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CLINICAL RESEARCH ARTICLE

Prediction of acute kidney injury, sepsis and mortality in children with urinary CXCL10

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BACKGROUND: To determine the associations of urinary CXC motif chemokine 10 (uCXCL10) with AKI, sepsis and pediatric intensive care unit (PICU) mortality in critically ill children, as well as its predictive value for the aforementioned issues.
METHODS: Urinary CXCL10 levels were serially measured in 342 critically ill children during the first week after PICU admission. AKI diagnosis was based on the criteria of KDIGO. Sepsis was diagnosed according to the surviving sepsis campaign's international guidelines for children.
RESULTS: Fifty-two (15.2%) children developed AKI, 132 (38.6%) were diagnosed with sepsis, and 30 (12.3%) died during the PICU stay. Both the initial and peak values of uCXCL10 remained independently associated with AKI, sepsis, septic AKI and PICU mortality. The AUCs of the initial uCXCL10 for predicting AKI, sepsis, septic AKI and PICU mortality were 0.63 (0.53–0.72), 0.62 (0.56–0.68), 0.75 (0.64–0.87) and 0.77 (0.68–0.86), respectively. The AUCs for prediction by using peak uCXCL10 were as follows: AKI 0.65 (0.56–0.75), sepsis 0.63 (0.57–0.69), septic AKI 0.76 (0.65–0.87) and PICU mortality 0.84 (0.76–0.91).
CONCLUSIONS: Urinary CXCL10 is independently associated with AKI and sepsis and may be a potential indicator of septic AKI and PICU mortality in critically ill children.

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IMPACT:

- Urinary CXC motif chemokine 10 (uCXCL10), as an inflammatory mediator, has been proposed to be a biomarker for AKI in a specific setting. AKI biomarkers are often susceptible to confounding factors, limiting their utility as a specific biomarker, especially in heterogeneous population.
- This study revealed that uCXCL10 levels are independently associated with increased risk for AKI, sepsis, septic AKI and PICU mortality.
- A higher uCXCL10 may be predictive of septic AKI and PICU mortality in critically ill children.

INTRODUCTION

Acute kidney injury (AKI) is a common complication in clinical settings and is associated with high morbidity and mortality,[1–3] especially in critically ill children.[4,5] During recent decades, studies on AKI have focused on identifying novel biomarkers capable of detecting kidney injury before an increase in serum creatinine (SCr) is observed, which could optimize and improve the outcomes of AKI. However, although many urinary biomarkers have been reported in children,[6,7] these early biomarkers have not been widely accepted and adopted in clinical practice. Strong evidence is still needed to confirm that early urinary biomarkers of AKI have beneficial effects on clinical outcomes in a general pediatric intensive care unit (PICU) population.

The CXC motif chemokine ligand 10 (CXCL10), also known as IFN-γ-inducible protein 10 (IP-10), is released by inflammatory cells and various epithelial cells, including renal tubular epithelial cells, and upregulated in kidney tissue exposed to ischemic, nephrotoxic, or inflammatory stress.[8–12] It has been reported that CXCL10 levels in urine (uCXCL10) are elevated in adult patients with biopsy-proven acute tubular injury after renal transplantation[13] and patients undergoing cardiac surgery,[14] and associated with AKI after hematopoietic cell transplantation (HCT) in children.[15] In addition, a clinical cross-sectional study indicated that the concentrations of uCXCL10 were significantly higher in patients with documented AKI and performed well in differentiating between adult patients with and without AKI.[16] Taken together, however, research on the relationship between uCXCL10 and AKI is relatively limited and conducted in a specific setting.[13–15] It remains unclear whether uCXCL10 could be used to discriminate AKI in critically ill children because AKI biomarkers are often

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susceptible to confounding factors, limiting their utility as a specific biomarker in the prediction of AKI, especially in heterogeneous populations. AKI is frequently associated with sepsis, and sepsis is the most common cause of AKI in critically ill patients, which may limit the use of uCXCL10 as a specific biomarker of AKI.[17] Several studies have shown that elevation of serum CXCL10 is an early diagnostic marker of sepsis in adults.[18,19] However, there is scarce evidence for using urinary CXCL10 as a marker for sepsis in children. There has been little previous evidence for the association between uCXCL10 and sepsis. In this study, we first investigated clinical variables potentially associated with uCXCL10 levels and determined the associations of uCXCL10 with AKI, sepsis and PICU mortality in critically ill children. Moreover, we evaluated whether uCXCL10 could serve as an early predictor of AKI, septic AKI or mortality in this population, independent of potential confounders.

METHODS

Study design

This was a prospective study conducted from September to December 2016 and from December 2017 to January 2018 in the PICU, and critically ill children aged between 1 month and 18 years old were eligible for enrollment in the study. Exclusion criteria included known congenital abnormality of the kidney and a failure to collect urine samples before discharge from the PICU or death. This study was approved by the Institutional Review Board at the Children's Hospital of Soochow University and performed in accordance with the Declaration of Helsinki. Informed parental consent was obtained on admission.

