Derivation and Validation of Urinary TIMP-1 for Prediction of Acute Kidney Injury and Mortality in Critically Ill Children

Hui Huang
Soochow University Affiliated Children's Hospital https://orcid.org/0000-0003-1835-6657
Qiang Lin
Soochow University Affiliated Children's Hospital
Xiaomei Dai
Soochow University Affiliated Children's Hospital
Jiao Chen
Soochow University Affiliated Children's Hospital
Zhenjiang Bai
Soochow University Affiliated Children's Hospital
Xiaozhong Li
Soochow University Affiliated Children's Hospital
Fang Fang
Soochow University Affiliated Children's Hospital
Yanhong Li (lyh072006@hotmail.com)
Soochow University Affiliated Children's Hospital https://orcid.org/0000-0003-0882-6408

Research

Keywords: Acute kidney injury, Critically ill children, Mortality, Urinary TIMP-1

Posted Date: July 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-673067/v1

Version of Record: A version of this preprint was published at Journal of Translational Medicine on February 23rd, 2022. See the published version at https://doi.org/10.1186/s12967-022-03302-0.

Abstract

Background: Acute kidney injury (AKI) is associated with high morbidity and mortality. Multiple urinary biomarkers have been identified to associate with the prediction of AKI and outcomes. However, the accuracy of these urinary biomarkers for AKI and associated outcomes has not been clearly defined, especially in heterogeneous populations. The aims of the study were to compare the ability of 10 existing or potential urinary biomarkers for prediction of AKI and pediatric intensive care unit (PICU) mortality, and identify and validate the best biomarker of urinary tissue inhibitor of metalloproteinases-1 (uTIMP-1) for early prediction in heterogeneous critically ill children.

Methods: A derivation-validation approach with separate critically ill cohorts was designed. We first conducted a prospective cohort study to determine the ability of 10 candidate urinary biomarkers serially measured in 123 children during the first 7 days of PICU stay and identify the best biomarker for predicting AKI and PICU mortality (derivation study). The best biomarker of uTIMP-1 from derivation was validated in a separate cohort of 357 critically ill children (validation study). AKI diagnosis was based on KDIGO classification with serum creatinine and urine output.

Results: In the derivation cohort, 17 of 123 (13.8%) children developed AKI stage 3 or died during PICU stay, and both the initial and peak uTIMP-1 displayed the highest AUC of 0.87 (0.79-0.94) and 0.90 (0.84-0.96), respectively, for predicting AKI stage 3 or death. In the validation cohort, 47 of 357 (13.2%) developed AKI during the first week after admission, and 38 (10.6%) died during PICU stay. The initial uTIMP-1 level was validated to be independently associated with AKI (AOR=1.88, P=0.001), severe AKI (AOR=2.35, P<0.001), AKI stage 3 (AOR=2.87, P<0.001) and PICU mortality (AOR=1.92, P=0.019) after adjustment for potential confounders. The predictive values of uTIMP-1 for AKI, severe AKI, AKI stage 3 and PICU mortality were 0.82 (0.75-0.88), 0.84 (95%CI 0.77-0.91), 0.87 (0.81-0.94) and 0.83 (0.76-0.89), respectively.

Conclusions: Urinary TIMP-1 level has been identified and validated to be independently associated with AKI and PICU mortality in independent prospective cohorts, and may be an early potential indicator of AKI and PICU mortality in critically ill children.

Introduction

Acute kidney injury (AKI) is a common clinical complication and associated with high morbidity and mortality in critically ill patients [1, 2]. Thus, early and accurate diagnosis of AKI is crucial to initiate timing therapeutic intervention to potentially improve clinical outcomes [3]. During recent decades, multiple urinary biomarkers, characterized as noninvasive and early indicators of AKI, have been identified and various attempts have been made to associate the concentrations of urinary biomarkers with the prediction of AKI and outcomes in various clinical settings [4–6]. However, the accuracy of these urinary biomarkers in the clinical diagnosis of AKI and in the perdition of associated patient outcomes, especially in more heterogeneous populations such as general intensive care unit (ICU) patients, has not

been clearly defined [3, 7–9]. None of these urinary biomarkers is routinely used in pediatric clinical practice and adds a clear value beyond the traditional approach in clinical decision making in children, especially in critically ill children, with AKI.

