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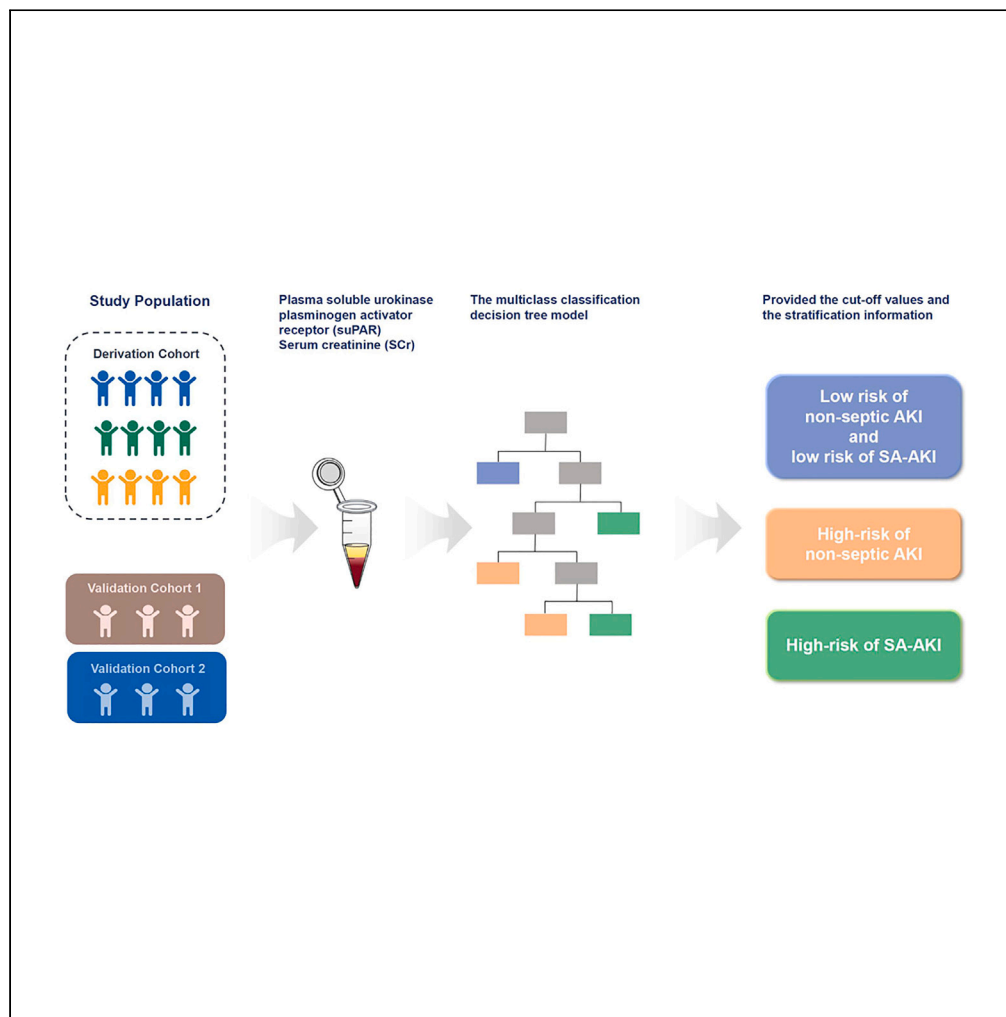
Utility of plasma suPAR to identify AKI and sepsis associated AKI in critically ill children

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Highlights

Plasma suPAR was associated with AKI and SA-AKI in critically ill children

The multiclass classification model provided the cutoffs for plasma suPAR and SCr

Provided stratification information to identify high risk of non-septic AKI and SA-AKI

Article

Utility of plasma suPAR to identify AKI and sepsis associated AKI in critically ill children

Jing Xu,[1,8] Jiao Chen,[2,3,8] Min Li,[4] Zhen Jiang,[5] Fang Fang,[6] Junlong Hu,[1] Yueying Zhou,[1] Huiwen Li,[1] Zhenjiang Bai,[2] Xiaozhong Li,[1] Guoping Lu,[7,*] and Yanhong Li[1,6,9,*]

SUMMARY

Current biomarkers for sepsis-associated acute kidney injury (SA-AKI) lack specificity. The role of soluble urokinase plasminogen activator receptor (suPAR) in discriminating AKI and SA-AKI in children remains elusive. This prospective multicenter study was conducted in critically ill children cohorts using a derivation-validation design, and plasma samples were collected within first 24 h after admission. Plasma suPAR was independently associated with AKI, SA-AKI, and PICU mortality, even after adjustment for confounding variables. This multiclass classification model had the micro-average AUC of 0.89 with specificity of 97.6% for discriminating non-septic AKI, and specificity of 99.0% for discriminating SA-AKI, based on the cut-off values of 1.5 and 2.3-fold baseline in serum creatinine (SCr) and 4.5 and 11.2 ng/mL in plasma suPAR. The multiclass classification model provides the cutoffs for plasma suPAR and SCr and specifically discriminates critically ill children at high risk of non-septic AKI and SA-AKI, which can facilitate clinical utility.

INTRODUCTION

Acute kidney injury (AKI) as an independent risk factor for increased morbidity and mortality is a syndrome of critical illness characterized by a sudden decrease in renal function.[1,2] AKI occurs in 20% of hospitalized children and 35% in pediatric intensive care unit (PICU) settings.[3] Sepsis, which is defined as a clinical syndrome characterized by life-threatening organ dysfunction caused by a dysregulated response to infection, is a common and crucial contributing factor to AKI.[4] Approximately half of patients with AKI are associated with sepsis.[5] Patients with sepsis-associated AKI (SA-AKI) have a significantly higher ICU mortality compared to non-septic AKI patients or septic patients without AKI.[6] Early accurate identification of AKI and SA-AKI may lead to earlier effective therapeutic measures and improve patient outcomes.

In recent decades, a variety of biomarkers have been identified and attempted to predict AKI and SA-AKI in different clinical situations. It has been reported that urinary neutrophil gelatinase-associated lipocalin and the combination of urinary tissue inhibitor of metalloproteinase-2 with insulin-like growth factor binding protein-7 had good performance for predicting severe AKI in pediatric patients.[7,8] However, they have been demonstrated to be highly sensitive but not specific biomarkers of SA-AKI.[9] There remains a dire need to find specific biomarkers that may have the ability to discriminate SA-AKI in critically ill children.