Clinical data collection

We reviewed and recorded demographic and clinical characteristics, including age, body weight, sex, clinical diagnosis, the use of mechanical ventilation (MV), hemofiltration, furosemide, antibiotics, steroids, and inotrope during the PICU stay. The diagnosis of multiple organ dysfunction syndrome (MODS), shock, and disseminated intravascular coagulation (DIC) that developed during the PICU stay were defined clinically and diagnosed by the attending PICU physicians according to the criteria described previously.[20] The duration of MV and the length of PICU stay were also recorded. In addition, the pediatric risk of mortality III (PRISM III) score was calculated within 24 h after PICU admission to assess the severity of illness in critically ill children, as described previously.[21]

Diagnosis of AKI

The diagnosis of pediatric AKI that developed during the first 7 days after PICU admission was based on the increase in SCr and/or the reduction in urine output according to the criteria of Kidney Disease: Improving Global Outcome (KDIGO).[22] The baseline SCr was defined as the lowest level obtained within 3 months prior to PICU admission. If the baseline SCr value was not available, admission SCr was used. For critically ill children with SCr ≥1.2 mg/dL (106.0 μmol/L) at PICU admission, the lowest value of SCr within 2 weeks during the PICU stay was considered the baseline SCr, which was in accordance with our previous studies.[21,23] The SCr level was routinely measured daily during the first week after PICU admission, followed by measurement every 48–72 h during the PICU stay.

Diagnosis of sepsis

Sepsis was diagnosed by a chief physician and two attending physicians specializing in pediatric critical care medicine who were completely blinded to the urinary biomarker results retrospectively in accordance with the international guidelines for the management of septic shock and sepsis-associated organ dysfunction in children (Surviving Sepsis Campaign International Guidelines).[24] Critically ill children with sepsis can be identified as those with confirmed or suspected infection with any pathogen who had acute organ dysfunction defined by a Pediatric Sequential Organ Failure Assessment (pSOFA) score of 2 points or more.[24,25]

Clinical outcomes

The primary outcome measure was PICU mortality, defined as all-cause death occurring during the PICU stay, including death resulting from therapy withdrawal.

Measurement of urinary CXCL10

Urine samples were collected at predetermined time points for all children: within 24 h after PICU admission, followed by every 48–72 h within the first week of the PICU stay. All samples were immediately stored at −80 °C until testing. The urine samples were centrifuged for 15 min at 1500 × *g* at 4 °C, and the supernatants were used for measurement of uCXCL10 and urine creatinine (uCr). The concentration of uCXCL10 was measured by ELISA (human CXCL10/IP-10 Duo Set ELISA DIP100, R&D Systems). The minimum detectable level of CXCL10 was <1.67 pg/mL, and the intra- and interassay coefficients of variation were less than 5% and 10%, respectively. The uCXCL10 for analysis was adjusted by uCr (ng/mg uCr). The concentration of uCr was determined by sarcosine oxidase assay using an automatic biochemical analyzer (Hitachi 7600, Tokyo).

Statistical analysis

All data were analyzed using SPSS statistical software Version 22. The assumptions of normality and homogeneity of variance were confirmed. Continuous data are presented as the median and interquartile range (IQR), as they were not normally distributed. Categorical data are presented as counts and percentages. To compare differences among groups, the Mann–Whitney *U* test or Kruskal–Wallis *H* test was used for continuous variables, and the chi-square test or Fisher's exact test was used for categorical variables. Spearman's correlation tests were performed to investigate correlations between uCXCL10 and clinical variables. To determine clinical variables potentially associated with the levels of uCXCL10, variables with $P < 0.05$ in Spearman's test were analyzed by the generalized linear model (GLM), and the beta coefficient (β), standard error (SE), and odds ratio (OR) with a 95% confidence interval (CI) were calculated. Continuous variables were log-transformed (base10) to meet approximate normality. Multicollinearity of variables was evaluated via tolerance and variance inflation factor (VIF), and tolerance ≤0.5 and a VIF value ≥2 indicated the presence of multicollinearity. Univariate and multivariate logistic regression analyses were used to evaluate the association between uCXCL10 and AKI, sepsis, septic AKI, and mortality. The predictive values of uCXCL10 for AKI, sepsis, septic AKI, and mortality were assessed by the receiver operating characteristic (ROC) curve and area under the curve (AUC). The sensitivity, specificity, and Youden index were also calculated, and the optimal cutoff values were determined by the maximum Youden index. A two-tailed $P < 0.05$ was considered significant.

RESULTS

Patient characteristics

A total of 351 critically ill children were admitted to the PICU during the study period. Nine were excluded including one child who was admitted to the PICU at an age of <1 month, four children who had a failure in collecting urine samples before discharge from the PICU or death, and four children with multiple PICU admissions during one hospitalization, and only the last admission to the PICU was used for analysis. Admission diagnoses included respiratory diseases (42.4%), neurological diseases (16.1%), hematologic/oncologic diseases (9.9%), gastrointestinal diseases (9.7%), trauma or postoperative acute care (8.8%), and others (13.1%).

Among the 342 critically ill children, 52 (15.2%) developed AKI during the first week after admission, including 23 with KDIGO stage 1, 14 with stage 2, and 15 with stage 3. A total of 132 (38.6%) critically ill children were diagnosed with sepsis during their PICU stay. A comparison of the demographic and clinical characteristics and outcomes according to subgroups of patients with and without AKI or sepsis is presented in Table 1.