The possible reasons for suboptimal biomarker performance in critical care setting might be due to the fact that AKI is a heterogeneous clinical syndrome and has multiple etiologies and variable pathogenesis [3, 10]. The population of pediatric ICU (PICU) is also heterogeneous and AKI etiology and timing are largely unknown. Here we designed a derivation-validation approach with separate critically ill cohorts and report the results of the prospective investigation in which 10 existing or potential urinary biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), tissue inhibitor of metalloproteinase-2 (TIMP-2), insulin-like growth factor-binding protein 7 (IGFBP7), [TIMP-2]•[IGFBP7], fatty-acid-binding protein-1 (FABP-1), tissue inhibitor of metalloproteinase-1 (TIMP-1), renin, interferon inducible protein-10 (IP-10) and trefoil factor-3 (TFF-3), were compared in critically ill children for predation of AKI and PICU mortality; and the best biomarker of urinary TIMP-1 (uTIMP-1) was identified and subsequently validated in an independent cohort of heterogeneous critically ill children for early prediction of AKI and adverse outcome.

Methods

Study design and population

We conducted a two-stage prospective cohort study in which we first collected urine samples from a cohort to identify the best biomarker for prediction of AKI and PICU mortality among 10 candidate urinary biomarkers, including novel potential candidates and previously described biomarkers (derivation study). A separate independent cohort was used to validate the performance value of the best biomarker identified from the derivation (validation study).

The overall study design is shown in Figure 1. Both two cohorts were conducted in the PICU of a single tertiary children's hospital and included critically ill children aged between 1 month and 16 years. The derivation cohort was conducted from September to December 2016 and the validation cohort was preformed from December 2017 to January 2018 and September to December 2019. Exclusion criteria were as follows: known congenital abnormality of the kidney and a failure to collect urine samples before discharge from the PICU or death. Children had multiple PICU admissions within a single hospital stay, only their last admission was included in the analysis. The 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. Written consent forms were obtained from their parents involved in this study.

Clinical data collection

In both derivation and validation cohort studies, the medical records of eligible patients were reviewed. Demographic characteristics, including age, body weight and gender, admission diagnosis, clinical status as defined by illness severity, medication and therapeutic interventions were recorded daily until

PICU discharge or death. Sepsis, multiple organ dysfunction syndrome (MODS), shock and disseminated intravascular coagulation (DIC) that developed during the PICU stay were diagnosed by the treating physicians, according to the criteria described previously [11].

Assessment of illness severity

The score of the pediatric risk of mortality III (PRISM III), which was calculated on the day of PICU admission, was used to assess illness severity of critically ill children In both derivation and validation cohorts, according to methods described in the original study [12] and in accordance with our previous studies [11, 13].

Diagnosis of AKI

The diagnosis of AKI was based on the increase of serum creatinine (sCr) and/or the reduction of the urine output within the first 7 days after PICU admission, according to the criteria of Kidney Disease: Improving Global Outcome (KDIGO) [14]. When the baseline sCr measurement was unavailable, the sCr value at hospital or PICU admission was used. For children with increased sCr ≥1.2 mg/dL (106.1 μmol/L) at admission, the lowest sCr value within 2 weeks while in the PICU was considered as the baseline sCr, Sin accordance with our previous studies [11, 13]. The sCr level was measured daily during the first week after PICU admission, followed by routine measurement every 48-72 hours during the PICU stay. Severity of AKI was characterized by KDIGO staging, and KDIGO stages 2 and 3 were defined as severe AKI.

Clinical outcomes

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

Urine sample collection

In the derivation cohort, urine samples were collected within the first 24 h after PICU admission and followed by every 48-72 h during the first 7 days of PICU stay. In the validation cohort, the urine samples were only collected within 24 h after PICU admission. All acquired urine samples were collected using a plastic bag and immediately frozen and stored at -80°C. The samples were centrifuged at 1,500 g at 4°C for 10 min and the supernatants were aliquoted for the measurement.

Measurement of urinary biomarkers

In the derivation cohort study, six biomarkers (KIM-1, FABP-1, TIMP-1, renin, IP-10 and TFF-3) in urine were measured using multiplex bead assays incorporated in human kidney injury panel 1 (HKI1MAG-99K, MILLIPLEX MAP kit, Millipore, Billerica, USA) run on the Luminex FlexMAP 3D instrument according to manufacturer's instructions. The calibration curve was calculated using a five-parameter logistic fit and the concentration of urinary biomarkers was determined. The Human Lipocalin 2/NGAL (ab113326,

Abcam, USA), TIMP-2 (DY971, R&D Systems, USA) and IGFBP7 (DY1334-05, R&D Systems, USA) ELISA kits were used for the measurement of NGAL, TIMP-2 and IGFBP7 in urine, respectively. In the ELISA assays, the samples were diluted 10-fold to 1000-fold in Reagent Diluent to ensure that the enzymatic reaction was maintained within the linear range. The coefficient of variation of intra-assay and inter-assay within and between ELISA tests were less than 10%. In the validation cohort, the concentration of uTIMP-1 was measured by means of ELISA (DTM100, R&D Systems, USA). The minimum detectable level of TIMP-1 was <0.08 ng/mL, and the coefficient of variation of intra-assay and inter-assay were less than 5% and 4.9%, respectively.

