with sensitivities, specificities and cut-off values of 81.8%, 85.5% and 1.72 ng/mL for uKIM-1, respectively, and 100%, 63.2% and 9.07 ng/mL for uNGAL respectively. The AUC values of Scr levels on day 0 and day 1 were low (AUC = 0.447, $p = 0.574$ and AUC = 0.676, $p = 0.060$ respectively), suggesting that they did not appreciably predict the development of AKI.

Combination of uKIM-1 and uNGAL in Predicting Vancomycin-associated AKI

We combined the results obtained for uKIM-1 and uNGAL at the same time points in a fitted multiple logistic regression model (Table IV). Their AUC values, corresponding sensitivities and specificities, and positive and negative predictive values of the combined biomarkers, are shown in Table V. In brief, the highest AUC of the combined biomarkers was 0.852 (95% CI 0.754-0.949) on day 2 of vancomycin therapy,

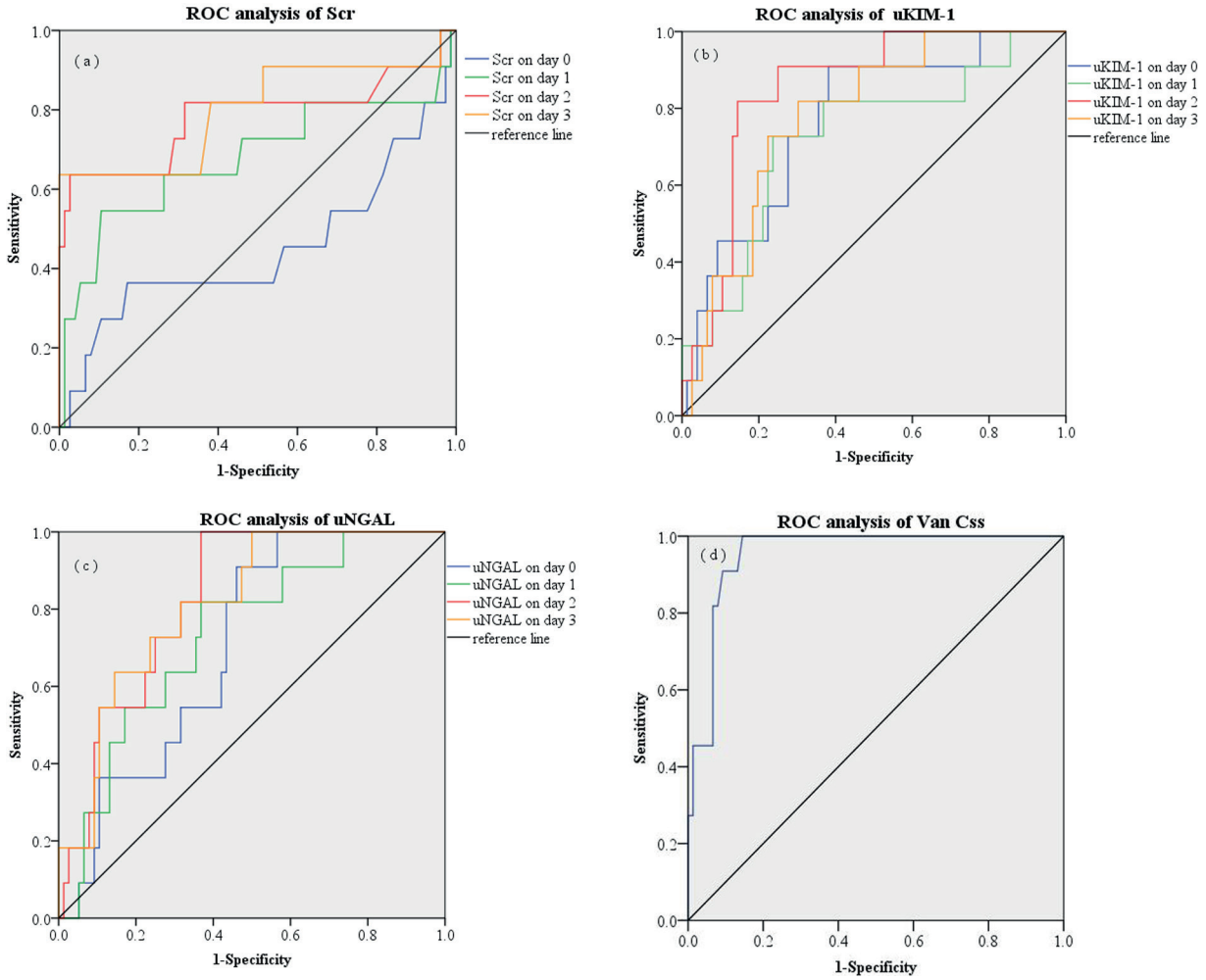

Figure 1. Receiver Operating Characteristic (ROC) analysis of Scr, uKIM-1, uNGAL and Van Css for predicting vancomycin-associated AKI. ***(a):*** ROC analysis of Scr; ***(b):*** ROC analysis of uKIM-1; ***(c):*** ROC analysis of uNGAL; ***(d):*** ROC analysis of Van Css.