Urinary CXCL10

There were 706 urinary samples collected within the first week after PICU admission. Of the 342 critically ill children, 111 (32.4%) had one sample, 140 (40.9%) had two samples, and 90 (26.3%) had three or more samples. Urinary CXCL10 values were detectable in 689 (97.6%) samples. The concentrations of undetectable uCXCL10 were assumed to be 1.67 pg/mL, which were equivalent to the mean minimum detectable dose of the assay. Seven (1.0%)

Table 1. Comparison of demographic and clinical characteristics between patients with and without AKI or sepsis.

	Non-AKI $n = 290$	AKI $n = 52$	*P* value	Non-sepsis $n = 210$	Sepsis $n = 132$	*P* value
Age, months	14.0 [3.0–46.3]	37.5 [11.5–81.8]	0.001	20.0 [3.0–60.0]	12.5 [3.0–45.0]	0.114
Body weight, kg	10.0 [6.0–16.0]	13.5 [9.3–22.5]	0.003	11.3 [6.5–19.0]	10.0 [6.0–15.8]	0.050
Male, *n*	192 (66.2)	34 (65.4)	0.908	140 (66.7)	86 (65.2)	0.773
PRISM III score	3 [0–8]	10 [5–16]	<0.001	2 [0–8]	6 [2–13]	0.001
AKI[a], *n*		52 (100)	NA	30 (14.3)	22 (16.7)	0.551
AKI stage 1, *n*		23 (44.2)	NA	16 (7.6)	7 (5.3)	0.370
AKI stage 2, *n*		14 (26.9)		6 (2.9)	8 (6.1)	
AKI stage 3, *n*		15 (28.8)		8 (3.8)	7 (5.3)	
Sepsis[b], *n*	110 (37.9)	22 (42.3)	0.551		132 (100)	NA
MODS[b], *n*	18 (6.2)	17 (32.7)	<0.001	16 (7.6)	19 (14.4)	0.044
Shock/DIC[b], *n*	18 (6.2)	16 (30.8)	<0.001	10 (4.8)	24 (18.2)	<0.001
MV[b], *n*	69 (23.8)	25 (48.1)	<0.001	39 (18.6)	55 (41.7)	<0.001
Duration of MV[b], hours	0 [0–0]	0 [0–100.3]	0.001	0 [0–0]	0 [0–131.8]	<0.001
Hemofiltration[b], *n*	9 (3.1)	8 (15.4)	<0.001	12 (5.7)	5 (3.8)	0.425
Furosemide[b], *n*	22 (7.6)	17 (32.7)	<0.001	21 (10.0)	18 (13.6)	0.303
Steroid[b], *n*	164 (56.6)	29 (55.8)	0.917	101 (48.1)	92 (69.7)	<0.001
Antibiotic[b], *n*	234 (80.7)	39 (75.0)	0.346	148 (70.5)	125 (94.7)	<0.001
Inotrope[b], *n*	24 (8.3)	10 (19.4)	0.015	16 (7.6)	18 (13.6)	0.070
PICU LOS, hours	91.0 [48.0–158.3]	106.0 [41.2–200.0]	0.553	71.6 [43.6–133.4]	132.4 [78.0–210.6]	<0.001
PICU death, *n*	14 (4.8)	16 (30.8)	<0.001	16 (7.6)	14 (10.6)	0.342
Initial uCXCL10, ng/mg uCr	0.096 [0.040–0.257]	0.186 [0.054–1.068]	0.004	0.079 [0.032–0.227]	0.151 [0.064–0.453]	<0.001
Peak uCXCL10, ng/mg uCr	0.117 [0.055–0.382]	0.500 [0.078–1.806]	0.001	0.104 [0.042–0.334]	0.192 [0.083–0.767]	<0.001

Values are median [interquartile range]. Numbers in parentheses denote percentages.
AKI acute kidney injury, *DIC* disseminated intravascular coagulation, *LOS* length of stay, *MODS* multiple organ dysfunction syndrome, *MV* mechanical ventilation, *N/A* not applicable, *PICU* pediatric intensive care unit, *PRISM III* pediatric risk of mortality III. *uCXCL10* urinary CXCL10, *uCr* urinary creatinine.
[a]Developed during the first week after PICU admission.
[b]Administered and developed during PICU stay.

urinary samples were diluted 2, 5, or 20 times to maintain the enzymatic reactions within a linear range.

The uCXCL10 level from the first 24 h after admission to the PICU was denoted as the initial uCXCL10 level. The highest uCXCL10 level among all collected samples during the first 7 days after PICU admission was denoted the peak uCXCL10. The initial and peak values of uCXCL10 were used for analysis.

Correlation of urinary CXCL10 levels with clinical variables

All variables in Table 1 were analyzed for association with uCXCL10. Spearman's correlation analysis showed that both the initial and peak levels of uCXCL10 were significantly correlated with age, body weight, PRISM III score, AKI, AKI stage, sepsis, MODS, shock/DIC, MV, duration of MV, and the use of furosemide, steroids, and antibiotics ($P < 0.05$).

Variables with $P < 0.05$ under Spearman's test were included in the GLM to confirm factors significantly associated with the uCXCL10 levels after checking the multicollinearity. As listed in Table 2, the initial uCXCL10 was independently associated with body weight ($P = 0.020$), PRISM III score ($P = 0.007$), sepsis ($P = 0.026$), and AKI stage ($P = 0.047$); the peak uCXCL10 was independently associated with body weight ($P = 0.003$), PRISM III score ($P = 0.007$), sepsis ($P = 0.038$), AKI stage ($P = 0.006$), and shock/DIC ($P = 0.046$).