In both derivation and validation cohort studies, the concentration of urinary biomarkers was expressed in nanograms per milligram of urinary Cr (ng/mg uCr). The uCr level from
the aliquoted sample was measured automatically on an automatic biochemical analyzer (Hitachi 7600, Tokyo, Japan) by using the sarcosine oxidase method. For urinary [TIMP-2]•[IGFBP7], the concentrations of TIMP-2 and IGFBP-7 in the urine were multiplied and then divided by 1000 to convert them into international general units, $(ng/mL)^2/1000$, in accordance with our previous study [15] and study by others [16].

In addition, the initial and the peak values of urinary biomarkers were used for data analysis in the derivation study. For each child, the level of urinary biomarkers from the sample collected in the first 24 h after PICU admission was denoted as the initial value. The highest level among collected samples during the first 7 days after PICU admission was denoted the peak value.

Statistical analysis

SPSS statistics software Version 22 and GraphPad software Inc. Prism Version 8 were used for statistical analyses. Continuous data were presented as median and interquartile range (IQR), as they were not-normally distributed. Categorical data were presented as counts and percentage. Continuous variables among groups were compared using the Mann-Whitney U test or Kruskal-Wallis H test, and categorical variables using the chi-square test or Fisher’ s exact test, as appropriate. Univariate and stepwise multivariate linear regression analyses were performed to investigate factors potentially associated with the levels of uTIMP-1 in the validation cohort. Multicollinearity of variables was evaluated via tolerance and variance inflation factor (VIF), and tolerance ≤ 0.5 and the VIF value ≥ 2 indicate the presence of multicollinearity. In both derivation and validation cohorts, the multivariate logistic regression analyses were performed to investigate the associations between urinary biomarker and AKI and PICU mortality after adjustment for potential confounders. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate the model fit. Subsequently, the predictive values of urinary biomarkers for AKI and PICU mortality were assessed by the receiver operating characteristic (ROC) curves. The area under the ROC curves (AUC) with the corresponding 95% confidence interval (CI) was recorded. In the validation cohort, the predictive accuracy was further assessed by sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) at the optimal cutoff values, which were determined by the maximum Youden index. For all analyses, a 2-tailed $P<0.05$ was considered significant.

Results

Derivation cohort characteristics

The derivation cohort study involved 123 critically ill children. Of a total of 125 children were admitted to the PICU during the study period, 2 children had multiple PICU admissions within a single hospital stay, only their last admission was included in the analysis. The leading cause of PICU admission in the cohort was respiratory diseases (30.9%), followed by neurologic diseases (14.6%), poison/trauma/accident (14.6%) and hematologic diseases (11.4%). None of the children had any known congenital abnormality of the kidney and received aminoglycosides during the PICU stay.

Of the 123 children, 29 (23.5%) developed AKI during PICU stay, including 16 with AKI stage 1, 8 with stage 2, and 5 with AKI stage 3. All the AKI occurred during the first week after PICU admission. The comparison of the demographic and clinical characteristics and the initial and peak levels of urinary biomarkers among children with non-AKI and AKI stage 1, 2, and 3 is displayed in Additional file 1: Table S1. The PICU mortality in the whole cohort with or without AKI was 15 (12.2%). The comparison of the characteristics and the levels of urinary biomarkers between survivors and non-survivors is displayed in Additional file 1: Table S2.

Association of urinary biomarkers with AKI stage 3 or death in the derivation cohort

Since there was no significant difference in the initial levels of urinary biomarkers among children with non-AKI, AKI stage 1 and AKI stage 2 (except KIM-1 and TIMP-2), as shown in Additional file 1: Table S1, the association of urinary biomarkers with AKI stage 3 or death developed during PICU stay was analyzed in Additional file 1: Table S3, by using univariate and multivariate logistic regression analyses. The comparison of the demographic and clinical characteristics and the initial and peak levels of urinary biomarkers among survivors with non-AKI (n = 86), survivors with AKI stage 1 or 2 (n = 20), and survivors with AKI stage 3 or non-survivors (n = 17) is displayed in Additional file 1: Table S4. In addition, the distributions of the initial and peak levels of urinary biomarkers among these groups are displayed in Additional file 2: Figure S1 (A and B).

Comparison of urinary biomarkers in predicting AKI stage 3 or death in the derivation cohort

The performance of both initial and peak urinary biomarkers in predicting AKI stage 3 or death developed during PICU stay is displayed in Fig. 2 and Additional file 1: Table S5. As shown in Fig. 2, both the initial and the peak uTIMP-1 displayed the highest AUC of 0.87 (95%CI 0.79–0.94, $P < 0.001$) and 0.90 (95%CI 0.84–0.96, $P < 0.001$) for the prediction. Therefore, the associations between uTIMP-1 levels and AKI or PICU mortality were sought to confirm in the validation study.

