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Identification of Biomarkers for the Diagnosis of Sepsis-Associated Acute Kidney Injury and Prediction of Renal Recovery in the Intensive Care Unit

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Purpose: Acute kidney injury (AKI) following sepsis is associated with higher mortality; however, reliable biomarkers for AKI development and recovery remain to be elucidated.

Materials and Methods: Patients with sepsis admitted to the medical intensive care unit (ICU) of Severance Hospital between June 2018 and May 2019 were prospectively analyzed. Patients were divided into those with and without AKI within 48 hours. Patients with septic AKI were subdivided into AKI-recovery and non-recovery groups based on whether their kidney injury recovered within 7 days.

Results: A total of 84 patients were enrolled. The baseline creatinine (2.9 mg/dL vs. 0.8 mg/dL vs. 1.2 mg/dL, $p<0.001$), Charlson Comorbidity Index (4.5 vs. 2.0 vs. 3.0, $p=0.002$), Sequential Organ Failure Assessment (10.0 vs. 6.5 vs. 8.0, $p<0.001$), and Acute Physiology and Chronic Health Evaluation II scores (32.0 vs. 21.5 vs. 30.5, $p=0.004$) were higher in the non-recovery AKI group compared to the non-AKI and AKI-recovery groups. The Kaplan-Meier curves revealed that non-recovery from AKI was associated with lower survival ($p<0.001$). High-lactate ($p\leq0.05$) and kynurenine levels ($p\leq0.05$) were associated with non-recovery of renal function following AKI. The areas under the curve for predicting non-recovery from AKI were 0.693 and 0.721 for lactate and kynurenine, respectively. The survival rate was lower in the high-kynurenine ($p=0.040$) and high-lactate ($p=0.010$) groups.

Conclusion: The mortality of patients who recovered from AKI was comparable to that of patients without AKI. Lactate and kynurenine could be useful biomarkers for the diagnosis and recovery of AKI following sepsis.

Key Words: Intensive care units, biomarkers, sepsis, acute kidney injury

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INTRODUCTION

Sepsis is an important factor that contributes to acute kidney injury (AKI).[1,2] The development of AKI following sepsis and septic shock is reportedly associated with high morbidity and mortality.[3] The mortality rate of patients with septic AKI is estimated to be 35%.[4] Moreover, non-recovery from AKI is associated with a decreased long-term survival.[5]

The diagnosis of septic AKI is based mainly on the clinical status and serum creatinine (Scr) in clinical practice. However, the Scr concentration may be influenced by non-renal factors, such as sex, age, medications, and the amount of muscle mass.[6] Therefore, biomarkers for identification of the features

of septic AKI and renal recovery are warranted. Amino acid profiling using liquid chromatography–tandem mass spectrometry (LC-MS/MS) was used for investigating the biomarkers.

Tryptophan (TRP) is an essential amino acid that plays an important role in the biosynthesis of proteins.[7] Several studies[8-14] have focused on the role of TRP catabolism in numerous immune-regulatory processes, including protein synthesis, inflammation, tissue injury, and systemic infections. TRP is a precursor of several physiologically important metabolites.[7] Kynurenine (KYN), the first degradation product of TRP,[11,13,15] is synthesized metabolically by indoleamine 2,3-dioxygenase (IDO) and TRP 2,3-dioxygenase.[7] IDO is activated during inflammation[16] leading to elevated levels of downstream metabolites, including KYN. KYN metabolites have been implicated in the pathophysiologies of various immunomodulatory disorders, including sepsis.[16]

The current study aimed to investigate potential biomarkers for the development and recovery of AKI following sepsis. We sought to identify biomarkers, including amino acids, TRP metabolites, and inflammatory markers, such as C-reactive protein (CRP), and procalcitonin.

MATERIALS AND METHODS

Study population and design

This study prospectively analyzed the samples of adult patients (aged ≥18 years) who were diagnosed with sepsis and admitted to the medical intensive care unit (ICU) of Severance Hospital, a 2500-bed (30-bed medical ICU) tertiary referral hospital in South Korea, between June 2018 and May 2019. The study protocol involved medical ICU cohorts. The inclusion criteria for the cohorts were an age ≥18 years and fulfillment of at least two criteria of systemic inflammatory response syndrome or the presence of sepsis within 24 hours of admission to the ICU (D0). The exclusion criteria were age under 18 years and pregnant patients. Participants who met the revised sepsis 3 definition were included in the current study. Participants were divided into the AKI and non-AKI groups, based on the development of AKI within 48 hours At D0. Patients with septic AKI were subdivided into AKI-recovery and non-recovery groups, based on whether the kidney injury recovered within 7 days of the precipitating event.

Data collection

The following demographic, clinical, and laboratory data were collected: baseline data [age, sex, body mass index, and the Charlson Comorbidity Index (CCI),[17] Acute Physiology and Chronic Health Evaluation II (APACHE II), and Sequential Organ Failure Assessment (SOFA) assessment scores]; laboratory parameters [Scr, estimated glomerular filtration rate (eGFR), albumin, lactate, CRP, procalcitonin]; sepsis severity (sepsis or septic shock); AKI severity (stage 1, 2, or 3); and clinical outcomes [renal replacement therapy (RRT), length of stay (LOS) in the hospital and ICU (in days), and mortality].