Association between urinary CXCL10 and AKI

The initial and peak uCXCL10 levels were significantly higher in AKI than in non-AKI (Table 1). Comparisons of the initial and peak uCXCL10 levels among different stages of AKI in critically ill children are displayed in online Supplementary Fig. S1a and b.

Univariate and multivariate logistic regression analyses were performed to identify whether uCXCL10 levels were independently associated with AKI in critically ill children. As shown in Table 3 and Fig. 1a, b, both the initial and peak uCXCL10 levels remained significantly associated with AKI (initial: AOR = 1.791, 95% CI 1.152–2.785, $P = 0.010$; peak: AOR = 2.002, 95% CI 1.284–3.123, $P = 0.002$) after adjustment for body weight, PRISM III score, and sepsis. The AUCs of the initial and peak uCXCL10 for predicting AKI were 0.63 (0.53–0.72) and 0.65 (0.56–0.75), respectively.

Association between urinary CXCL10 and sepsis

The sepsis group in critically ill children had higher initial and peak levels of uCXCL10 than the non-sepsis group (Table 1). Comparisons of the initial and peak uCXCL10 levels between non-sepsis and sepsis in critically ill children are displayed in online Supplementary Fig. S2a, b. To identify the association between uCXCL10 and sepsis, univariate and multivariate logistic regression analyses were performed. As shown in Table 3 and Fig. 1a, b, the initial and peak uCXCL10 were both independently associated with sepsis (initial: AOR = 1.679, 95% CI 1.189–2.371, $P = 0.003$; peak: AOR = 1.752, 95% CI 1.231–2.494, $P = 0.002$) after adjustment for body weight, PRISM III score, and AKI stage. The AUCs of the initial and peak uCXCL10 in predicting sepsis were 0.62 (0.56–0.68) and 0.63 (0.57–0.69), respectively.

Association between urinary CXCL10 and septic AKI

Since the initial and peak uCXCL10 levels were both independently associated AKI and sepsis (Table 2), their relationship between uCXCL10 and septic AKI was further investigated.

Table 2. Generalized linear model analysis of urinary CXCL10 with clinical variables.

	β	SE	OR	95% CI	P value
Initial uCXCL10					
Body weight, kg	−0.270	0.116	0.763	0.607–0.958	0.020
PRISM III score	0.013	0.005	1.014	1.004–1.024	0.007
AKI stage[a]	0.105	0.053	1.111	1.001–1.232	0.047
Sepsis[b]	0.173	0.077	1.188	1.021–1.383	0.026
MODS[b]	0.255	0.144	1.290	0.972–1.711	0.078
Shock/DIC[b]	0.179	0.137	1.196	0.913–1.565	0.193
Duration of MV[b], hours	−0.027	0.044	0.973	0.893–1.060	0.535
Furosemide[b]	0.057	0.122	1.059	0.834–1.345	0.639
Steroid[b]	0.082	0.074	1.086	0.940–1.254	0.262
Antibiotic[b]	0.055	0.094	1.056	0.879–1.269	0.558
Peak uCXCL10					
Body weight, kg	−0.333	0.112	0.717	0.576–0.893	0.003
PRISM III score	0.013	0.005	1.013	1.004–1.023	0.007
AKI stage[a]	0.141	0.051	1.152	1.042–1.273	0.006
Sepsis[b]	0.155	0.075	1.167	1.009–1.351	0.038
MODS[b]	0.201	0.139	1.223	0.932–1.606	0.147
Shock/DIC[b]	0.264	0.132	1.303	1.005–1.688	0.046
Duration of MV[b], hours	0.003	0.042	1.003	0.923–1.089	0.949
Furosemide[b]	0.226	0.118	1.253	0.996–1.578	0.055
Steroid[b]	0.074	0.071	1.077	0.937–1.237	0.298
Antibiotic[b]	0.093	0.090	1.097	0.920–1.309	0.303

Variables in Table 1 with a *P* value <0.05 (Spearman's analysis) were taken into the generalized linear model analysis after checking the multicollinearity. Continuous variables were log-transformed.
AKI acute kidney injury, *DIC* disseminated intravascular coagulation, *MODS* multiple organ dysfunction syndrome, *MV* mechanical ventilation, *PRISM III* pediatric risk of mortality III.
[a]Developed during the first week after PICU admission.
[b]Administered and developed during PICU stay.

Children were divided into four groups according to the presence/absence of AKI and sepsis: AKI/sepsis (septic AKI, $n = 22$), non-AKI/sepsis ($n = 110$), AKI/non-sepsis ($n = 30$), and non-AKI/non-sepsis ($n = 180$). As displayed in Fig. 2a, b, both the initial and peak uCXCL10 levels in the septic AKI group were the highest. Comparisons of clinical characteristics and uCXCL10 levels among the four groups are shown in online Supplementary Table S1.