Validation Cohort Characteristics

The validation cohort study involved 357 critically ill children. Of a total of 368 children admitted to the PICU during the study period, 11 were excluded because of age of less than 28 days, a failure in collecting urine samples before discharge from the PICU or death and repetitive admission to PICU within a single hospital stay, as displayed in Fig. 1. The leading cause of PICU admission in the validation cohort was respiratory diseases (42.4%), followed by neurological diseases (16.1%), hematologic/oncologic diseases (9.9%) and gastrointestinal diseases (9.7%).

Among the 357 critically ill children, 47 (13.2%) developed AKI during the first week after admission, including 13 with AKI stage 1, 11 with stage 2, and 23 with AKI stage 3. A comparison of demographic and clinical characteristics among non-AKI and AKI status in the validation cohort is depicted in Table 1. The level of uTIMP-1 was significantly higher in critically ill children with AKI than that in those without AKI (1.79 [0.95–4.87] vs. 20.98 [6.08-160.35], $P < 0.001$). With the severity of AKI increasing, the levels of uTIMP-1 were higher, which is displayed in Fig. 3A.

Table 1
Comparison of demographic and clinical characteristics among non-AKI and AKI status in validation cohort

	Non-AKI n = 310	AKI Stage 1 n = 13	AKI Stage 2 n = 11	AKI Stage 3 n = 23	P value
Age, months	20.0 [4.0–53.0]	57.0 [22.0-151.5]*	52.0 [11.0–92.0]	37.0 [13.0–71.0]*	0.004
Body weight, kg	11.0 [7.0-17.5]	18.0 [13.0-47.5]*	13.0 [9.0–21.0]	13.0 [10.0–20.0]	0.006
Male, n	200 (64.5)	12 (92.3)*	6 (54.5)	14 (60.9)	0.120
PRISM III, score	3.0 [0.0–9.0]	10.0 [4.0–24.0]*	8.0 [4.0–16.0]*	13.0 [11.0–23.0]*&	0.001
MV[a], n	75 (24.2)	6 (46.2)	4 (36.4)	14 (60.9)*	0.001
Sepsis[a], n	52 (16.8)	4 (30.8)	4 (36.4)	12 (52.2)*	0.001
MODS[a], n	13 (4.2)	2 (15.4)	3 (27.3)*	13 (56.5)*#	< 0.001
Shock/DIC[a], n	16 (5.2)	2 (15.4)	1 (9.1)	11 (47.8)*&	< 0.001
Antibiotic[a], n	253 (81.6)	10 (76.9)	8 (72.7)	18 (78.3)	0.870
Inotrope[a], n	26 (8.4)	4 (30.8)*	1 (9.1)	7 (30.4)*	0.002
Furosemide[a], n	69 (22.3)	6 (46.2)	4 (36.4)	16 (69.6)*	< 0.001
Steroid[a], n	146 (47.1)	4 (30.8)	4 (36.4)	6 (26.1)	0.157
Hemofiltration[a], n	11 (3.5)	3 (23.1)*	2 (18.2)	8 (34.8)*	< 0.001
LOS of PICU, hours	96.0 [48.0-170.3]	101.0 [36.0-166.0]	96.0 [48.0-288.0]	123.0 [72.0-218.0]	0.927
PICU Mortality, n	15 (4.8)	6 (46.2)*	3 (27.3)*	14 (60.9)*	< 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* multi-organ dysfunction syndrome, *MV* mechanical ventilation, *PICU* pediatric intensive care unit, *PRISM III* pediatric risk of mortality III.

[a]Administered or developed during PICU stay. *P < 0.05 vs. non-AKI, #P < 0.05 vs. AKI Stage 1, &P < 0.05 vs. AKI Stage 2.

In the validation cohort, 38 (10.6%) died during PICU stay. The comparison of the uTIMP-1 level between survivors and non-survivors is displayed in Fig. 3B. These characteristic comparisons between survivors and non-survivors are summarized in Additional file 1: Table S6.

Correlation of uTIMP-1 levels with clinical variables in the validation cohort

All variables in Table 1 were analyzed for association with uTIMP-1. On univariate linear regression analysis, uTIMP-1 was significantly correlated with PRISM III score, AKI stage, sepsis, MODS, Shock/DIC and the use of mechanic ventilation, inotrope, furosemide, steroid and hemofiltration. To investigate factors independently associated with uTIMP-1 levels, variables with $P < 0.05$ under the univariate analysis were entered into the stepwise multivariate linear regression analysis after checking the multicollinearity. As listed in Table 2, the uTIMP-1 level was independently associated with PRISM III score ($P < 0.001$), AKI stage ($P < 0.001$) and hemofiltration ($P = 0.047$).