Definition of variables

Sepsis and septic shock were defined using the third international consensus definition.[18] The diagnosis of AKI was based on the Kidney Disease Improving Global Outcomes criteria.[19] Patients were categorized as having stage 1, 2, or 3 AKI based on the maximum Scr measured within 48 hours of D0. Participants who received RRT were defined as having stage 3 AKI. Renal recovery was defined as the return of Scr levels to within 150% of baseline or less without the need for RRT based on the international consensus criteria.[20] The AKI-recovery status was assessed within 7 days of the precipitating event.

Blood sample collection and amino acids using the metabolomics approach

The patients' serum samples were collected at D0. All blood samples were centrifuged at 3000 rounds per minute for 10 and 15 minutes, respectively. The supernatants were transferred to Eppendorf tubes and stored at -80°C. All samples were thawed immediately prior to analysis. For the measurement of the 22 amino acids, the Zivak amino acids kit (Zivak Technologies, Istanbul, Turkey), a quantitative LC-MS/MS analysis kit specific for amino acids in biological fluids, was used. First, whole blood samples were vortexed for 30 s, and centrifuged at 12700×g for 5 min. For each sample, the pellet was discarded, and 100 μL of serum was added to 400 μL of acetonitrile and vortexed for 30 s. The mixture was centrifuged at 12700×g for 10 min. Finally, 300 μL of the supernatant was analyzed using a LC-MS/MS. An equal volume of 0.1% trifluoroacetic acid aqueous solution was added to each serum extract prior to LC-MS/MS analysis. Pure amino acids solution was prepared for mass comparison and quantitative LC-MS/MS. The 22 amino acids measured were as follows: alpha-aminobutyric acid, alanine, arginine, asparagine, aspartic acid, citrulline, glutamic acid, glutamine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine, valine, and TRP. The serum samples were processed in strict accordance with the manufacturer's instructions. The KYN concentration was determined using the same LC-MS/MS system using our laboratory-developed method. As for the repeatability of the assay, the correlation of variance was 3.0%–4.8%.

Statistical analysis

Data were presented as the number of cases (%) and median [interquartile range (IQR)]. Categorical data were compared using the Pearson's chi-squared test or Fisher's exact test. Continuous variables were compared using the Mann–Whitney test or t-test. One-way analysis of variance was used to compare the three groups. The differences in the cumulative survival obtained by Kaplan–Meier curves were identified using the log-

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rank test. Multivariate logistic regression analysis was used to identify the risk factors associated with sepsis-induced AKI. The odds ratios (ORs) and 95% confidence intervals (CI) were also calculated. *P*-values<0.05 were considered significant for all analyses. Data analysis was performed using SPSS version 25 (IBM Corp., released 2017, Armonk, NY, USA). The receiver operating characteristic (ROC) curves were plotted, and the area under the curve (AUC) was analyzed using the MedCalc

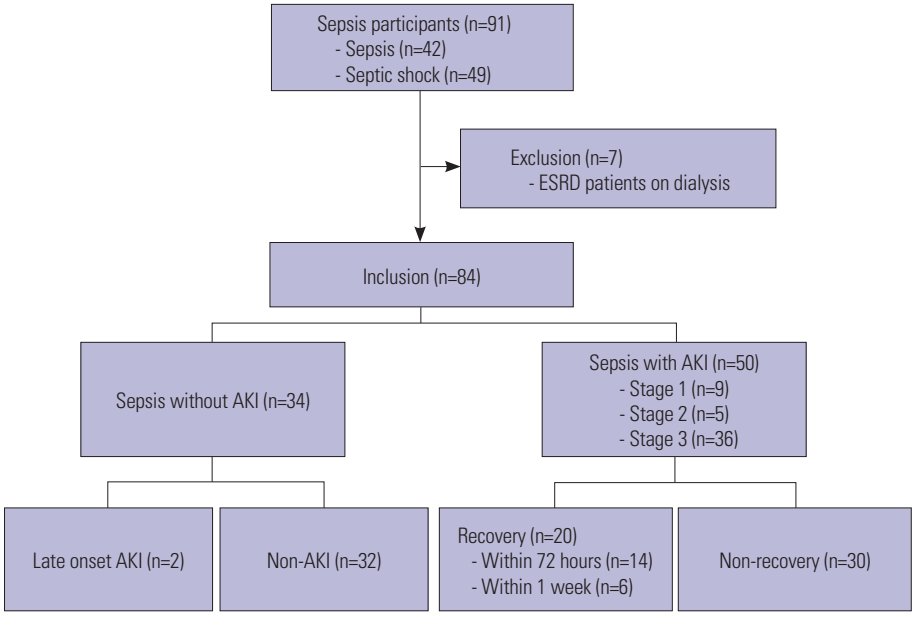

Fig. 1. Flowchart of the study population. ESRD, end-stage renal disease; AKI, acute kidney injury.