Systemic inflammation and oxidative stress are particularly involved in the pathogenesis of kidney injury.[10,11] Soluble urokinase plasminogen activator receptor (suPAR), as a marker of activation of immune and inflammatory systems involved in modulation of cellular bioenergetics and increased oxidative stress, was found to involved in the pathogenesis of AKI by sensitizing kidney proximal tubules to injury.[12] Previous studies confirmed that elevated level of plasma suPAR was a strong predictor of AKI in adult cohorts.[13,14] Circulating levels of suPAR may discriminate the highest AKI stage, varying AKI courses, poor renal outcome in critically ill adult patients with sepsis at any time within 7 days of sepsis diagnosis.[15] To date, there is no evidence reporting the discriminative ability of suPAR in the pediatric AKI population. Whether plasma suPAR has an effective discriminative performance of AKI or SA-AKI in critically ill children is unclear.

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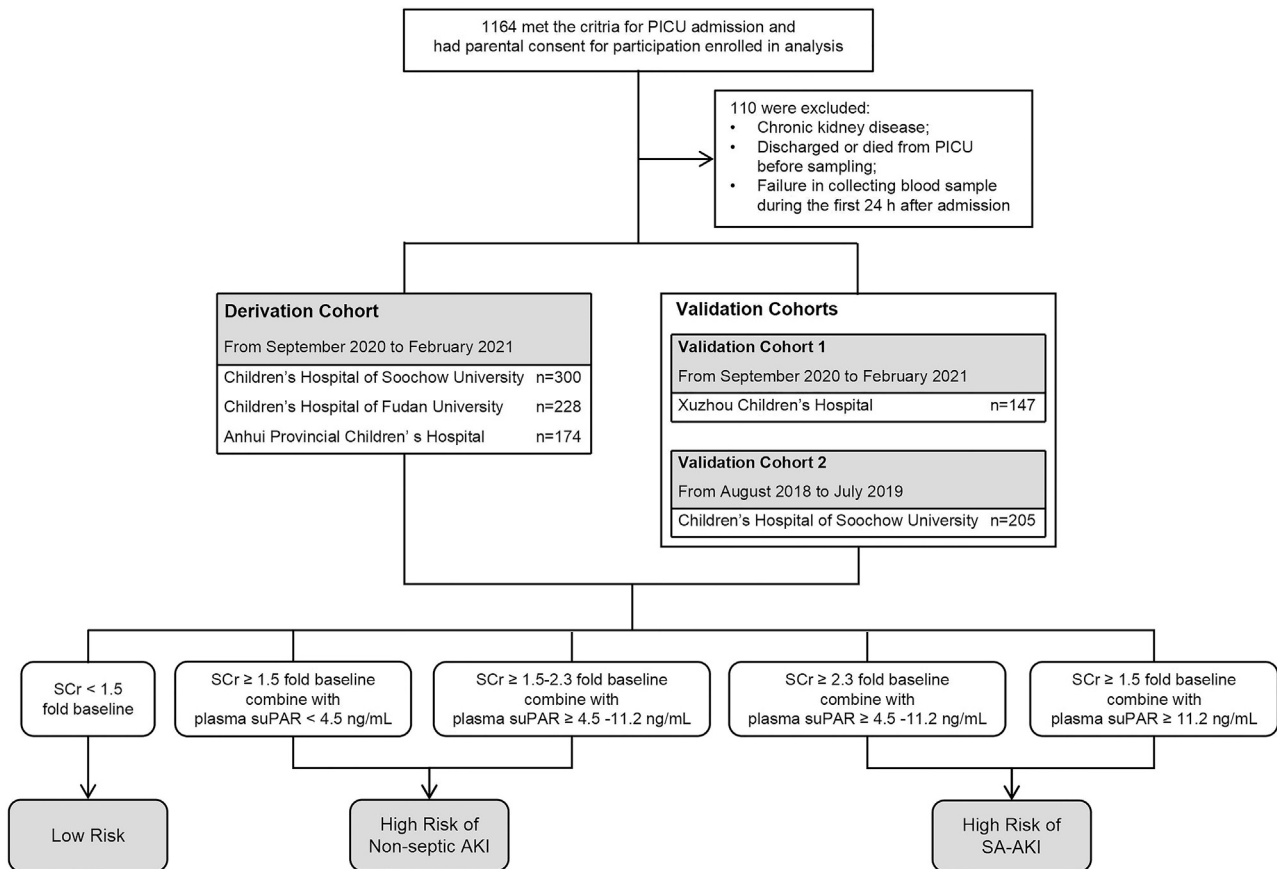[7]Pediatric Intensive Care Unit, Children's Hospital of Fudan University, Shanghai, P.R. China
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Figure 1. Flow diagram of study design and the cut-off value of non-septic AKI and SA-AKI
AKI, acute kidney injury; SA-AKI sepsis-associated AKI; SuPAR, soluble urokinase plasminogen activator receptor; SCr, serum creatinine.

This study aims to explore the clinical utility of plasma suPAR as a discriminative biomarker for AKI and SA-AKI developed during the PICU stay, and derive and validate a multiclass classification decision tree model based on plasma suPAR and serum creatinine (SCr), to discriminate those at high risk of non-septic AKI and SA-AKI in critically ill children.

RESULTS

Patient characteristics

The prospective multicenter study total involved 1054 critically ill children in the derivation and validation cohorts. Of the 1164 children who met the criteria for PICU admission and had parental consent for participation during the study period, 110 were excluded. The details of the study were shown in Figure 1. A total of 702 critically ill children were included in the derivation cohort and 352 patients were included in the two validation cohorts.

A comparison of the demographic and clinical characteristics and outcomes according to subgroups of children with and without AKI or sepsis in the derivation cohort was shown in Table 1. Eighty-eight critically ill children had SA-AKI in the derivation cohort. Children who had SA-AKI were the youngest and had the highest severity of illness. In addition, comparisons of the levels of plasma suPAR and SCr at admission among the children with and without AKI or sepsis were displayed in Figure 2. It was found that both plasma suPAR and SCr levels in the SA-AKI group were the highest.

Correlation of plasma suPAR with clinical variables in the derivation cohort

Clinical variables were analyzed for correlation with plasma suPAR. On univariate linear regression analysis, plasma suPAR was significantly correlated with age, body weight, PRISM III score, AKI, sepsis, MODS, shock/DIC, SIC, and the use of mechanical ventilation, renal replacement therapy (RRT), and vasoactive agents. Variables with $p < 0.05$ under univariate analysis were entered into stepwise multivariate linear regression analysis to investigate independent correlation of plasma suPAR with variables. As displayed in Table 2, the plasma suPAR level was independently correlated with body weight ($p < 0.001$), AKI ($p = 0.001$), sepsis ($p < 0.001$), MODS ($p < 0.001$), SIC ($p < 0.001$), and RRT ($p = 0.023$).