Table 2
Univariate and stepwise multivariate linear regression analysis for identifying clinical variables potentially associated with initial urinary TIMP-1 level in validation cohort

	Univariate regression[a]			Multivariate regression[b]		
	B	SE	P value	B	SE	P value value
Age, months	0.100	0.073	0.172	N/A		
Body weight, kg	0.139	0.161	0.387	N/A		
Sex	-0.116	0.100	0.246	N/A		
PRISM III score	0.052	0.005	< 0.001	0.035	0.006	< 0.001
AKI stage	0.261	0.061	< 0.001	0.260	0.060	< 0.001
MV	0.494	0.103	< 0.001	0.184	0.110	0.095
Sepsis	0.487	0.116	< 0.001	0.198	0.109	0.069
MODS	1.099	0.159	< 0.001	0.191	0.191	0.319
Shock/DIC	0.769	0.167	< 0.001	-0.203	0.183	0.266
Antibiotic	0.116	0.121	0.340	N/A		
Inotrope	0.583	0.151	< 0.001	-0.068	0.160	0.668
Furosemide	0.411	0.106	< 0.001	-0.072	0.107	0.503
Steroid	-0.239	0.095	0.012	-0.141	0.085	0.097
Hemofiltration	1.021	0.182	< 0.001	0.360	0.180	0.047

AKI acute kidney injury, *DIC* disseminated intravascular coagulation, *MODS* multi-organ dysfunction syndrome, *MV* mechanical ventilation, *N/A* not applicable, *PRISM III* pediatric risk of mortality III. Continuous variables were log-transformed in the linear regression analyses.

[a]All variables in Table 1 were analyzed in the univariate linear analysis, [b]Variables with P < 0.05 were entered into the multivariate analysis after checking the multicollinearity by variance inflation factor and tolerance values.

Association Between Utimp-1 And Aki In The Validation Cohort

Univariate and multivariate logistic regression analysis were performed to validate whether uTIMP-1 levels were independently associated with AKI, severe AKI, or AKI stage 3 in critically ill children in the validation cohort in Table 3. The uTIMP-1 levels remained significantly associated with AKI (AOR = 1.88, 95%CI 1.29–2.74, P = 0.001), severe AKI (AOR = 2.35, 95%CI 1.55–3.58, P < 0.001), and AKI stage 3 (AOR

= 2.87, 95%CI 1.71–4.83, P < 0.001) after adjustment for body weight, sex, PRISM III score, mechanic ventilation, sepsis, MODS and shock/DIC.

Table 3
Association of initial urinary TIMP-1 with AKI and PICU mortality in validation cohort

	AKI	Severe AKI	AKI stage 3	PICU mortality
OR[a] (95% CI)	2.77 (2.02–3.79)	3.03 (2.14–4.29)	3.57 (2.36–5.40)	3.07 (2.19–4.31)
P value	< 0.001	< 0.001	< 0.001	< 0.001
AOR[a, b] (95% CI)	1.88 (1.29–2.74)	2.35 (1.55–3.58)	2.87 (1.71–4.83)	1.92 (1.11–3.30)
P value	0.001	< 0.001	< 0.001	0.019
AUC (95% CI)	0.82 (0.75–0.88)	0.84 (0.77–0.91)	0.87 (0.81–0.94)	0.83 (0.76–0.89)
P value	< 0.001	< 0.001	< 0.001	< 0.001
Optimal cutoff, ng/mg uCr	5.38	6.65	11.79	11.79
Sensitivity, %	80.9	82.4	82.6	71.1
Specificity, %	76.5	78.0	82.3	84.0
PPV, %	77.4	78.9	82.4	81.6
NPV, %	80.0	81.6	82.6	74.4

AKI acute kidney injury, *AOR* adjusted OR, *AUC* the area under the ROC curve, *CI* confidence interval, *NPV* negative predictive value, *OR* odds ratio, *PICU* pediatric intensive care unit, *PPV* positive predictive value, *uCr* urinary creatinine.

Severe AKI was defined as KDIGO stage 2 or 3. Urinary TIMP-1 levels were log-transformed in the logistic regression because of the variation in the concentration.

[a]Odds ratio represents the increase in risk per log increase in urinary TIMP-1 levels, [b]Adjustment for body weight, sex, PRISM III score, mechanical ventilation, multi-organ dysfunction syndrome, and shock/disseminated intravascular coagulation.