Univariate and multivariate logistic regression analyses were used to determine the association between uCXCL10 and septic AKI ($n = 342$). As shown in Table 3 and Fig. 1a, b, the initial and peak uCXCL10 were both independently associated with septic AKI after adjustment for body weight and PRISM III (initial: AOR = 3.281, 95% CI 1.788–6.018, $P < 0.001$; peak: AOR = 3.172, 95% CI 1.735–5.799, $P < 0.001$). The AUCs of the initial and peak uCXCL10 in predicting septic AKI were 0.75 (95% CI 0.64–0.87) and 0.76 (95% CI 0.65–0.87), respectively. The ROC curves of the initial and peak uCXCL10 and PRISM III for predicting septic AKI are displayed in Fig. 3a.

In addition, univariate and multivariate logistic regression analyses indicated that uCXCL10 was robustly associated with septic AKI in critically ill children with sepsis ($n = 132$), as shown in online Supplementary Table S2. The performances of the initial and peak uCXCL10 in predicting septic AKI were 0.72 (95% CI 0.59–0.85) and 0.73 (95% CI 0.60–0.85), respectively, in this population in online Supplementary Table S2.

Association between urinary CXCL10 and mortality

A comparison of demographic and clinical characteristics between survivors and non-survivors is shown in online Supplementary Table S3. Out of the 342 children, 30 (8.8%) died during the PICU stay. The values of uCXCL10 were significantly higher in those who died within the PICU than in survivors, as displayed in online Supplementary Fig. S3a and b. To explore whether uCXCL10 levels were independently associated with mortality in critically ill children, univariate and multivariate logistic regression analyses were performed. After adjustment for body weight, PRISM III score, sepsis, and AKI stage, the initial and peak uCXCL10 levels remained independently associated with mortality, with adjusted odds ratios of 2.779 (95% CI 1.487–5.195, $P = 0.001$) and 3.965 (95% CI 2.037–7.717, $P < 0.001$), as listed in Table 3 and Fig. 1a and b.

The initial and peak uCXCL10 displayed AUCs of 0.77 (95% CI 0.68–0.86) and 0.84 (95% CI 0.76–0.91), and the PRISM III score displayed an AUC of 0.83 (95% CI 0.75–0.91) in predicting mortality. The ROC curves of the initial and peak uCXCL10 and PRISM III for predicting mortality are shown in Fig. 3b.

DISCUSSION

This study prospectively assessed uCXCL10 in a typical heterogeneous PICU population. Our data revealed that elevated uCXCL10 was independently associated with AKI and sepsis after adjustment for confounding factors, and uCXCL10 could be a potential indicator of septic AKI and PICU mortality in critically ill children.

Under normal conditions, CXCL10 is expressed at low levels in the kidney, but its expression levels are upregulated in renal tubular cells after insult, involving in the pathogenesis of AKI.[10,11,26] In a mouse model of AKI, Kirita et al. used a scRNA sequencing method to profile proinflammatory and profibrotic signaling from the renal proximal tubule. The results of their study suggested that a wide range of proinflammatory and profibrotic cytokines, including CXCL10, might have been implicated in the pathogenesis and progression of AKI, and attenuation of the cytokine expression may represent a new therapeutic target to improve survival in AKI patients.[27] Higher uCXCL10 levels were found among critically ill children with AKI in our study, which was consistent with a prospective nested case-control study performed by Ho et al.[14]. They compared urinary proteomes of adults before, during, and after cardiopulmonary bypass surgery, discovering that uCXCL10 levels were upgraded in patients with AKI postoperatively.[14] In a recent study on urinary biomarkers of AKI, uCXCL10 was discovered and validated to be associated with AKI in children after HCT.[15] However, these data are limited to small studies originally designed for AKI prediction in a specific setting. Our results confirmed the significant association of uCXCL10 with AKI regardless of illness severity and the presence of sepsis in critically ill children.

The discriminative power of uCXCL10 for AKI in our study was lower than that in the study of Vaidya et al.[16]. Our results suggested that uCXCL10 may not be a good predictor of AKI in critically ill children. In contrast to the patients without AKI in the present study, 102 individuals without AKI in the previous study were of three types: 50 healthy volunteers, 39 patients undergoing cardiac catheterization, and 13 patients admitted to the intensive care unit.[16] Healthy volunteers accounted for half of those who were non-AKI, which may partly explain the discrepancy. In critically ill patients, AKI development is characterized by heterogeneity, and the predictive value of AKI is highly dependent on the underlying conditions. Our study indicates that although it is undisputed that uCXCL10 is associated with kidney injury, a similar association has been identified between uCXCL10 and sepsis, which makes uCXCL10 measurements a poor diagnostic tool in complex contexts in critically ill children. Moreover, the levels of uCXCL10 were influenced by age and body weight, which

Table 3. Association of urinary CXCL10 with AKI, sepsis, septic AKI, and PICU mortality.