Table 1. Comparison of demographic and clinical characteristics in the derivation cohort

Variables	non-Sepsis		Sepsis		p value
	non-AKI *n* = 407	AKI *n* = 88	non-AKI *n* = 120	AKI *n* = 87	
Age, months	46.0 [18.0–96.0]	25.0 [5.5–103.3][a]	49.0 [16.5–89.3]	16.0 [4.0–120.0][a]	0.004
male, n	227 (55.8)	53 (60.2)	74 (61.7)	54 (62.1)	0.521
body weight, kg	16.0 [11.0–27.7]	13.0 [6.6–25.0][a]	15.8 [10.0–23.6]	10.8 [5.9–33.0][a]	0.001
PRISM III, score	3.0 [2.0–6.0]	6.0 [2.0–10.0][a]	8.0 [5.0–12.8][a,b]	12.0 [8.0–19.0][a,b,c]	<0.001
MODS, n	23 (5.7)	25 (28.4)[a]	35 (29.2)[a]	62 (71.3)[a,b,c]	<0.001
Shock/DIC, n	25 (6.1)	9 (10.2)	20 (16.7)[a]	47 (54.0)[a,b,c]	<0.001
SIC, n	46 (11.3)	22 (25.0)[a]	60 (50.0)[a,b]	51 (58.6)[a,b]	<0.001
MV, n	101 (24.8)	35 (39.8)[a]	41 (34.2)	55 (63.2)[a,b,c]	<0.001
RRT, n	3 (0.7)	18 (20.5)[a]	3 (2.5)[b]	22 (25.30)[a,c]	<0.001
Vasoactive agents, n	18 (4.4)	24 (27.3)[a]	22 (18.3)[a]	39 (44.8)[a,c]	<0.001
PICU length of stay, day	3.6 [1.8–7.6]	5.5 [2.6–13.0][a]	7.8 [3.9–17.2][a,b]	8.5 [4.1–16.1][a,b]	<0.001
PICU Mortality, n	14 (3.4)	10 (11.4)[a]	12 (10.0)[a]	29 (33.3)[a,b,c]	<0.001

Values were median [interquartile range]. Numbers in parentheses denoted percentages.
AKI, acute kidney injury; PRISM III, pediatric risk of mortality III; MODS, multiple organ dysfunction syndrome; DIC, disseminated intravascular coagulation; SIC, sepsis-induced coagulopathy; MV, mechanical ventilation; RRT, renal replacement therapy; PICU, pediatric intensive care unit.
[a]$P < 0.05$, vs. non-Sepsis/non-AKI.
[b]$P < 0.05$, vs. non-Sepsis/AKI.
[c]$P < 0.05$, vs. Sepsis/non-AKI.

We further assessed the incidence rate of comorbidities in the study population according to quartile of plasma suPAR level (Figure 3). There was a stepwise rise in the incidence of AKI, ranging from 6.9% to 45.7%. Patients with higher plasma suPAR levels were also more likely to have severe AKI, sepsis, MODS, shock/DIC, SIC, and PICU mortality, as shown in Figure 3.

Association of plasma suPAR with risk of AKI in the derivation cohort

To discriminate whether plasma suPAR was independently associated with AKI, the univariate and multivariate logistic regression analyses were performed in Figure 4A. The association between plasma suPAR and AKI remained significant after adjustment for body weight, sex male, and PRISM III score in all critically ill children (AOR = 1.10, $p < 0.001$, $n = 702$) and in children without sepsis (AOR = 1.07, $p = 0.028$, $n = 495$). However, when adjusted for body weight, sex male, PRISM III score, MODS, SIC, and RRT, the association of plasma suPAR with AKI remained significant in all critically ill children (AOR = 1.06, $p = 0.005$, $n = 702$), but not in children without sepsis (AOR = 1.04, $p = 0.117$, $n = 495$).

The AUC of plasma suPAR in discriminating AKI was 0.72, both in critically ill children ($p < 0.001$, $n = 702$) and in children without sepsis ($p < 0.001$, $n = 495$) respectively (Figures 4B and 4C), which were similar to the SCr for the discrimination of AKI. However, when combining plasma suPAR with the SCr, the discriminative performance did not improve significantly both in all critically ill children (ΔAUC = 0.05, $p = 0.066$, $n = 702$) and in those without sepsis (ΔAUC = 0.03, $p = 0.403$, $n = 495$).

Association of plasma suPAR with risk of SA-AKI in the derivation cohort

Since that plasma suPAR was independently associated with AKI, we further explored the relationship between plasma suPAR and SA-AKI. SA-AKI was diagnosed in 12.4% (87/702) of all critically ill children, in 49.7% (87/175) of those with AKI, and 42.0% (87/207) of those with sepsis. In univariate analysis, each unit increase in plasma suPAR was associated with a 1.15-fold increase in the odds of SA-AKI in all critically ill children ($p < 0.001$, $n = 702$), 1.12-fold increase in those with AKI ($p = 0.001$, $n = 175$), and 1.10-fold increase in the odds of SA-AKI in those with sepsis ($p < 0.001$, $n = 207$) (Figure 4A). The association of plasma suPAR with SA-AKI remained significant after adjustment for body weight, sex male, and PRISM III score in all critically ill children (AOR = 1.10, $p < 0.001$), in children with AKI (AOR = 1.09, $p = 0.011$), and in children with sepsis (AOR = 1.09, $p = 0.004$). When it is adjusted for body weight, sex male, PRISM III score, MODS, SIC, and RRT, the AORs were 1.07 in all critically ill children ($p < 0.001$) and 1.08 in children with sepsis ($p = 0.018$), respectively.

The AUC for plasma suPAR in discriminating SA-AKI was 0.77 in all critically ill children ($n = 702$; Figure 4D), which is higher than that in those with AKI (AUC = 0.68, $n = 175$, Figure 4E), and in those with sepsis (AUC = 0.64, $n = 207$; Figure 4F). The SCr achieved AUCs of 0.67 for discriminating SA-AKI in all children (Figure 4D) and 0.74 in children with sepsis ($n = 207$; Figure 4F), whereas the SCr could not discriminate SA-AKI in children with AKI (AUC = 0.51, $n = 175$; Figure 4E). The AUC was improved for discriminating SA-AKI in children with sepsis when plasma suPAR was combined with SCr, compared to plasma suPAR alone (ΔAUC = 0.13, 95% CI, 0.21 to 0.50, $p = 0.001$, $n = 207$; Figure 4F). However,
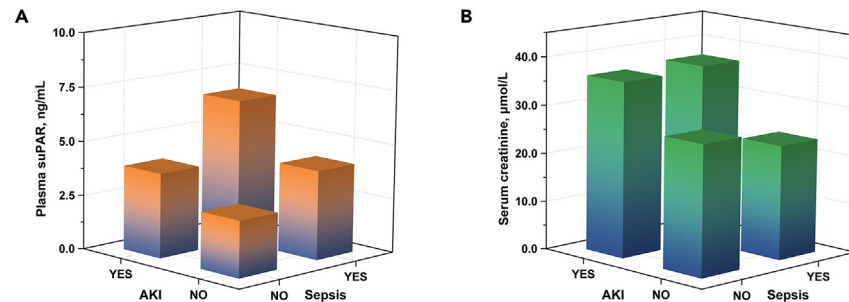
Figure 2. Comparison of median plasma suPAR and serum creatinine levels among children with and without AKI or sepsis in the derivation cohort
(A) plasma suPAR.
(B) serum creatinine.
AKI, acute kidney injury. SuPAR, soluble urokinase plasminogen activator receptor.

the discriminative performance of the combination did not significantly improve over that of plasma suPAR alone in critically ill children (ΔAUC = 0.02, *p* = 0.325, *n* = 702; Figure 4D) and in children with AKI (ΔAUC = 0.01, *p* = 0.579, *n* = 175; Figure 4E).