As shown in Table 3, the predictive values of uTIMP-1 for AKI, severe AKI and AKI stage 3 were 0.82 (95%CI 0.75–0.88), 0.84 (95%CI 0.77–0.91) and 0.87 (95%CI 0.81–0.94), respectively. The ROC curves for the abilities of uTIMP-1 to predict AKI, severe AKI and AKI stage 3 are displayed in Fig. 4. We also calculated the optimal cutoff values of uTIMP-1 for prediction in Table 3. Urinary TIMP-1 had sensitivity of 80.9% and specificity of 76.5% at the optimal cutoff value of 5.38 ng/mg uCr to predict AKI.

Association between uTIMP-1 and PICU mortality in the validation cohort

To validate whether uTIMP-1 levels were independently associated with PICU mortality in critically ill children, univariate and multivariate logistic regression analysis were performed in the validation cohort. After adjustment for body weight, sex, PRISM III score, mechanic ventilation, sepsis, MODS and shock/DIC, the uTIMP-1 remained independently associated with PICU mortality (AOR = 1.92, 95%CI 1.11–3.30, P = 0.019), as listed in Table 3.

The performance of uTIMP-1 in predicting PICU mortality is also shown in Table 3 and Fig. 4. The uTIMP-1 level was predictive of mortality with an AUC of 0.83 (0.76–0.89), and had a sensitivity of 71.1% and a specificity of 84.0% at the optimal cutoff of 11.79 ng/mg uCr to predict PICU mortality in critically ill children in Table 3. The ROC curve for the ability of uTIMP-1 to predict PICU mortality is displayed in Fig. 4.

Discussion

The diagnostic approach to AKI is currently based on sCr and urine output, which, however, do not directly reflect cell injury but rather delayed functional consequences of the kidney injury. This has greatly impeded early identification and therapy. A key step for the application of novel biomarkers of AKI in clinical practice is the good predictive performance with sensitivity and specificity in heterogeneous population. In this study, the derivation and validation cohorts were performed in a mixed heterogeneous PICU. We not only detected urinary biomarkers of AKI, including NGAL, KIM-1, TIMP-2, IGFBP7, [TIMP-2]•[IGFBP7], FABP-1, TIMP-1, renin, IP-10 and TFF-3 to identify the best biomarker when compared with others, but also validated that uTIMP-1 had useful values in early prediction of AKI and PICU mortality.

In our derivation cohort study, these urinary biomarkers had overall poorer discriminative performance in AKI stages 1 and 2, which might result from more variable urinary biomarker excretion in a PICU population limited size. This is particularly problematic in view of the high prevalence and incidence of critically ill children with mild AKI [2]. Nevertheless, biomarkers have shown better diagnostic performance in severe form of AKI, as compared to mild AKI. Severe AKI, corresponding to KDIGO stage 3, is associated with a significantly increased incidence of mortality in critically ill children [2, 17]. The PICU mortality in the critically ill children with AKI stage 3 in the derivation cohort was up to 60%. Therefore, it is reasonable that we evaluated the predictive values of these urinary biomarkers for AKI stage 3 or PICU mortality in the derivation study. Our results of derivation cohort highlight the utility and importance of initial and peak urinary TIMP-1 in critically ill children, which is similar to urinary NGAL, KIM-1 and TIMP-2 and has an increased predictive value relative to other urinary biomarkers, such as FABP-1, IGFBP7, IP-10, renin, and TTF-3, as assessed by AUCs, for the prediction of AKI stage 3 or death. In addition, a separate cohort with larger number samples were performed in turn to validate the main result from the derivation study.

Moreover, our results of derivation cohort are consistent with previous studies conducted in children, indicating that urinary NGAL is a useful AKI biomarker for the prediction of the development of severe AKI and mortality in a heterogeneous group of patients with unknown timing of kidney injury [18, 19]. The

increased urinary levels of KIM-1, FABP-1, IGFBP7, [TIMP-2]•[IGFBP7], renin and IP-10 have also been reported in children with AKI [20–25]. We verified the impact of these biomarkers on predicting AKI stage 3 or mortality in a general PICU population. In our study, urinary TIMP-2 had better performance than urinary IGFBP7 for the prediction. This difference might be explained by assuming that urinary IGFBP7 was superior to urinary TIMP-2 in surgical patients while urinary TIMP-2 was best in sepsis-induced AKI [16]. The cell-cycle arrest biomarker of urinary [TIMP-2]•[IGFBP7] is suggested to be better than any existing biomarkers for predicting the development of moderate or severe AKI [16, 26]. Westhoff et al. reported that upregulated urinary [TIMP-2]•[IGFBP7] had a good performance of predicting mortality in neonatal and pediatric AKI [21]. However, the heterogeneity of the diagnostic value of urinary [TIMP-2]•[IGFBP7] for AKI has been reported and mainly influenced by different population settings and AKI thresholds [26], which may be the most likely explanation for the lower discriminative power in our study. Our result emphasizes that biomarker of AKI must be interpreted in the specific clinical context. In addition, the measurement by using multiplex bead assays in a small sample size may be another reason for the discrepancy between our data and others. Unlike the above biomarkers, there was no study to evaluate TTF-3, as a urinary biomarker for predicting AKI in children. Only one study so far has evaluated urinary TFF-3 as a biomarker of nephrotoxicity in humans [27]. Urinary TFF-3 levels were associated with death in patients with coexistent kidney disease, and predicted all-cause mortality [28]. The roles of urinary TFF-3 in AKI and associated outcomes merit additional investigation.