	AKI	Sepsis	Septic AKI	PICU mortality
Initial uCXCL10				
OR (95% CI)	2.127 (1.413–3.202)	1.835 (1.322–2.546)	3.679 (2.062–6.564)	4.091 (2.387–7.012)
P value	<0.001	<0.001	<0.001	<0.001
AOR (95% CI)	1.791 (1.152–2.785)[a]	1.679 (1.189–2.371)[b]	3.281 (1.788–6.018)[c]	2.779 (1.487–5.195)[d]
P value	0.010	0.003	<0.001	0.001
AUC (95% CI)	0.63 (0.53–0.72)	0.62 (0.56–0.68)	0.75 (0.64–0.87)	0.77 (0.68–0.86)
Optimal cutoff value, ng/mg	0.40	0.09	0.15	0.54
Sensitivity	42.3%	70.5%	81.8%	56.7%
Specificity	82.4%	52.4%	60.3%	86.5%
Peak uCXCL10				
OR (95% CI)	2.319 (1.542–3.485)	1.908 (1.378–2.641)	3.570 (2.008–6.345)	5.947 (3.292–10.741)
P value	<0.001	<0.001	<0.001	<0.001
AOR (95% CI)	2.002 (1.284–3.123)[a]	1.752 (1.231–2.494)[b]	3.172 (1.735–5.799)[c]	3.965 (2.037–7.717)[d]
P value	0.002	0.002	<0.001	<0.001
AUC (95% CI)	0.65 (0.56–0.75)	0.63 (0.57–0.69)	0.76 (0.65–0.87)	0.84 (0.76–0.91)
Optimal cutoff value, ng/mg	0.45	0.09	0.94	0.54
Sensitivity	53.8%	75.0%	59.1%	73.3%
Specificity	79.0%	43.8%	87.8%	81.1%

AKI acute kidney injury, *AOR* adjusted odds ratio, *AUC* the area under the ROC curve, *CI* confidence interval.
[a]After adjustment for body weight, pediatric risk of mortality III (PRISM III) score, and sepsis. [b]After adjustment for body weight, PRISM III score, and AKI stage.
[c]After adjustment for body weight and PRISM III score. [d]After adjustment for body weight, PRISM III score, sepsis, and AKI stage.

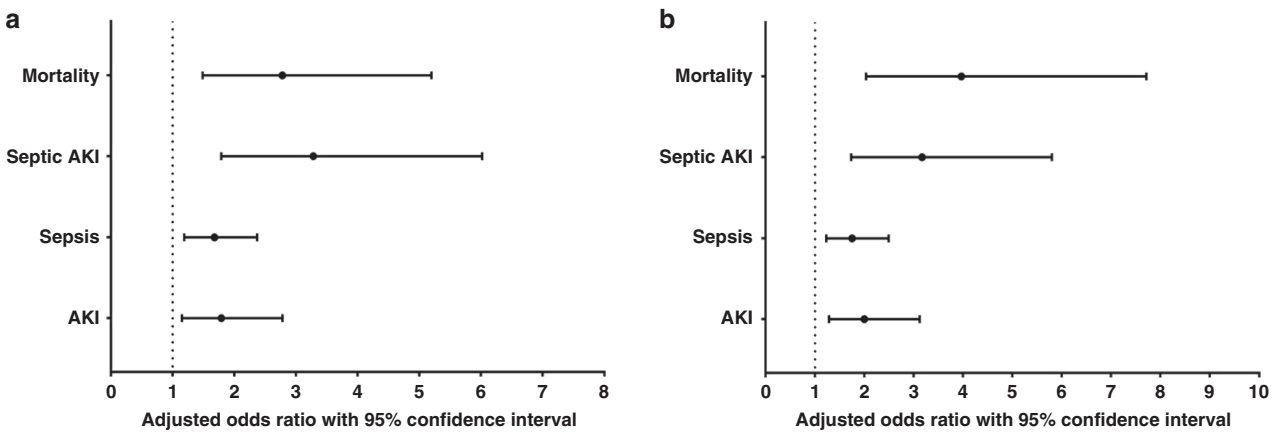

Fig. 1 Forest plot of the correlation between the uCXCL10 and AKI, sepsis, septic AKI, and mortality in multivariate logistic regression models as described in Methods. Adjusted odds ratio: AKI, after adjustment for body weight, PRISM III score and sepsis. Sepsis, after adjustment for body weight, PRISM III score and AKI stage. Septic AKI, after adjustment for body weight and PRISM III score. Mortality, after adjustment for body weight, PRISM III score, sepsis and AKI stage. **a** Initial uCXCL10, **b** peak uCXCL10. *AKI* acute kidney injury, *PRISM III* pediatric risk of mortality III, *uCXCL10* urinary CXCL10.

might be another explanation for the poor performance in the clinical utility of uCXCL10 as an AKI biomarker in the PICU population.

Sepsis is a well-known main cause of AKI in critically ill patients.[17,28] CXCL10 has been reported to regulate the pathogenesis of sepsis.[29,30] In our present study, uCXCL10 levels were higher in critically ill children with sepsis, suggesting that the levels of uCXCL10 were influenced by inflammation and infection. Urinary CXCL10 remained independently associated with both sepsis and AKI, indicating that the increases in uCXCL10 due to AKI and sepsis are additive. These results were supported by animal research in which a marked upregulation of CXCL10 was detected within the kidney in a septic acute renal failure model.[31]

In this study, uCXCL10 had the ability to discriminate septic AKI in critically ill children. These findings suggest that uCXCL10 could be diagnostic of septic AKI. Considering that septic AKI is a frequent complication in critically ill patients and associated with a higher risk of in-hospital mortality,[28,32] the identification of early biomarkers of septic AKI is extremely important. The urinary biomarker neutrophil gelatinase-associated lipocalin and cell cycle biomarkers of tissue inhibitor of metalloproteinases-2 and insulin-like growth factor-binding protein 7 are frequently investigated and have been shown to predict septic AKI in patients with sepsis.[32–34] Our findings emphasize that uCXCL10, as any other biomarker, must be interpreted in the specific clinical context. Although, to our knowledge, this is the first report about the