Association of plasma suPAR with risk of mortality in the derivation cohort

We explored the relationship between plasma suPAR and PICU mortality in Figures 3 and 4. The PICU mortality rate increased with increasing quartile of plasma suPAR, ranging from 2.9% (lowest quartile) to 19.4% (highest quartile) (Figure 3). Univariate analysis showed that the plasma suPAR was associated with mortality (*p* < 0.001), and the association remained significant after adjustment for body weight, sex male, and PRISM III score in multivariate analysis (*p* = 0.004; Figure 4A). In addition, the AOR for associating plasma suPAR levels with PICU mortality were 1.04 (95% CI: 1.00–1.08, *p* = 0.046) after adjustment for body weight, sex male, PRISM III score, and the presence of sepsis and AKI in critically ill children.

The AUC for plasma suPAR in discriminating PICU mortality in all children is shown in Figure 4G. When combining plasma suPAR with SCr, the discriminative performance was not superior to that of plasma suPAR alone for PICU mortality (ΔAUC = 0.01, *p* = 0.681; Figure 4G).

Derivation of the multiclass classification decision tree model

The multiclass classification decision tree model was used to estimate the risk of non-septic AKI and SA-AKI among critically ill children in the derivation cohort, and is displayed in Figure 5. The top node of the model which is the root node provides the total number of subjects and the number of children with different classes. The model had 5 TN which reflect the final assignment of risk to an individual case. The multiclass classification model showed that the SCr ≥ 1.5-fold baseline and plasma suPAR level ≥ 11.2 ng/mL (TN5) were the predictor of SA-AKI, with 95.0% critically ill children had SA-AKI. When SCr ≥ 2.3-fold baseline combines with plasma suPAR ≥ 4.5–11.2 ng/mL (TN4), the model was able to reliably estimate the high risk of SA-AKI (66.7%). In addition, TN2 and 3 identified 61 critically ill children with AKI, 75.4% (46/61) of whom developed non-septic AKI. TN1 were deemed to have low risk of non-septic AKI (5.9%) and SA-AKI (7.1%). The final stratification information was integrated into flow diagram to facilitate its clinical utility, as shown in Figure 1.

The multiclass classification decision tree model in Figure 5 showed good overall performance in multiclass classification, with the micro-average AUC of 0.89 (95% CI, 0.87 to 0.91) and the macro-average AUC of 0.73 (95% CI, 0.66 to 0.77), displayed in the Additional file: Figure S1. The specificity of the model for discriminating non-septic AKI in all critically ill children was 97.6% and the NPV was 93.4%, as shown in the Additional file: Table S1. The specificity of plasma suPAR combined with SCr to discriminate SA-AKI in model was 99.0%, which was higher than that of traditional combination method of the two biomarkers (74.8%) in all critically ill children. In addition, when limited to those with low (TN1) and high (TN2 and 3) risk of non-septic AKI (*n* = 667), the model demonstrated a specificity of 97.4% for discriminating non-septic AKI with the NPV of 94.1%. When restricted to those with a low (TN1) and high (TN4 and 5) risk of SA-AKI (*n* = 641), the specificity of the model for discriminating SA-AKI was 98.9% and the NPV was 92.9%.

Validation of the multiclass classification decision tree model

A total of 147 critically ill children were included in validation cohort 1, in which 27 (17.2%) were non-septic AKI and 14 (8.9%) were SA-AKI. Validation cohort 2 involved 205 children, of which 19 (9.3%) were non-septic AKI and 29 (14.1%) were SA-AKI. The model performance was validated in two separate cohorts in the Additional file: Figures S1–S3. Moreover, the multiclass classification model had a stable performance in combined validation cohorts, in Table 3, with micro average AUC of 0.87 (95% CI, 0.84 to 0.90) and macro average AUC of 0.74 (95% CI, 0.67 to 0.83). Among children classified as a low (TN1) and high (TN2 and 3) risk of non-septic AKI, the specificity and the NPV of the model for discriminating non-septic AKI (*n* = 320) were 96.4% and 93.6%. Additionally, the specificity and the NPV of discriminating SA-AKI among all critically ill children were 95.4% and 91.3%, and among children classified as a low (TN1) and high (TN4 and 5) risk of SA-AKI (*n* = 315) were 95.0% and 93.6%, respectively, as shown in Table 3.

Table 2. Associated of clinical variables with plasma suPAR level in the derivation cohort

	Univariate regression[a]			Multivariate regression[b]		
	B	SE	p value	B	SE	p value
Age, months	−0.142	0.018	<0.001	N/A	–	–
Male	−0.037	0.023	0.102	N/A	–	–
Body weight, kg	−0.265	0.034	<0.001	−0.256	0.03	<0.001
PRISM III, score	0.014	0.002	<0.001	N/A	–	–
AKI	0.234	0.024	<0.001	0.081	0.025	0.001
Sepsis	0.257	0.022	<0.001	0.124	0.023	<0.001
MODS	0.27	0.026	<0.001	0.119	0.028	<0.001
Shock/DIC	0.178	0.031	<0.001	N/A	–	–
SIC	0.243	0.024	<0.001	0.124	0.024	<0.001
MV	0.083	0.024	<0.001	N/A	–	–
RRT	0.247	0.044	<0.001	0.095	0.041	0.023
Vasoactive agents	0.213	0.03	<0.001	N/A	–	–

AKI, acute kidney injury; PRISM III, pediatric risk of mortality III; MODS, multiple organ dysfunction syndrome; DIC, disseminated intravascular coagulation; SIC, sepsis-induced coagulopathy; MV, mechanical ventilation; RRT, renal replacement therapy; suPAR, soluble urokinase plasminogen activator receptor.
[a]All variables in Table 1 were analyzed in the univariate linear analysis.
[b]Variables with $p < 0.05$ were entered into the multivariate stepwise analysis.