Table 1. Demographic and Clinical Characteristics of Participants Enrolled in the Study

	Total (n=84)	Sepsis			*p* value
		Non-AKI (n=34)	AKI		
			Recovery (n=20)	Non-recovery (n=30)	
Age, yr	71.0 (60.9–80.2)	73.5 (57.0–82.3)	66.0 (60.0–78.5)	70.0 (64.0–80.0)	0.961
Sex, male	50 (59.5)	20 (58.8)	11 (55.0)	19 (63.3)	0.836
Body mass index, kg/m^2	22.5 (19.8–25.6)	23.5 (20.4–25.9)	21.9 (19.1–24.1)	23.1 (20.1–25.7)	0.556
CCI	3.0 (2.0–5.8)	2.0 (1.0–4.0)	3.0 (2.0–5.0)	4.5 (2.0–7.25)	0.002
SOFA	8.0 (6.0–10.8)	6.5 (4.0–9.0)	8.0 (7.0–10.8)	10.0 (7.8–13.0)	<0.001
APACHE II	28.5 (20.3–33.0)	21.5 (17.0–30.3)	30.5 (25.3–33.8)	32.0 (22.8–38.0)	0.004
Scr, mg/dL	1.2 (0.6–2.8)	0.8 (0.5–1.2)	1.2 (0.8–2.7)	2.9 (1.5–3.4)	<0.001
eGFR (CKD-EPI), mL/min/1.73 m^2	47.5 (19.4–94.2)	86.0 (48.8–114.3)	61.0 (21.0–87.5)	18.0 (13.0–38.8)	<0.001
Albumin, g/dL	2.5 (2.2–2.8)	2.6 (2.2–2.9)	2.7 (2.2–2.9)	2.3 (2.1–2.7)	0.137
Lactate, mmol/L	2.1 (1.5–4.9)	1.7 (1.2–2.2)	2.2 (1.5–4.8)	4.6 (2.1–9.2)	<0.001
CRP, mg/L	148.6 (67.9–247.4)	133.7 (71.7–221.4)	175.9 (50.5–320.0)	158.6 (81.2–243.7)	0.363
Procalcitonin, ng/mL	3.6 (0.6–14.6)	0.9 (0.3–3.3)	6.4 (1.5–24.7)	12.3 (2.6–28.5)	0.004
Sepsis severity					<0.001
Sepsis	39 (46.4)	26 (76.5)	7 (32.0)	6 (20.0)	
Septic shock	45 (53.6)	8 (23.5)	13 (65.0)	24 (80.0)	
AKI severity					<0.001
Stage 1	9 (10.7)	0	8 (40.0)	1 (3.3)	
Stage 2	5 (6.0)	0	4 (20.0)	1 (3.3)	
Stage 3	36 (42.9)	0	8 (40.0)	28 (93.3)	
RRT	31 (36.9)	0	5 (25.0)	26 (86.7)	<0.001

AKI, acute kidney injury; CCI, Charlson Comorbidity Index; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; RRT, renal replacement therapy.
Data are presented as numbers (%) or median (interquartile range).

software (version 16.4.3; MedCalc, Oostende, Belgium). GraphPad Prism 7 (Graph-Pad, San Diego, CA, USA) was used for graphical plotting. For predicting the development of AKI, AUC-ROC analysis was performed by comparing patients with AKI to those without. Also, for predicting the biomarker of AKI recovery, the AKI-recovery and non-recovery AKI groups were compared.

Ethics statement

The study protocol was reviewed and approved by the Institutional Review Board of Severance Hospital (IRB No. 4-2017-0654). Written consent was obtained from the patients or their guardians. All procedures were performed in accordance with the relevant guidelines and regulations.

RESULTS

Baseline characteristics of the study population

A total of 91 patients diagnosed with sepsis were admitted to the medical ICU during the study period (Fig. 1). Seven patients with end-stage renal disease who required dialysis were excluded from the study. Thirty-four (40.5%) of the 84 patients who met the inclusion criteria were classified as having sepsis without AKI, and 50 (59.5%) as sepsis with AKI. Of the non-AKI patients, only two patients developed delayed AKI after 48 hours. Of the patients with sepsis with AKI, 20 (40%) recovered within 7 days following the precipitating event, whereas 30 (60%) patients did not recover during that period.

Table 1 shows the clinical and laboratory characteristics of the participants enrolled in this study. The non-recovery AKI group showed higher CCI (4.5 vs. 2.0 vs. 3.0 vs., p=0.002), SOFA (10.0 vs. 6.5 vs. 8.0 vs., p<0.001), and APACHE II (32.0 vs. 21.5 vs. 30.5 vs., p=0.004) scores compared to the non-AKI and AKI-recovery groups. The Scr (2.9 vs. 0.8 vs. 1.2, p<0.001), eGFR (18.0 vs. 86.0 vs. 61.0, p<0.001), serum lactate (4.6 vs. 1.7 vs. 2.2, p<0.001), and procalcitonin (12.3 vs. 0.9 vs. 6.4, p=0.004) levels obtained during the initial laboratory test were also the highest for the non-recovery AKI group.