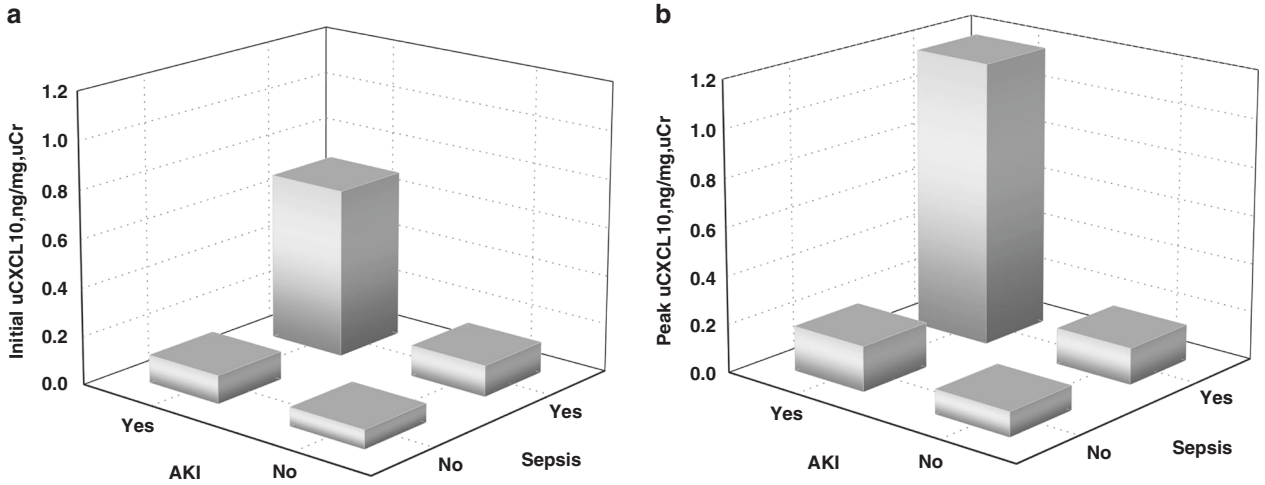

Fig. 2 Median uCXCL10 differences among children with and without AKI and with and without sepsis. a Initial uCXCL10, **b** peak uCXCL10. *AKI* acute kidney injury, *uCXCL10* urinary CXCL10, *uCr* urinary creatinine.

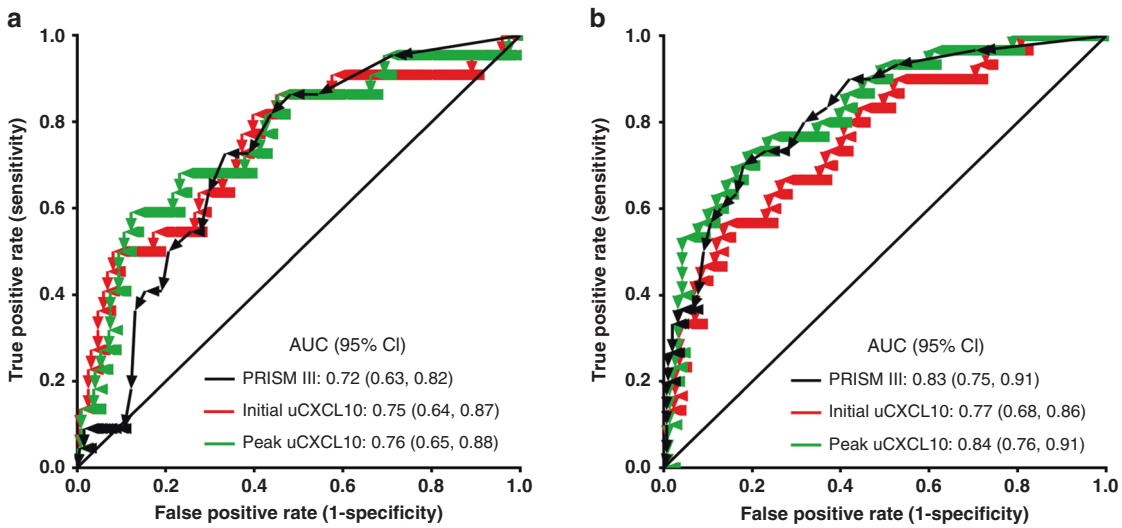

Fig. 3 ROC curves for the abilities of PRISM III and the initial and peak uCXCL10 to predict septic AKI and PICU mortality in critically ill children. a Septic AKI, **b** mortality. *AKI* acute kidney injury, *PICU* pediatric intensive care unit, *PRISM III* pediatric risk of mortality III, *uCXCL10* urinary CXCL10.

relationship of uCXCL10 and septic AKI, this was a single cohort study, and 22 (6.4%) critically ill children had septic AKI. A multicenter study with more cases is needed to confirm our findings. Additional studies are also required to delineate the specific mechanism of CXCL10 in septic and non-septic AKI.