DISCUSSION

This multicenter prospective study was attempted to determine plasma suPAR as an early biomarker to discriminate AKI and SA-AKI in critically ill children. The increased level of plasma suPAR on the first day at admission was independently associated with AKI and SA-AKI developed during the PICU stay. Subsequently, the multiclass classification decision tree model derived and validated in the study provided the cut-offs for plasma suPAR and SCr and the stratification information to specifically discriminate critically ill children at high risk of non-septic AKI and SA-AKI.

The suPAR, as the soluble form of uPAR, has become a stronger predictive for decline renal function.[13,16–19] The uPAR, expressed at low levels in normal conditions, can be shed from the cell surface by a specific phospholipase in response to inflammation stimuli, and detected in plasma and urine.[20] Plasma suPAR has been implicated in the pathogenesis of kidney disease, with prolonged exposure directly contributing to tubular cell injury, proteinuria, and eventually kidney dysfunction.[12,21] Clinical studies reporting the association between suPAR and AKI or SA-AKI limited mainly to adult patients.[13–15,22–25] In general, critically ill adults, plasma suPAR is an independent predictive biomarker of AKI.[13,14,22] Moreover, elevated circulating levels of suPAR are associated with SA-AKI disease severity, and predict RRT supporting renal function in adult patients.[15,24,25] In children, Franz et al. study demonstrated that serum suPAR level was associated with a more rapid decline in kidney function in CKD.[16] Compare to previous studies, our study included critically ill children from multicenter PICUs. This study complemented recent work conducted in adult populations and proved the independence of the association of suPAR level in plasma with AKI and SA-AKI in critically ill children.

To date, this is the first study implied that non-septic AKI and SA-AKI developed during the PICU stay, may be discriminated early through the utilization of the plasma suPAR and SCr collected in the first 24 h. For estimating the risk of non-septic AKI and SA-AKI, we used a decision tree model approach which has been successfully applied in SA-AKI,[26] and other cause of AKI.[27] The decision tree model based on the CART algorithm is a supervised multivariate approach used to discriminate target outcome measures. The strengths to the model are its ability to visualize characteristics and provide insight regarding stratification information with easy interpretability. It was notable that the multiclass classification model in the study could determinate the cut-offs for plasma suPAR and SCr and discriminate the high risk of non-septic AKI and SA-AKI in critically ill children. The cut-off values in SCr were 1.5 and 2.3-fold baseline, 4.5 and 11.2 ng/mL in plasma suPAR. Plasma suPAR cut-off value of 4.184 ng/mL has been reported associated with the incidence of AKI after coronary angiography.[12] The results of this study further indicate that similar plasma suPAR cut-off value combined with SCr≥1.5– to 2.3-fold baseline could discriminate the high risk of non-septic AKI with a specificity of 97.6%, and combined with SCr≥2.3-fold baseline could discriminate the high risk of SA-AKI with a specificity of 99.0% in critically ill children. An overall 52.3% sensitivity for non-septic AKI and 33.3% sensitivity for SA-AKI in this model may be viewed as being relatively low. Sensitivity is often used as screening test, because it is helpful for ruling out a diagnosis in patients when the result is negative, while test with high specificity is used to rule in a disease where a positive result indicates a high probability that the patient has the disease. The multiclass classification decision tree model in this study performed well in discriminating high risk of non-septic AKI and SA-AKI in critically ill children.

Previous approaches to classifying sepsis shock in children mainly aimed at discriminating children who developed severe SA-AKI at day 3 and were based on several variables and thresholds,[26,28] including the CART prediction model developed by Atreya et al. that had an AUC of
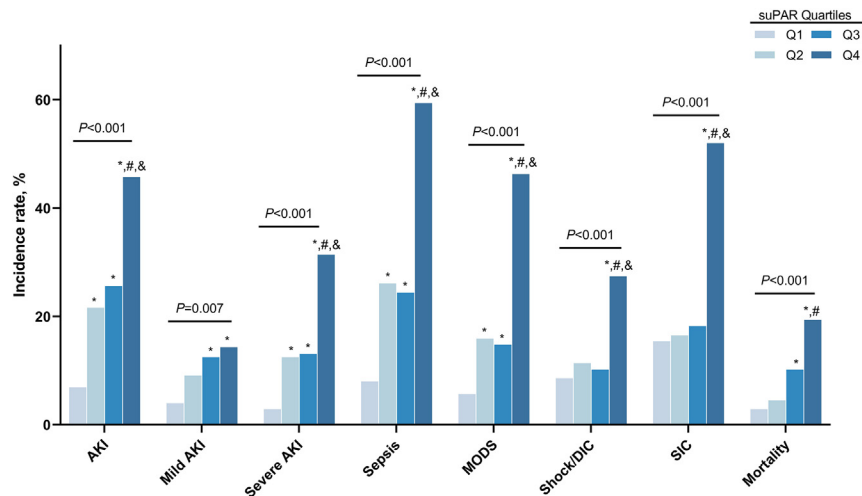
Figure 3. Comparison of the incidence rate according to plasma suPAR quartile in the derivation cohort

AKI stage1 was defined as mild AKI. AKI stages 2 and 3 were defined as severe AKI. Q1 indicated a suPAR level less than 2.27 ng/mL; Q2: 2.27–3.27 ng/mL; Q3: 3.27–5.11 ng/mL; Q4: ≥5.11 ng/mL *$p < 0.05$, vs. Q1. #$p < 0.05$, vs. Q2. &$p < 0.05$, vs. Q3. AKI, Acute kidney injury; MODS, multi-organ dysfunction syndrome; DIC, shock/disseminated intravascular coagulation; SIC, sepsis-induced coagulopathy.

0.90.[28] Similar to their findings, the model in our study had good overall performance in multiclass classification, with the micro-average AUC of 0.89. Our multiclass classification decision tree model is only based on two variables at admission to discriminate critically ill children with SA-AKI and non-septic AKI which developed during the PICU stay. This finding may not be unexpected from a clinical perspective, but it has not been previously reported in pediatric population. The importance of early recognition of the causes of AKI and proactive intervention in children at risk for non-septic AKI and SA-AKI is emphasized by its association with poor outcomes and the lack of effective disease-modifying therapies.[6,29] Few attempts thus far have yielded effective strategies for discriminating non-septic AKI and SA-AKI.

We further demonstrated that plasma suPAR is independently associated with PICU mortality, even after adjustment, which is consistent with the previous studies conducted in adults.[17,22] The findings strengthen the evidence for a relationship between plasma suPAR and mortality. Critically ill children with high levels of plasma suPAR at PICU admission were more likely to develop worse clinical outcomes. The recognition of early risk factors for PICU mortality may help in implementing treatment measures to prevent adverse outcomes.