Treatment outcomes

Table 2 shows the clinical outcomes of the participants enrolled in the study. The hospital LOS (44.5 days vs. 29.5 days, p=0.028), in-hospital mortality (50.0% vs. 23.5%, p=0.022, ICU mortality (42.0% vs. 14.7%, p=0.009), and 90-day mortality (48.0% vs. 20.6%, p=0.012) were higher in the AKI group compared to the non-AKI group. Also, when comparing the non-recovery AKI group and the recovery-AKI group, we found that the non-re-

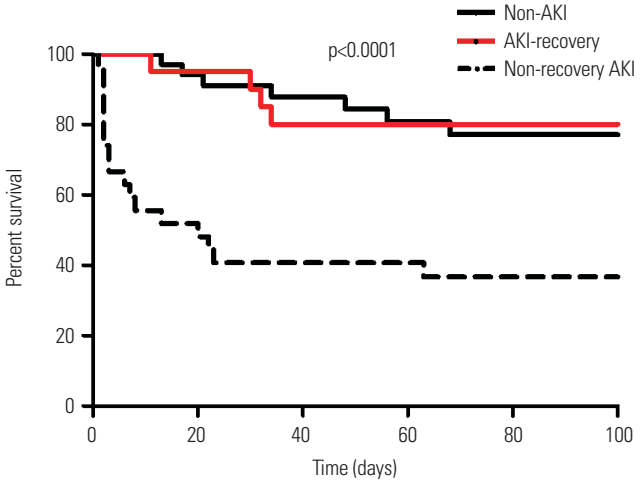

Fig. 2. Kaplan-Meier survival analysis stratified by recovery status. AKI, acute kidney injury.

Table 2. Clinical Outcomes of Participants Enrolled in the Study

(A) Comparison of clinical outcomes between non-AKI and AKI groups				
	Total (n=84)	Sepsis		*p* value
		Non-AKI (n=34)	AKI (n=50)	
Hospital LOS, days	35.0 (21.3–62.8)	44.5 (28.5–73.3)	29.5 (18.3-45.3)	0.028
ICU LOS, days	9.0 (4.0–16.0)	10.5 (7.8–18.3)	8.0 (3.0-16.0)	0.160
In-hospital mortality	33 (39.3)	8 (23.5)	25 (50.0)	0.022
ICU mortality	26 (31.0)	5 (14.7)	21 (42.0)	0.009
90-day mortality	31 (36.9)	7 (20.6)	24 (48.0)	0.012
(B) Comparison of clinical outcomes between recovery and non-recovery AKI groups				
	Total (n=50)	AKI		*p* value
		Recovery (n=20)	Non-recovery (n=30)	
Hospital LOS, days	29.5 (18.3–45.3)	33.0 (23.5–90.0)	23.5 (7.8–44.3)	0.106
ICU LOS, days	8.0 (3.0–16.0)	11.5 (7.0–16.0)	5.5 (3.0–15.3)	0.066
In-hospital mortality	25 (50.0)	3 (15.0)	22 (73.3)	<0.001
ICU mortality	21 (42.0)	2 (10.0)	19 (63.3)	<0.001
90-day mortality	24 (48.0)	4 (20.0)	20 (66.7)	0.002

AKI, acute kidney injury; LOS, length of stay; ICU, intensive care unit.
Data are presented as numbers (%) or median (interquartile range).

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Table 3. Metabolites of Participants Enrolled in the Study