Table III. The predictive performance of biomarkers and Van Css to predict AKI.

	AUC-ROC (95% CI)	*p*-values	Cut-off values	Sensitivity (%)	Specificity (%)
Scr on day 0	0.447 (0.222-0.673)	0.574	–		
Scr on day 1	0.676 (0.461-0.892)	0.060	–		
Scr on day 2	0.782 (0.582-0.981)	0.003**	99.50 (μmol/L)	63.6	97.4
Scr on day 3	0.799 (0.617-0.981)	0.001**	114.00 (μmol/L)	63.6	100.0
uKIM-1 on day 0	0.769 (0.629-0.910)	0.004**	0.72 (ng/mL)	90.9	61.8
uKIM-1 on day 1	0.724 (0.556-0.892)	0.017*	1.52 (ng/mL)	72.7	76.3
uKIM-1 on day 2	0.849 (0.750-0.948)	0.000**	1.72 (ng/mL)	81.8	85.5
uKIM-1 on day 3	0.781 (0.658-0.904)	0.003**	1.55 (ng/mL)	81.8	69.7
uNGAL on day 0	0.703 (0.575-0.831)	0.030*	9.33 (ng/mL)	90.9	53.9
uNGAL on day 1	0.733 (0.590-0.877)	0.013*	13.48 (ng/mL)	81.8	63.2
uNGAL on day 2	0.824 (0.726-0.922)	0.001**	9.07 (ng/mL)	100.0	63.2
uNGAL on day 3	0.812 (0.698-0.927)	0.001**	13.81 (ng/mL)	81.8	68.4
Van Css	0.953 (0.911-0.996)	< 0.000**	15.40 (mg/L)	100.0	85.5

AUC-ROC: Area under the receiver operating characteristic curve; CI: Confidence interval; Scr: serum creatinine; uKIM-1: urinary kidney molecule-1; uNGAL: urinary neutrophil gelatinase-associated lipocalin; Van Css: vancomycin steady-state concentration; **: $p < 0.005$; *: $p < 0.05$.

with a sensitivity and specificity of 90.9% and 75.0%, respectively, and the positive and negative predictive values were 34.4% and 98.3%, respectively.

ROC Analysis of Van Css for Diagnosing Vancomycin-associated AKI

We also performed the ROC analysis of Van Css, which is shown in Figure 1 (d). The AUC of Van Css reached up to 0.953 (95% CI [0.911-0.996]), and the cut-off was 15.4 mg/L, with a preferable sensitivity and specificity of 100.0% and 85.5% (Table III).

Correlations Between Biomarkers and Van Css

Because the best predictive efficiency and most of the Van Css values were obtained on day 2 of vancomycin therapy, we probed the correlations between the two biomarkers as well as biomarkers and Van Css on the same day. The results are shown in Table VI. Our findings indicated that there were weak positive correlations between the two variables. For each of the two biomarkers, the correlation between uKIM-1 and uNGAL was the most prominent (the related coefficient [r] = 0.543, $p < 0.001$), whereas for biomarkers and Van Css, Scr *vs.* Van Css was better (r = 0.485, $p < 0.001$).

Discussion

Preclinical trials have shown the diagnostic value of uKIM-1 and uNGAL for subacute kidney toxicity in vancomycin-treated rats[29]. To the best of our knowledge, this is the first study investigating the predictive performance of uKIM-1 and uNGAL individually and in combination for clinical vancomycin-associated AKI.

In this study, we found that elevated uKIM-1 and uNGAL could be detected at least 2 days earlier than a significant rise in Scr among those who subsequently developed AKI. These data were in accordance with previous studies regarding AKI patients admitted to the ICU with surgery or trauma[10,23,30,31] which showed the same

Table IV. Formula for the fitted multiple logistic regression model for the combination of uKIM-1 and uNGAL.