In conclusion, the high level of plasma suPAR, serving as an early discriminative biomarker, maintains an independent association with AKI, SA-AKI, and PICU mortality, even after adjustment for confounding variables. The multiclass classification decision tree model proposed in this study provides the cutoffs for plasma suPAR and sCr and the insight regarding stratification information to specifically discriminate critically ill children at high risk of non-septic AKI and SA-AKI. Further large-sample studies are needed to verify the clinical utility of the multiclass classification model in this population.

Limitations of the study

Our study has several limitations. First, since plasma suPAR and SCr were not measured daily in children, which may limit our ability to determine the exact time of incidence and grade of AKI and SA-AKI. Secondly, plasma suPAR and SCr were included into the multiclass classification model to discriminate the high risk of non-septic AKI and SA-AKI. Although the specificity of the model in our study is high, whether plasma suPAR combined with other biomarkers can further improve the sensitivity and the AUC of the model remains to be explored. Second, although the specificity of the model in our study is high, it remains to be explored whether plasma suPAR and SCr, when combined with other biomarkers, can further improve the model's sensitivity and AUC. Additionally, blood collection in critically ill children can be challenging and highly invasive compared to urine sampling. Further investigation is needed to determine whether the multiclass classification model can be adapted for urinary suPAR or combined with other biomarkers. Third, although this study included critically ill children from multicenters, only 88 (12.5%) were diagnosed non-septic AKI and 87 (12.4%) with SA-AKI. Nevertheless, well-performed external validations, with a prospective multicenter study design, provided adequate power for exploring the model discriminating non-septic AKI and SA-AKI in critically ill children. Further studies are needed to verify and generalize our findings, especially in patients outside the PICU setting. Fourth, the outcome of non-septic AKI and SA-AKI cases that occurred on the first day of admission seemed likely to be a large driver of the overall study results, which may be a bias in this study toward positive outcomes. However, this is a common phenomenon in clinical settings with most cases of AKI occurred on the first 24 h of admission.[30] Additionally, this multiclass classification decision tree model could discriminate the occurrence of non-septic AKI and SA-AKI during the PICU stay but not the exactly occurring timing of them. Further study is required to investigate the prediction of the timing of non-septic AKI and SA-AKI occurring, especially in the time window of 24 h or 48 h prior to their onset.
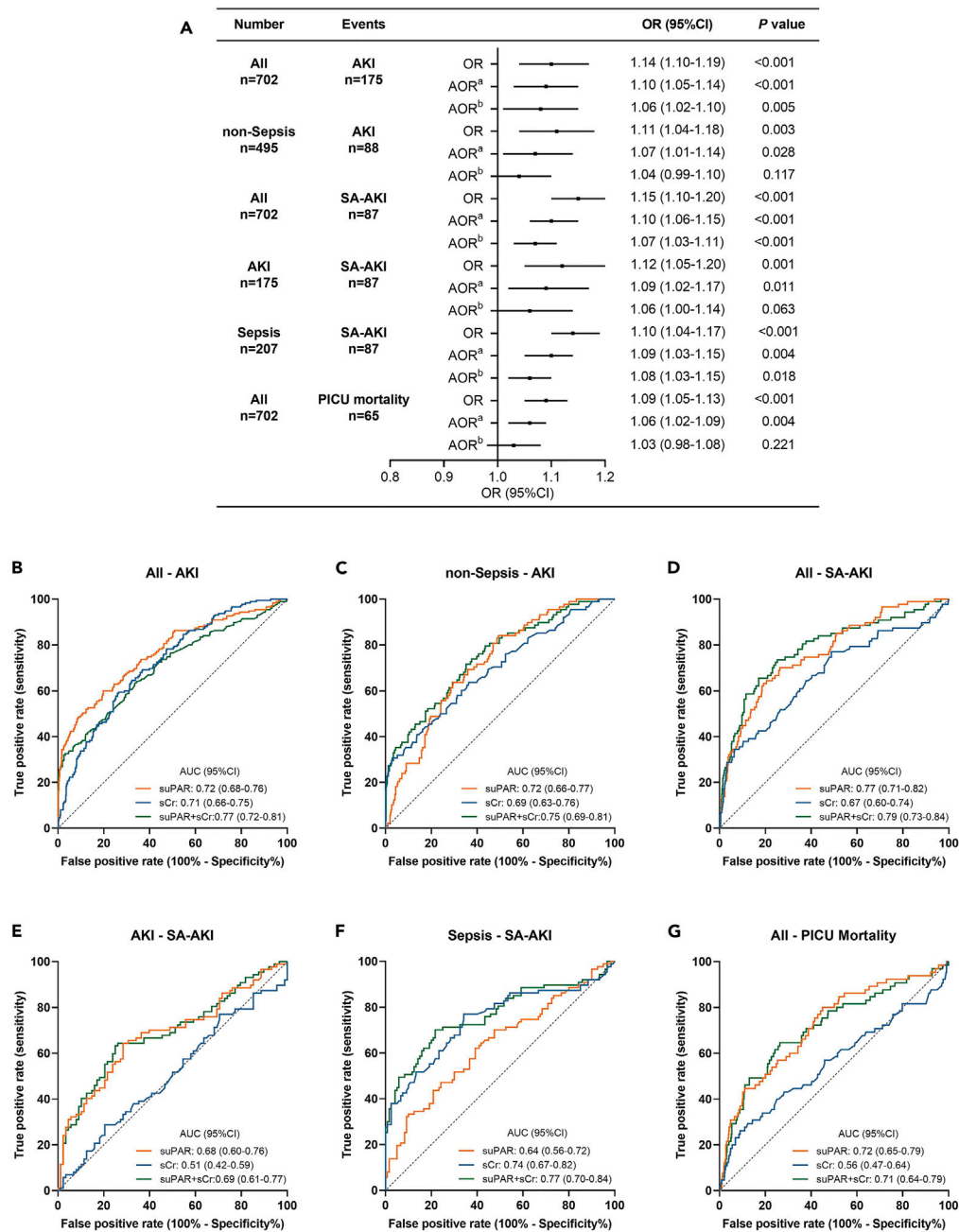
Figure 4. The association between plasma suPAR and AKI, sepsis, SA-AKI, and PICU mortality

(A) Forest plot in logistic regression analyses. [a]After adjustment body weight, sex male, PRISM III score; [b]After adjustment body weight, sex male, PRISM III score, MODS, SIC, RRT.

(B–G) ROC curves for the abilities of the plasma suPAR and SCr to predict clinical outcomes.

(B) AKI in all critically ill children.

(C) AKI in patients without sepsis.