(A) Comparison of metabolites between non-AKI and AKI groups			
	Sepsis		*p* value
	Non-AKI (n=34)	AKI (n=50)	
Alpha-aminobutyric acid	22.2 (13.9–28.8)	21.2 (14.1–32.3)	0.985
Arginine	80.5 (60.2–102.9)	49.9 (31.4–96.5)	0.083
Asparagine	49.6 (41.0–64.9)	52.5 (38.5–68.2)	0.483
Aspartic acid	41.6 (32.8–56.0)	37.4 (26.6–53.4)	0.262
Citrulline	19.9 (12.1–28.8)	17.8 (12.2–26.7)	0.729
Glutamic acid	124.6 (102.2–155.6)	124.3 (100.2–19.3)	0.799
Glutamine	458.2 (353.6–518.3)	446.6 (339.1–599.0)	0.636
Glycine	267.4 (198.8–349.5)	254.6 (215.7–388.4)	0.560
Histidine	55.5 (46.8–74.7)	62.3 (45.9–95.1)	0.348
Hydroxyproline	11.3 (9.9–14.4)	13.7 (9.5–19.8)	0.111
Isoleucine	55.6 (44.0–72.5)	53.5 (33.3–66.5)	0.357
Leucine	119.1 (94.7–146.8)	114.3 (78.2–140.5)	0.636
Lysine	151.2 (119.6–229.4)	164.1 (127.3–241.5)	0.572
Methionine	28.5 (17.1–40.2)	29.2 (19.5–41.9)	0.529
Ornithine	96.1 (73.3–144.1)	97.0 (64.1–137.0)	0.792
Phenylalanine	144.8 (106.7–192.7)	160.1 (125.6–233.3)	0.153
Proline	130.4 (87.1–227.3)	158.8 (105.9–257.4)	0.330
Serine	133.4 (108.8–174.3)	116.2 (87.4–144.6)	0.076
Threonine	101.4 (65.0–118.7)	90.6 (67.6–132.1)	0.964
Tyrosine	58.5 (48.6–79.4)	68.6 (49.7–94.9)	0.183
Valine	200.0 (170.4–239.2)	184.2 (154.6–255.2)	0.357
Kynurenine	3.3 (2.1–6.8)	5.2 (3.6–7.3)	0.009
Tryptophan	28.8 (15.9–36.5)	28.1 (19.2–36.1)	0.931
Kynurenine/Tryptophan ratio	0.1 (0.1–0.3)	0.2 (0.1–0.3)	0.029
(B) Comparison of metabolites between recovery and non-recovery AKI groups			
	AKI		*p* value
	Recovery (n=20)	Non-recovery (n=30)	
Alpha-aminobutyric acid	26.7 (15.6–37.6)	20.1 (12.0–29.0)	0.212
Arginine	58.7 (22.3–128.1)	49.9 (34.6–87.6)	0.953
Asparagine	57.1 (40.3–62.4)	51.0 (37.2–77.9)	0.663
Aspartic acid	45.2 (28.2–59.6)	34.4 (23.5–45.8)	0.109
Citrulline	15.4 (11.1–26.1)	20.2 (15.1–29.9)	0.166
Glutamic acid	134.6 (101.8–171.7)	117.4 (97.4–144.2)	0.267
Glutamine	439.3 (302.8–550.8)	454.0 (373.6–676.3)	0.322
Glycine	255.8 (233.6–396.7)	254.6 (211.0–391.1)	0.797
Histidine	60.2 (45.2–84.9)	68.4 (46.9–104.7)	0.593
Hydroxyproline	12.6 (9.6–15.9)	14.7 (9.3–30.4)	0.259
Isoleucine	55.2 (38.1–69.0)	51.9 (29.9–65.2)	0.539
Leucine	118.9 (84.6–148.6)	112.4 (77.5–141.8)	0.579
Lysine	171.5 (125.1–230.4)	160.1 (127.3–312.4)	0.539
Methionine	30.0 (19.7–38.9)	26.3 (19.5–45.4)	0.968
Ornithine	110.8 (69.4–134.9)	90.4 (59.6–155.5)	0.313
Phenylalanine	152.7 (122.2–220.4)	160.7 (133.3–260.1)	0.464
Proline	140.0 (108.6–217.7)	164.5 (95.7–304.4)	0.579
Serine	138.8 (116.0–154.6)	98.9 (76.2–131.7)	0.008
Threonine	96.9 (73.0–132.4)	82.7 (65.9–135.6)	0.722
Tyrosine	62.4 (40.1–86.0)	78.8 (51.9–109.1)	0.220
Valine	205.8 (159.6–262.7)	172.6 (142.4–245.7)	0.235
Kynurenine	4.3 (2.7–5.8)	6.6 (4.0–8.9)	0.009
Tryptophan	27.3 (20.2–35.8)	29.0 (17.4–36.9)	0.968
Kynurenine/Tryptophan ratio	0.1 (0.1–0.2)	0.2 (0.2–0.3)	0.013

AKI, acute kidney injury.
Data are presented as the median (interquartile range). Results are expressed in μmol/L.

covery AKI group showed higher in-hospital mortality (73.3% vs. 15.0%, p<0.001), ICU mortality (63.3% vs. 10.0%, p<0.001), and 90-day mortality (66.7% vs. 20.0%, p=0.002). The Kaplan-Meier curves also revealed that non-recovery from AKI was associated with lower survival (p<0.001). The survival in the AKI-recovery group was similar to that of the non-AKI group (p=0.753) (Fig. 2).

Difference in amino acid concentrations

The amino acids profiling using LC-MS/MS revealed that the KYN (5.2 vs. 3.3, p=0.009) and KYN-to-TRP ratio (K/T ratio) (0.2 vs. 0.1, p=0.029) concentrations at D0 were significantly higher in the AKI group than in the non-AKI group (Table 3A). Also, serine (98.9 vs. 138.8, p=0.008), KYN (6.6 vs. 4.3, p=0.009), and K/T ratio concentration (0.2 vs. 0.1, p=0.013) at D0 were significantly different between the non-recovery AKI group and the recovery-AKI group (Table 3B). Fig. 3 depicts the potential kidney markers stratified by the recovery status of renal function. High baseline levels of lactate (p≤0.05) and KYN (p≤0.05) were associated with non-recovery of renal function following AKI. The baseline KYN-to-TRP ratio (K/T ratio) was also significantly higher in the non-recovery AKI group compared to the non-AKI group (p≤0.01), although it did not differ significantly from the K/T ratio of the AKI-recovery group.

Lactate and KYN as biomarkers for the prediction of AKI and renal recovery

ROC curves were generated to compare the predictive value of lactate and KYN for AKI (Fig. 4A and C) and renal recovery (Fig. 4B and D). The AUC for predicting AKI was 0.777 (95% CI, 0.680–0.874) and 0.668 (95% CI, 0.545–0.790) for lactate and KYN, respectively. The combination of lactate and Scr yielded the highest AUC value (0.913; 95% CI, 0.850–0.975). The combination of KYN and eGFR yielded the highest AUC value (0.792, 95% CI, 0.697–0.887) when calculating the ROC curve using eGFR instead of Scr. The AUC for the prediction of renal recovery was higher for KYN (0.721, 95% CI, 0.577–0.865) than that for lactate (0.693; 95% CI, 0.545–0.842). Moreover, the combination of lactate, KYN, and Scr yielded the highest AUC value (0.820; 95% CI, 0.702–0.938) when calculated based on Scr, although not when based on eGFR.