For AKI (n = 11) *vs.* non-AKI (n = 76) combined	
Baselines (day 0)	$[0.001 \times \text{uKIM-1} + 2 \times 10^{-5} \times \text{uNGAL} - 2.610]$
1 day after vancomycin therapy	$[0.001 \times \text{uKIM-1} + 3 \times 10^{-6} \times \text{uNGAL} - 3.047]$
2 days after vancomycin therapy	$[0.001 \times \text{uKIM-1} + 4 \times 10^{-6} \times \text{uNGAL} - 3.684]$
3 days after vancomycin therapy	$[0.003 \times \text{uKIM-1} + 1.2 \times 10^{-5} \times \text{uNGAL} - 2.773]$

uKIM-1: urinary kidney molecule-1; uNGAL: urinary neutrophil gelatinase-associated lipocalin.

Table V. Predictive performance of the combined biomarkers with respect to vancomycin-associated AKI.

	AUC-ROC (95% CI)	*p*-values	Sensitivity (%)	Specificity (%)	PPV (%)[a]	NPV (%)[a]
uKIM-1 + uNGAL[b]	0.795 (0.690-0.901)	0.002**	100.0	57.9	25.5	100.0%
uKIM-1 + uNGAL[c]	0.731 (0.570-0.892)	0.014*	72.7	75.0	29.5	95.0%
uKIM-1 + uNGAL[d]	0.852 (0.754-0.949)	< 0.000**	90.9	75.0	34.4	98.3%
uKIM-1 + uNGAL[e]	0.812 (0.704-0.921)	0.001**	90.9	71.1	31.2	98.2%

AUC-ROC: Area under the receiver operating characteristic curve; CI: Confidence interval; uKIM-1: urinary kidney molecule-1; uNGAL: urinary neutrophil gelatinase-associated lipocalin; PPV: Positive predictive value; NPV: Negative predictive value; **: $p < 0.005$; * $p < 0.05$. [a]Positive predictive values and negative predictive values will vary depending on the prevalence of AKI. Te prevalence of AKI for this table is 12.6%; [b]Combined uKIM-1 and uNGAL at the baseline; [c]Combined uKIM-1 and uNGAL on 1 day after vancomycin therapy; [d]Combined uKIM-1 and uNGAL on 2 days after vancomycin therapy; [e]Combined uKIM-1 and uNGAL on 3 days after vancomycin therapy.

high urinary biomarkers at baseline. Our findings indicate that these urinary biomarkers also showed early differentiation of patients with or without AKI from the baseline in terms of vancomycin therapy. The cut-off values of these two biomarkers varied widely in different conditions of AKI. The uNGAL values reported in most studies[23,32,33] were higher than our results, while they were analogous to AKI induced by burn injury[34] or contrast injection[35] and much lower in amphotericin B-induced AKI[26] compared with our results. For uKIM-1, the values were higher in patients with burn injury[36] and analogous to those receiving percutaneous coronary intervention[37] compared with our results. There has been no study of vancomycin for comparison. The explanations for the discrepancies may be that they studied patients under different conditions, and the numbers of included patients and incidence of AKI varied widely. In addition, different time points and AKI definitions in these studies might account for the discrepancies.

It remains controversial whether it is necessary to normalize the urinary biomarkers by urine creatinine in practice[14,21,23,36,38]. Here, we did not normalize urinary biomarkers to urine creatinine, and we found that they still had a good predictive performance. This was in accordance with many other reports[14,23,36]. In addition, some believe that normalization to urine creatinine is less than ideal because of the non-steady state of creatinine balance in patients with AKI[21]. Our results provide some evidence that uncorrected urinary biomarkers could also be useful for predicting AKI.

In contrast, a previous work showed that uKIM-1 and uNGAL were not better than Scr for predicting paraquat-induced AKI[13]. Also, it reported much higher uKIM-1 levels (52.06 ng/mL *vs.* 1.79 ng/mL) and much lower uNGAL levels (2.84 ng/mL *vs.* 30.4 ng/mL) in AKI patients at 24 h compared with our study. This result was possibly affected by the very small number of patients in the prior research, and most of them were diagnosed with more serious AKI, which might cause higher uKIM-1 levels. In addition, most of the patients in the present paper were diagnosed with infectious diseases, which could generate high uNGAL levels[39]. The authors of the previous study hypothesized that patients with paraquat poisoning might regulate the expressions of uKIM-1 and uNGAL by a reactive oxy-

Table VI. Correlations of each two biomarkers and between biomarkers and vancomycin Css.