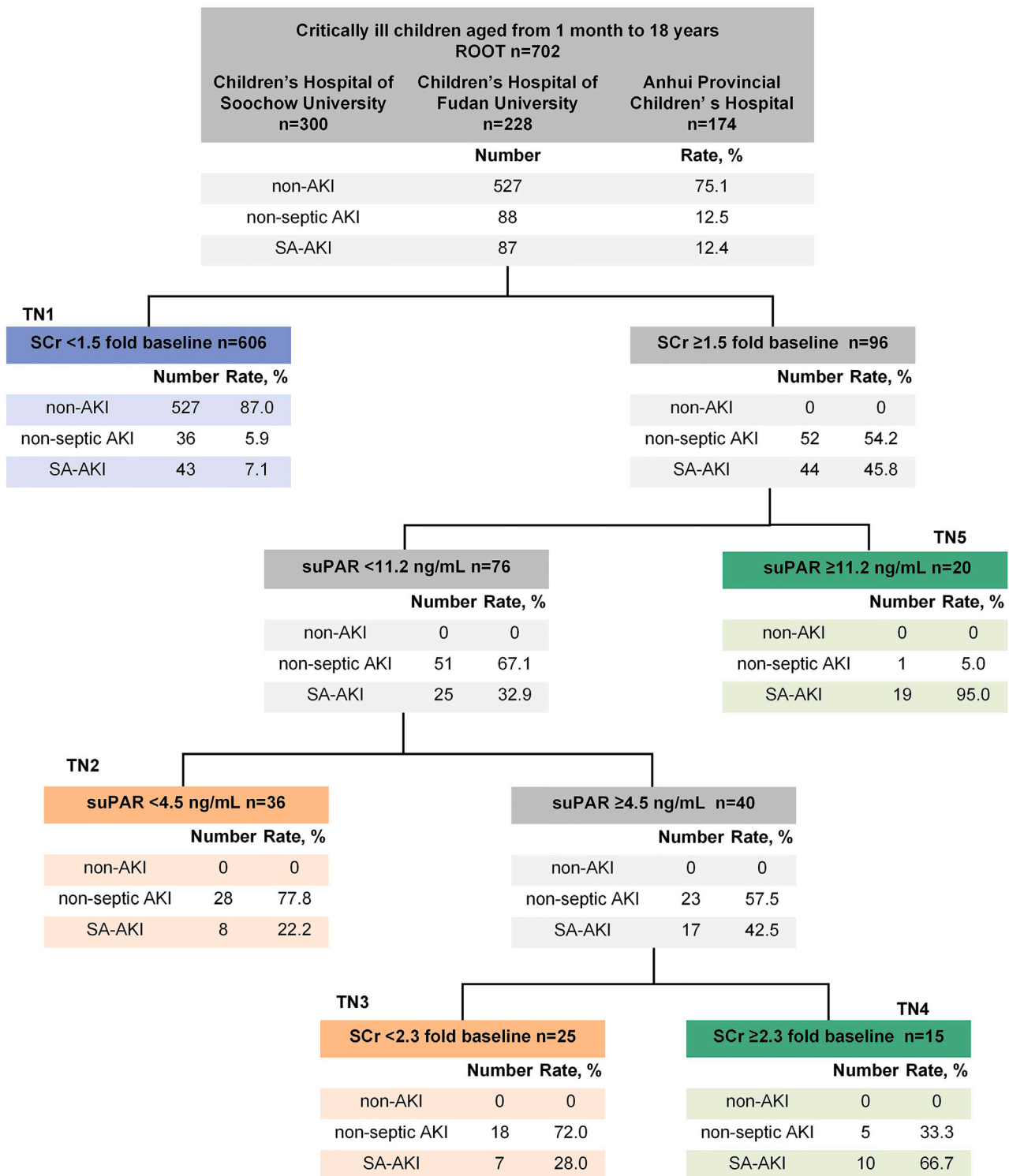(D) SA-AKI in all critically ill children.

(E) SA-AKI in AKI patients.

(F) SA-AKI in patients with sepsis.

(G) PICU mortality in all critically ill children.

AKI, acute kidney injury; SA-AKI, sepsis-associated AKI; ROC, receiver operating characteristic; OR, odds ratio; AOR, adjusted OR; CI, confidence interval; AUC, area under the receiver operating characteristic curve.

Figure 5. The multiclass classification decision tree model from the derivation cohort (n = 702)
The model consisted of two biomarker-based decision rules and eight daughter nodes. Each node provided the respective decision rule criterion and the total number of subjects in the node, and the number of non-AKI, non-septic AKI, and SA-AKI with the respective rates. TN2 and 3 were regarded as high risk of non-septic AKI (77.8% and 72.0%, respectively), shown as the color of orange. TN4 and 5 were considered high-risk of SA-AKI (66.7% and 95.0%, respectively), shown as the color of green. TN1 were considered to have low risk of non-septic AKI (5.9%) and low risk of SA-AKI (7.1%), shown as the color of blue.
TN, terminal node; AKI, acute kidney injury; SA-AKI, sepsis associated AKI.

Table 3. Performance of the multiclass classification decision tree model in validation cohorts

	All nodes		TN1, 2, and 3	TN1, 4, and 5
	non-septic AKI	SA-AKI	non-septic AKI	SA-AKI
Number of subjects	352	352	320	315
true positives, n	27	18	27	18
true negatives, n	283	292	265	265
false positives, n	10	14	10	14
false negatives, n	32	28	18	18
Sensitivity, %	45.8	39.1	60	50
Specificity, %	96.6	95.4	96.4	95
PPV, %	73	56.3	73	56.3
NPV, %	89.8	91.3	93.6	93.6
LR+	13.4	8.6	16.5	10
LR-	0.6	0.6	0.4	0.5

TN2 and 3 were regarded as high risk of non-septic AKI. TN4 and 5 were considered high-risk of SA-AKI. TN1 were considered to have low risk of non-septic AKI and low risk of SA-AKI.

TN, terminal node; AKI, acute kidney injury; SA-AKI, sepsis associated AKI; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, LiYanhong (lyh072006@hotmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon reasonable request.
- This paper does not report additional code.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon reasonable request.