Patients were divided into the high-KYN (≥4.7) and low-KYN (<4.7) groups (Fig. 5A) based on the optimal cut-off value. They were also classified into the high-lactate (≥2.0) and low-lactate (<2.0) groups (Fig. 5B). The high-KYN (p=0.040) and high-lactate (p=0.010) groups had lower survival rates compared to the low-KYN and low-lactate groups, respectively.

In addition, Table 4 shows the results of the univariate and multivariate logistic regression analyses that estimated the ORs of non-recovery from AKI. In the univariate analysis, CCI, SOFA

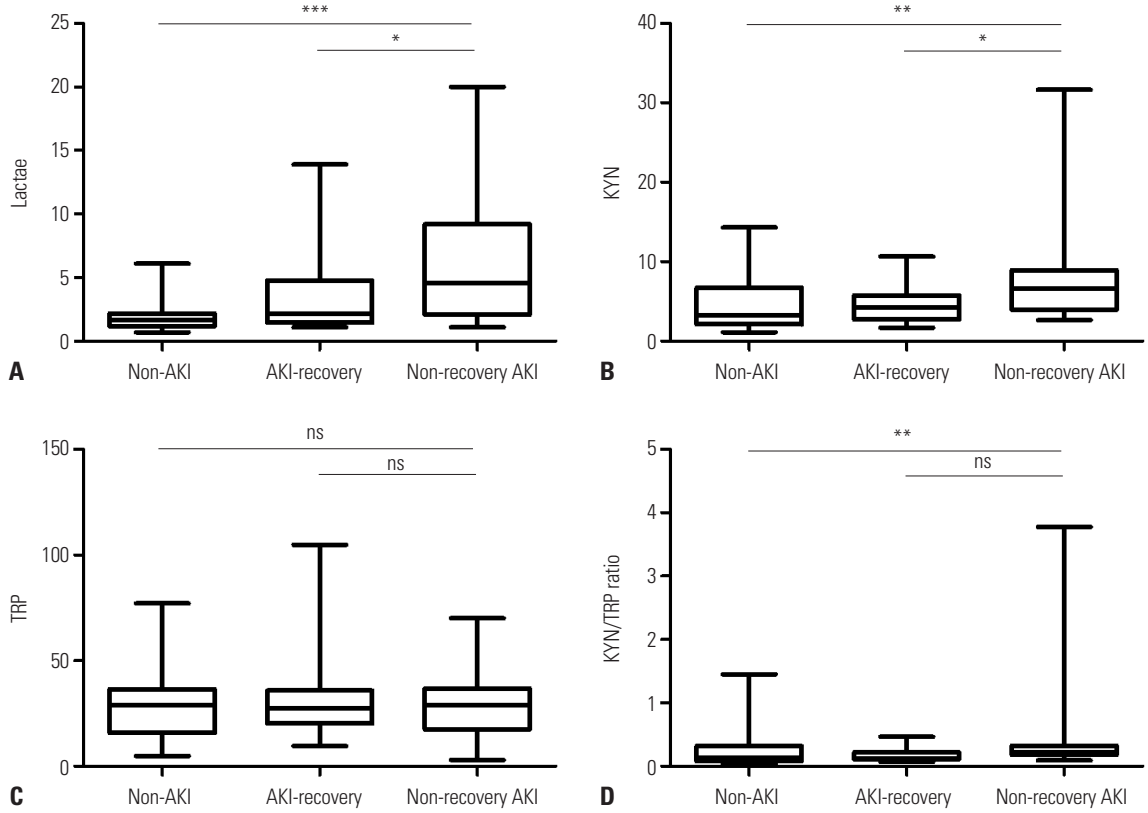

Fig. 3. Kidney markers stratified by the recovery status of renal function. (A) Lactate. (B) KYN. (C) TRP. (D) KYN/TRP ratio. Data as presented as the median (IQR). "ns" indicates a lack of significance (p>0.05), whereas the asterisk indicates statistically significant differences. *p<0.05, **p<0.01, ***p<0.001, analyzed by Student's unpaired two-tailed t test. AKI, acute kidney injury; KYN, kynurenine; TRP, tryptophan; IQR, interquartile range.