As uCXCL10 has been related to different conditions of inflammation and injury and proven to be a reliable marker for septic AKI, it has been consequentially evaluated as a prognostic marker. Our study first demonstrated that uCXCL10 levels had independent associations with PICU mortality in critically ill patients, even after adjusting for potential confounders, including the severity of illness assessed by the PRISM III score. Clinical studies on the association between CXCL10 and mortality have been limited and predominantly investigated in the blood rather than in the urine of adult patients.[19,35,36] Regarding CXCL10 in urine, a pilot study conducted in patients with ischemic AKI suggested that uCXCL10 might predict renal functional outcome at various times along the course of ischemic AKI and predict mortality of AKI patients within 3 months.[37] However, in adult patients with established AKI, uCXCL10 was a good biomarker of AKI, as mentioned earlier, but it was not predictive of in-hospital mortality.[16] Both AKI and sepsis are established risk factors for mortality, and AKI and sepsis synergistically increase mortality.[2,38] In our study, patients with septic AKI had a similar mortality compared to non-septic AKI, suggesting that non-septic etiologies may also contribute to the high mortality rate in AKI patients. The relationship between elevated uCXCL10 levels and increased risk of all-cause PICU death in our cohort was independent of the presence of sepsis and/or AKI and illness severity assessed by the PRISM III score, indicating that uCXCL10 is not simply associated with AKI and sepsis but is also a potential indicator of PICU mortality in critically ill children. Thus, elevated uCXCL10 levels in ICU patients should not be simply viewed as a risk indicator for AKI but rather prompt a thorough investigation of coexisting conditions, which will ultimately determine the prognosis of ICU patients. Furthermore, the predictive abilities of peak uCXCL10 for PICU mortality were better than those of initial uCXCL10. These results suggest that dynamic monitoring of uCXCL10 levels upon PICU admission is valuable for clinicians in guiding preventive strategies and improving prognosis.

There are some limitations in this study. First, the AKI incidence may be underestimated when SCr at PICU admission is considered a baseline, given that the majority of critically ill children did not have baseline SCr prior to PICU admission. Consistent with our previous studies,[21,23] the lowest SCr value within 2 weeks during the PICU stay was used as a baseline for patients with elevated SCr ≥106.1 μmol/L at PICU admission, which, however, has not been validated in critically ill children. Nevertheless, a previous study suggests that the lowest SCr within the first week in the ICU better approximates the true baseline distribution and leads to a more accurate diagnosis of AKI than the estimation methods of back-calculating baseline SCr.[39] Second, the original intention of our study was to evaluate the associations between uCXCL10 and AKI and sepsis; however, the predictive values of uCXCL10 for AKI and sepsis in a mixed heterogeneous PICU were not valuable. In a previous study conducted in adult patients at risk of kidney injury, Minie Sarwal's group[40] developed and validated a Kidney Injury Test (KIT) score based on six urinary biomarkers, including uCXCL10, for the early detection of kidney injury. Whether there are any additional benefits of profiling the KIT score in PICU settings merits further exploration. Third, although it is a challenge to evaluate the performance of biological indicators on the diagnosis of septic AKI based on the recommendations applied to children[24,32] and the relevance of this finding of uCXCL10 in predicting septic AKI is limited by the small sample size, the diagnostic accuracy of uCXCL10 persisted in all critically ill children and in a subgroup analysis of children with sepsis. Fourth, we did not perform an etiological analysis for developing AKI. Since our study was carried out in a general and mixed PICU population, it was difficult to distinguish the exact causes of AKI from the existence of complex comorbidities. Fifth, although the prognostic accuracy of the initial and peak uCXCL10 was not superior to the PRISM III score in predicting PICU mortality, elevated uCXCL10 levels were independently associated with PICU mortality, regardless of illness severity and the presence of AKI and sepsis. This result may prompt uCXCL10 to be used as an invasive biomarker to assess mortality risk or as an auxiliary indication for its simpler utility compared with PRISM III. Furthermore, it is unclear whether uCXCL10 is truly causatively involved in the pathophysiologic mechanisms of underlying conditions resulting in high mortality or whether it reflects general inflammation and injury in critical illness. Further studies are required to understand the biochemical properties and regulatory mechanisms of uCXCL10 in critical illness. Sixth, the single-center study design represents a main limitation. Although we added novel insights to the literature, we did not compare other kidney injury biomarkers and could not prove the statistical superiority of the initial uCXCL10 value over other standard parameters in the early prediction of adverse outcomes. A multicenter study with a larger quantity of samples is therefore necessary for further validation.

CONCLUSIONS

Urinary CXCL10 levels were independently associated with an increased risk for AKI, sepsis, septic AKI and PICU mortality even after adjustment for confounding factors. A higher uCXCL10 may be predictive of septic AKI and PICU mortality in critically ill children.

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AUTHOR CONTRIBUTIONS

H.H. performed the experiments and data analysis and drafted the manuscript. H.Z. participated in data analysis and revised the manuscript. W.W., J.C., and Z.B. had primary responsibility for diagnosis of sepsis and participated in clinical data collection. X.D. and W.L. participated in collecting the data and samples. J.P. and X.L. participated in data analysis and interpretation. J.W. participated in the design of the study and coordination. Y.L. had primary responsibility for study design, performing the experiments, data analysis, interpretation of data, and writing the manuscript. All authors read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Board of the Children's Hospital of Soochow university, and parental written informed consent was obtained for all participants.

ADDITIONAL INFORMATION

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