AUTHOR CONTRIBUTIONS

X.J. performed the data analyses, established the model, and drafted the manuscript. C.J. performed the experiments. L.M., J.Z., F.F., L.J., Z.Y., and L.H. participated in data collection. B.Z., L.X., and L.G. participated in the design of the study and coordination. L.Y. had primary responsibility for study design, data analyses, data interpretation, and writing the manuscript. X.J. and L.Y. had accessed and verified the data. All authors read and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Derivation cohort	This study	N/A
Validation cohort	This study	N/A
Biological samples		
Human plasma	Parental written informed consent was obtained for all participants.	N/A
Critical commercial assays		
SuPARnostic kit	ViroGates A/S Corporation, Denmark	NO.2108-E1-1
SCr	Hitachi7600, Tokyo	N/A
Software and algorithms		
SPSS version 26.0	IBM Corp.	https://www.ibm.com/spss
GraphPad Prism version9.0.2	GraphPad	https://www.graphpad.com/
R version 4.3.3	RStudio	https://www.r-project.org
Python version 3.6.5	Python Software Foundation	https://www.python.org

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study population

The prospective multicenter study was conducted in critically ill cohorts using a derivation-validation design. The derivation cohort consisted of 702 critically ill children from the three mixed PICUs of tertiary teaching hospitals (Children's Hospital of Soochow University, Children's Hospital of Fudan University, and Anhui Provincial Children's Hospital) between September 2020 and February 2021. All children who met the criteria for PICU admission and had a parental agreement for participation were allowed to enroll. The exclusion criteria included age<1 month or >18 years, chronic kidney disease (CKD), and failure to collect blood samples during the first day after PICU admission. The external data set included two separate cohorts for validation, consisting of a concurrent prospective study conducted in the PICU of Xuzhou Children's Hospital between September 2020 and February 2021 and a pilot, prospective previous study conducted in the PICU of Children's Hospital of Soochow University between August 2018 and July 2019. The inclusion and exclusion criteria were identical to those of the derivation cohort. The clinical and laboratory information from the Han Chinese critically ill children's electronic medical records, including age, sex, disease stage, and PRISM III score, were collected both in derivation cohort and validation cohorts. Information on gender and socioeconomic status was not collected.

Ethics

The study was approved by the Institutional Review Board/Ethical Committee of the four hospitals and performed following the Declaration of Helsinki and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline, with approvals of the Institutional Review Board of Children's Hospital of Soochow University (2020KS009), Children's Hospital of Fudan University [(2020) 404], Anhui Provincial Children's Hospital (EYLL-2020-023), and Xuzhou Children's Hospital (2020-1-3). Parental written informed consent was obtained for all participants.

METHOD DETAILS

Clinical data collection

Demographic and clinical data were recorded upon admission in both derivation and validation cohorts. Children's information, including clinical status, admitting diagnosis, comorbidities, medication situation, and therapeutic intervention, was recorded daily until PICU discharge or death. The diagnosis of multiple organ dysfunction syndrome (MODS), shock, disseminated intravascular coagulation (DIC), and sepsis-induced coagulopathy (SIC) that developed during the PICU stay were defined clinically and diagnosed by the attending physicians, according to the criteria described previously.[31,32] In addition, the severity of illness in critically ill children was assessed by the pediatric risk of mortality III (PRISM III) score at admission, as described in our previous studies.[2,33]

Diagnosis of AKI and SA-AKI

The diagnosis and stages of AKI developed during the PICU stay were based on the Kidney Disease Improving Global Outcome (KDIGO) criteria for SCr and urine output.[34] Baseline SCr was defined as the lowest level obtained within 3 months before PICU admission. If baseline SCr was unavailable, the Schwartz formula was used to estimate the baseline SCr: back calculation with the bedside Schwartz formula (GFR=k×height (cm)/SCr (mg/dL), k=0.413) assuming a GFR of 120 mL/min/1.73 m^2, following previous studies.[33–35] When the two criteria of SCr and urine output resulted in different AKI stages, the higher stage was chosen. KDIGO stage 1 was defined as mild AKI, and KDIGO stage 2 or 3 was defined as severe AKI. All children had SCr on the first day of PICU admission and the SCr was routinely measured 2-7 days during the PICU stay.

The diagnosis of sepsis during the PICU stays was in accordance with the Sepsis-3 criteria, defined as pediatric patients with confirmed or suspected infection who had an increase in a pediatric version of Sequential Organ Failure Assessment (pSOFA) score≥2.[36] SA-AKI was defined as the presence of combining sepsis and AKI during the PICU stay.[5,37]

Clinical outcome

The primary endpoint was the diagnosis of AKI and SA-AKI during the stay of PICU. The secondary outcome included PICU mortality, which was defined as all-cause mortality developed during the PICU stay, including death resulting from the withdrawal of treatment.

Blood sample collection and measurement of plasma suPAR

The initially available blood sample was obtained within 24 hours of admission to PICU, and drawn into a centrifuge tube containing EDTA anti-coagulant. The samples were centrifuged at 3,000 g at 4°C for 10 minutes, and the supernatant were aliquoted and immediately frozen and stored at -80°C. For the measurement, the plasma samples with dry ice were delivered to the Children's Hospital of Soochow University from other hospitals. The level of plasma suPAR was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (NO.2108-E1-1, ViroGates A/S Corporation, Denmark). The procedure was conducted strictly according to the manufacturer's instructions. The intra-assay and inter-assay coefficients of variation were less than 10%. The kit assay range was 0.7-13.2 ng/mL, and sample was diluted using dilution buffer when the suPAR concentrations were above the range. The level of SCr at admission were measured by the sarcosine oxidase method on an automatic biochemical analyzer (Hitachi7600, Tokyo).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analysis was performed using SPSS Statistical Software Version 26.0 and GraphPad Prism 9.0.2. Continuous variables, which were skewed distributions, were described as the median and inter-quartile range (IQR) and were compared using the Mann-Whitney U test or Kruskal-Wallis H test. Categorical variables were described as counts (percentage) and compared using the χ^2 test or Fisher's exact test. Univariate and stepwise multivariate linear regression analyses were performed to investigate correlations between plasma suPAR and clinical and laboratory variables. To satisfy approximate normality in the linear regression analyses, the levels of plasma suPAR were log10 transformed. Univariate and multivariate logistic regression analyses were performed and the odds ratio (OR) and adjusted OR (AOR) with a 95% confidence interval (CI) were calculated to investigate the association of plasma suPAR with AKI, SA-AKI, and PICU mortality. The discriminative strength was assessed with the area under the curve of the receiver operating characteristic (AUC) and values of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The non-parametric method of Delong was used to compare the difference between AUCs. Moreover, a multiclass classification decision tree model based on the classification and regression tree algorithm (CART) was derived and validated to discriminate non-septic AKI and SA-AKI in critically ill children, conducted by R version 4.3.3 (https://www.r-project.org). The ultimate risk assignment to each instance was reflected in the terminal nodes (TN) of the decision tree model, determined by the cutoffs of plasma suPAR and SCr. The overall performance of multiclass classification model was assessed by the macro-average AUC and micro-average AUC using Python version 3.6.5 (https://www.python.org). The macro-average AUC calculates the AUC separately for each class but treats each class equally regardless of its size or class imbalance. The micro-average AUC method first aggregates the true positives, false positives, true negatives, and false negatives for all classes, and then uses these aggregate values to calculate the AUC. For all analyses, a two-tailed P value<0.05 was considered statistically significant.