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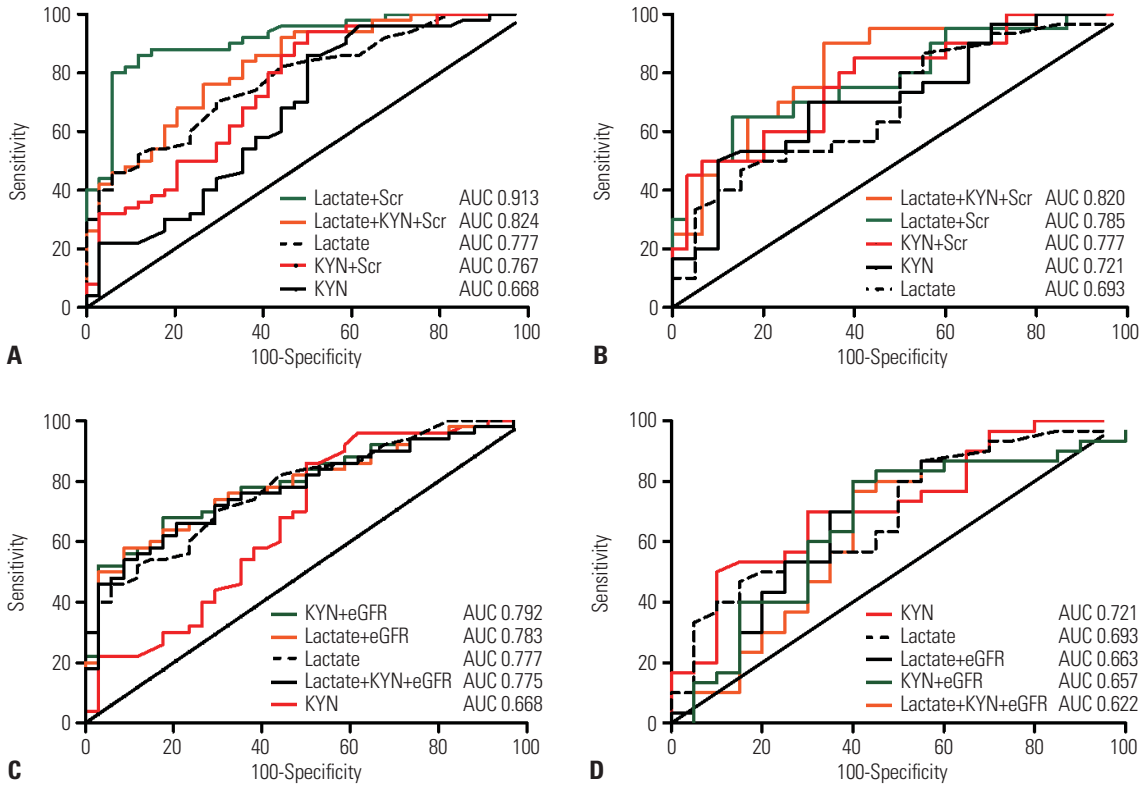

Fig. 4. ROC curves of renal markers. (A) For the prediction of AKI, using Scr. (B) For the prediction of failure to recover renal function, using Scr. (C) For the prediction of AKI, using eGFR calculated by the CKD-EPI formula. (D) For the prediction of failure to recover renal function, using eGFR calculated by the CKD-EPI formula. ROC, receiver operating characteristic; AUC, area under the curve; Scr, serum creatinine; KYN, kynurenine; AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

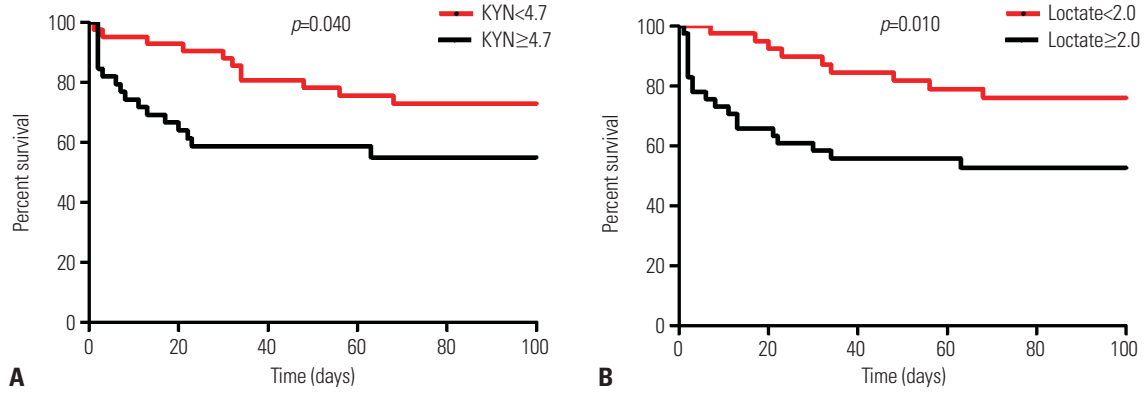

Fig. 5. Kaplan-Meier survival analysis. (A) Patients with baseline KYN concentration <4.7 μmol/L and ≥4.7 μmol/L. (B) Patients with baseline lactate concentration <2.0 μmol/L and ≥2.0 μmol/L. KYN, kynurenine.

score, lactate, and KYN were associated with non-recovery from AKI. However, none of the significant factors remained significant following the final multivariate analysis.

DISCUSSION

The present study observed that 59% of patients with sepsis developed AKI in the ICU, which was similar to the approximate 11%–64% range reported by previous studies.[21-26] Moreover, it was observed that the incidence of AKI increased with the severity of sepsis. The incidence of AKI in septic shock was higher than that in sepsis, which was consistent with the results of previous studies.[21,27] We also found that patients with septic AKI tended to have a higher burden of comorbidities and severity of illness than those without septic AKI. Organ dysfunction and failure, as represented by the SOFA scores, were also greater in patients with septic AKI. Similarly, patients with septic AKI had higher rates of in-hospital, ICU, and 90-day mortality. Moreover, recovery from AKI was identified as an important

Table 4. Univariate and Multivariate Logistic Regression Analyses for Non-Recovery from AKI