The major finding in this study was that a higher level of TIMP-1 in urine collecting during the first 24 h after PICU admission may be independently predictive of AKI and mortality developed during PICU stay in critically ill children. To our knowledge this is the first report of an AKI biomarker study performed in critically ill children that used a derivation-validation approach with separate patient cohorts. Urinary TIMP-1 was identified to be the best-performing marker and we tested its performance in a second group of critically ill children. Interesting, elevated uTIMP-1 levels showed robust relationship with AKI and PICU mortality.

The TIMP-1 is the first-discovered natural collagenase inhibitor and exhibits diverse biological function [29]. Several studies have revealed that TIMP-1 participates in kidney injury through regulating extracellular matrix synthesis and degradation, promoting tubulointerstitial fibrosis through inhibition of proteolytic matrix metalloproteinases and exacerbating inflammation and renal scaring [30–32]. Researches on urinary TIMP-1 for AKI were mainly about drug-induced AKI in animal models [31–33]. So far, our report is the first clinical study to attempt to use urinary TIMP-1 as an early biomarker for AKI in critically ill children. It has been indicated that serum TIMP-1 has a higher level in septic patients with AKI and is a good diagnostic biomarker of sepsis associated AKI [34]. In patients with sepsis after major abdominal surgery and sepsis-associated organ dysfunction, higher serum TIMP-1 levels were correlated with disease severity [35, 36], kidney injury and the use of vasopressors/inotropes [36]. Our results from the validation study further proved the independent correlations between TIMP-1 levels in urine and AKI and illness severity in critically ill children.

The positive correlation of uTIMP-1 with the PRISM III score in the study raises the question of whether uTIMP-1 levels are associated with clinical outcomes in critically ill children. Indeed, our data indicate that uTIMP-1 is an independent variable associated with PICU mortality, even after adjusting for potential confounders, including the severity of illness assessed by the PRISM III score. Compared with the discriminative ability of serum TIMP-1 for mortality in patients with sepsis [35], urinary TIMP-1 in our study had a good performance in predicting PICU mortality.

Our study has several limitations. First, the main limitation is that this was not a multicenter study. AKI occurred in the first week of PICU admission, with 60% in the first day, implying that critically ill children might be admitted later to the PICU. Nevertheless, our study is consistent with a previous study, indicating that the vast majority of children developed AKI within the first 24 hours of admission to the PICU [37]. Second, most critically ill children did not have baseline sCr prior to hospital admission. In consistent with our previous studies [11, 13], the lowest sCr value within the PICU stay was used as a baseline for children with elevated sCr ≥ 1.2 mg/dL (106.1 μmol/L) at admission, which, however, has not been validated in critically ill children. Third, the levels of some urinary biomarkers from the derivation cohort slightly lower in AKI stage 2 than in non-AKI and AKI stage 1. It is possible that the relatively small number of critically ill children could have added to the large variability in the data. Fourth, we did not perform an etiological analysis for developing AKI. Since AKI is not a single disease but a complex syndrome with multiple underlying etiologies, and 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.

Conclusions

Urinary TIMP-1 levels are identified and validated to be independently associated with increased risk for AKI and PICU mortality even after adjustment for confounding factors. A higher uTIMP-1 is predictive of AKI and PICU mortality in critically ill children. A large multicenter study is imperative to delineate the exact role and potential of urinary biomarkers in critically ill children.

Additional files

Additional file 1: Table S1. Comparison of demographic and clinical characteristics and the levels of urinary biomarkers among non-AKI and AKI status in derivation cohort. **Table S2.** Comparison of characteristics and the levels of urinary biomarkers between survivors and non-survivors in derivation cohort. **Table S3.** Association of urinary biomarkers with AKI stage 3 or death developed during PICU stay in derivation cohort. **Table S4.** Comparison of demographic, clinical characteristics and urinary biomarkers among patients with AKI status and/or death in derivation cohort. **Table S5.** Predictive characteristics of urinary biomarkers for AKI stage 3 or death in derivation cohort. **Table S6.** Comparison of demographic and clinical characteristics between survivors and non-survivors in validation cohort. (DOCX 64.7 KB)

Additional file 2: Figure S1. Comparison of the initial (A) and peak (B) urinary biomarkers among patients with AKI status and/or death in discovery cohort.