	Related coefficient (r)	*p*-values
uKIM-1 *vs.* Scr on 2 days	0.250	0.020*
uNGAL *vs.* Scr on 2 days	0.276	0.010*
uKIM-1 *vs.* uNGAL on 2 days	0.543	< 0.001**
uKIM-1 *vs.* Van Css on 2 days	0.334	0.002**
uNGAL *vs.* Van Css on 2 days	0.346	0.001**
Scr *vs.* Van Css on 2 days	0.485	< 0.001**

Van Css: Vancomycin steady state trough concentration; Scr: serum creatinine; uKIM-1: urinary kidney molecule-1; uNGAL: urinary neutrophil gelatinase-associated lipocalin; **: $p < 0.005$; *: $p < 0.05$.

gen species (ROS)-induced AKI model[13]. These differences suggested that different contexts of renal injury might lead to variable levels of these biomarkers.

The higher proportions of MODS and medians of Van Css as well as a lower level of hemoglobin in AKI patients of our study were consistent with the results of many previous investigations[4,40,41]. In addition, vancomycin trough concentration showed an excellent ability to assess AKI, which is a generally acknowledged fact[3]. These findings indicate that the patients in our study were at risk of vancomycin-induced AKI. Nevertheless, here, we investigated non-invasive biomarkers, and the results suggested that they could promote early detection of vancomycin-induced AKI. Moreover, we did not observe progressive increases in uKIM-1 and uNGAL following treatment in the AKI group. These findings might be explained by relatively mild and transient kidney damage in most of our patients, or we may have missed the earlier increases in urine biomarkers since they were reported to peak within 24 hours[8,42]. However, Scr increased significantly after 2 days compared with the baseline, which indicated decreased renal function in patients with AKI[22].

As for joint biomarkers, our data were similar to a previous study examining AKI following cardiopulmonary bypass[14] in which the combination of uKIM-1 and uNGAL only slightly improved the predictive performance. In contrast, Xue et al[10] reported a better predictive utility of these combined biomarkers compared with the single biomarker. These differences may be due to the inclusion of diverse patients, distinctions of AKI definition and different time points in these studies.

We found the correlations between Van Css and the two urinary biomarkers were similar, which was consistent with a recent experimental rat model[43]. It suggested that the relationships of vancomycin concentration and these two biomarkers in animals and humans were consistent. A stronger correlation of Scr and Van Css was found in our study, likely because the clearance of vancomycin was related to Scr[44]. In addition, the stronger correlation between the two urinary biomarkers than that between each of them and Scr was similar to the results reported by Sokolski et al[45]. This phenomenon confirmed that serum and urinary biomarkers represent different aspects of nephron dysfunction, indicating that Scr may be related to reduced renal function, while urinary concentration is more likely a marker of kidney injury[15].

The strengths of our research were as follows. First, this is the first prospective study to evaluate the role of urinary markers in the prediction of clinical vancomycin-associated AKI. Second, we tested a panel of uKIM-1 and uNGAL at multiple time points, and the time points we chose were convenient for clinical practice. Third, we preliminarily demonstrated that both uKIM-1 and uNGAL concentrations showed good discriminatory capacity to predict the development of AKI from the baseline and up to 3 days during the treatment period, which might promote the application of these biomarkers and guide the therapeutic options of vancomycin.

However, several limitations have to be noted. First, this is a single-center study with small number of patients exhibiting heterogeneous pathological and physiological conditions, which could have biased our results. Second, the low occurrence of vancomycin-associated AKI precluded obtaining robust estimates of risk regarding this endpoint. In addition, we could not confirm the relationships between biomarkers and the long-term outcomes. Moreover, we did not collect some important data, such as combination of other potential nephrotoxic drugs and the duration and dosage of vancomycin. Although our work did not address these issues, it provided a basis for later studies that may be exempt from these limitations. Large-scale and multi-center studies are necessary to confirm our results.

Conclusions

Urinary KIM-1 and NGAL can be used for early prediction of the occurrence of clinical vancomycin-associated AKI, and the efficiency was not substantially improved when combining these two biomarkers.

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Author Contributions

Hui-mei Pang, Xiao-ling Qin and Wen-xing Wei designed and performed the experiments; Hui-mei Pang analyzed the data and wrote the manuscript; Xiao-ling Qin assisted in collecting and analyzing the data, Tao-tao Liu, Daohai Cheng, and Hua Lu revised the manuscript; Qing Guo and Li Jing assisted in analyzing the data. All the authors approved the submission of this manuscript, and accepted the responsibility for the entire content of this submitted manuscript.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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