Variables	Unadjusted			Adjusted		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Age at diagnosis, yr	1.007	0.967–1.048	0.748	0.984	0.927–1.044	0.593
Sex, male	1.413	0.447–4.473	0.556	1.309	0.223–7.695	0.766
Body mass index, kg/m^2	1.062	0.932–1.209	0.367			
CCI	1.291	1.013–1.645	0.039	1.274	0.932–1.742	0.129
SOFA	1.229	1.017–1.485	0.033	1.187	0.902–1.563	0.222
APACHE II	1.015	0.951–1.083	0.660			
Lactate ≥2.0 mmol/L	4.000	1.143–13.995	0.030	3.076	0.633–14.947	0.164
Procalcitonin, ng/mL	1.004	0.988–1.020	0.651			
Hydroxyproline	1.050	0.988–1.115	0.115			
Serine	0.987	0.973–1.001	0.065	0.979	0.957–1.002	0.071
Kynurenine ≥4.7 umol/L	4.333	1.298–14.471	0.017	4.829	0.823–28.351	0.081

AKI, acute kidney injury; CCI, Charlson Comorbidity Index; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology And Chronic Health Evaluation II; OR, odds ratio; CI, confidence interval.

factor contributing towards a favorable prognosis, which was consistent with the findings of previous studies.[5,28,29] The survival rate of patients who recovered from AKI within 7 days was similar to that of sepsis without AKI.

Amino acids profiling was used to investigate potential biomarkers for the development and recovery of AKI following sepsis, using LC-MS/MS. We revealed significant differences in the serum lactate and KYN levels between patients with and without septic AKI, thus demonstrating a good predictive value of both markers. Furthermore, the elevation in these biomarkers during ICU admission was independently associated with a poor prognosis and poor renal recovery within 7 days. These markers were obtained on the day of admission to the ICU, which allowed early prediction of AKI development and renal function recovery before the Scr attained peak levels. Furthermore, the combination of lactate and Scr facilitated the maximization of the AUC value for AKI prediction, while the combination of lactate, KYN, and Scr maximized the AUC value for renal function recovery prediction.

Although several studies[13,16,30-33] have focused on plasma KYN as a potential biomarker of various biological processes, the association between KYN and renal function recovery prediction in septic AKI has not been well-established. The present study found that higher concentrations of KYN were associated with both AKI development and failure to recover following sepsis. This association could be related to the increased production and decreased elimination of KYN. Pro-inflammatory cytokines that contribute to the development of kidney injury and repair in sepsis may be responsible for KYN elevation in septic AKI owing to the up-regulation of the KYN metabolism.[8,34,35] Another possible explanation for the association between KYN elevation and septic AKI is impairment in excretion,[11] since renal excretion is the main route of KYN elimination.[36]

The K/T ratio, which represents the activity of indoleamine 2,3-dioxygenase, has been suggested as an indicator of TRP catabolism, and is known to be associated with the diagnosis of sepsis and other diseases.[15,16] We also showed that the K/T ratio could differentiate between non-AKI and AKI. However, the K/T ratio showed poor association with renal function recovery. Considering that the overall trend was consistent with the existing assumptions, we hypothesized that this limitation may be attributed to the small sample population.

This study also revealed that lactate may serve as a highly predictive biomarker for AKI development and renal function recovery. This finding was consistent with previous studies that demonstrated that serum lactate levels at ICU admission were associated with the occurrence of AKI in critically ill patients with sepsis.[37,38] Hsu and Hsu[39] also investigated the correlation between serum lactate levels and septic AKI in patients admitted to the emergency departments for sepsis. Hyperlactatemia may arise from tissue hypoperfusion and anerobic glycolysis in sepsis-induced AKI.[40,41] However, the pathogenesis of AKI in sepsis is distinct and relatively more heterogeneous than that of AKI of other etiologies.[42,43] Growing evidence supports that inflammation, diffuse micro-circulatory flow irregularities, and aerobic glycolysis secondary to stress are also pathophysiologic mechanisms associated with sepsis-induced AKI.[39,44,45] Therefore, further studies are needed to investigate the role of lactate in predicting septic AKI.

There were certain limitations to our study. First, this study was based on a single-center trial, and the results may not be generalizable to other settings. Second, the study enrolled patients from the medical ICU and excluded surgical patients. Third, other metabolites of the TRP pathway, such as kynurenate, quinolinate, and xanthurenate, were not evaluated in the study. The combination of more biomarkers could enhance the accuracy of the predictive power for the detection of septic AKI and renal function recovery. Moreover, additional urinary testing may be helpful, considering that the main route of KYN elimination is renal excretion. Lastly, this study only utilized data from the day of admission to the ICU without investigating the changes in serum biomarkers over time. The lactate and

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KYN levels analyzed in this study were measured immediately after admission to the ICU, and have value as early predictors. Further studies are needed to identify the differences between the levels of the respective biomarkers at various time points to permit a more accurate analysis of the association of these biomarkers.

In conclusion, this study suggested that serum lactate and plasma KYN could be utilized as reliable predictive biomarkers for the diagnosis and recovery of AKI in sepsis. Our data also indicated that lactate and KYN were associated with mortality. We believe that the findings of the current study could have substantial clinical implications; however, further research is warranted to validate our findings. Patients with sepsis who are not likely to recover from AKI based on the lactate and KYN concentrations may require more careful treatment strategies, such as closer follow-ups, and restricted exposure to nephrotoxic drugs or radiocontrast agents.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

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