AKI acute kidney injury, *FABP-1* fatty acid binding protein 1, *IGFBP7* insulin-like growth factor-binding protein 7, *IP-10* interferon inducible protein-10, *KIM-1* kidney injury molecule-1, *NGAL* neutrophil gelatinase-associated lipocalin, *TFF-3* trefoil factor-3, *TIMP-1* tissue inhibitor of metalloproteinases-1, *TIMP-2* tissue inhibitor of metalloproteinases-2. Each dot represents an individual patient; the horizontal lines indicate median with interquartile range. *P < 0.05 vs. survivors with non-AKI, #P < 0.05 vs. survivors with AKI stage 1 or 2. (TIF 4.19MB)

Abbreviations

AKI
acute kidney injury; AOR:adjusted odds ratio; AUC:area under the receiver operating characteristic curve; CI:confidence interval; Cr:creatinine; DIC:disseminated intravascular coagulation; FABP-1:fatty-acid-binding protein-1; ICU:intensive care unit; IGFBP7:insulin-like growth factor-binding protein 7; IP-10:interferon inducible protein-10; IQR:interquartile range; KDIGO:kidney disease:improving global outcomes; KIM-1:kidney injury molecule-1; LOS:length of stay; MODS:multiple organ dysfunction syndrome; MV:mechanical ventilation; NGAL:neutrophil gelatinase-associated lipocalin; NPV:negative predictive value; OR:odds ratio; PICU:pediatric intensive care unit; PPV:positive predictive value; PRISM III:pediatric risk of mortality III; ROC:receiver operating characteristic curve; sCr:serum creatinine; TFF-3:trefoil factor-3; TIMP-1:tissue inhibitor of metalloproteinase-1; TIMP-2:tissue inhibitor of metalloproteinase-2.

Declarations

Ethics approval and consent to participate

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 at enrollment.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by grants from the National Natural Science Foundation of China (81971432), Science and Technology Support Program of Jiangsu Province (BE2020660), Key talent of women's and children's health of JiangSu province (FRC201738). The funders had no role in study design, data collection, preparation of the manuscript, and decision to publish.

Authors' contributions

HH performed the experiments and data analysis and drafted the manuscript. QL participated in collecting data and samples and data analysis and revised the manuscript. XD collected clinical data and urine samples and participated in data analysis. JC, ZB and XL participated in the study design and interpretation of data and helped to draft the manuscript. FF participated in the statistical analysis and interpretation of data and revised the manuscript. YL had primary responsibility for study design, data analysis, interpretation of data and writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank the staff in biochemistry laboratory for technical assistance.

References

1. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. Lancet. 2019;394:1949–64.
2. Kaddourah A, Basu RK, Bagshaw SM, Goldstein SL, Investigators A. Epidemiology of acute kidney injury in critically ill children and young adults. N Engl J Med. 2017;376:11–20.
3. Kellum JA, Prowle JR. Paradigms of acute kidney injury in the intensive care setting. Nat Rev Nephrol. 2018;14:217–30.
4. Murray PT, Mehta RL, Shaw A, Ronco C, Endre Z, Kellum JA, Chawla LS, Cruz D, Ince C, Okusa MD, et al. Potential use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. Kidney Int. 2014;85:513–521.
5. Malhotra R, Siew ED. Biomarkers for the early detection and prognosis of acute kidney injury. Clin J Am Soc Nephrol. 2017;12:149–73.
6. Kestenbaum B, Seliger SL. Commentary on biomarkers for early detection and prognosis of AKI. Clin J Am Soc Nephrol. 2017;12:174–5.
7. Honore PM, Jacobs R, Joannes-Boyau O, Verfaillie L, De Regt J, Van Gorp V, De Waele E, Boer W, Collin V, Spapen HD. Biomarkers for early diagnosis of AKI in the ICU: ready for prime time use at the bedside? Ann Intensive Care. 2012;2:24.

8. Lameire NH, Vanholder RC, Van Biesen WA. How to use biomarkers efficiently in acute kidney injury. Kidney Int. 2011;79:1047–50.
9. Ronco C. Acute kidney injury: from clinical to molecular diagnosis. Crit Care. 2016;20:201.
10. McCaffrey J, Dhakal AK, Milford DV, Webb NJ, Lennon R. Recent developments in the detection and management of acute kidney injury. Arch Dis Child. 2017;102:91–6.
11. Li Y, Wang J, Bai Z, Chen J, Wang X, Pan J, Li X, Feng X. Early fluid overload is associated with acute kidney injury and PICU mortality in critically ill children. Eur J Pediatr. 2016;175:39–48.
12. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated pediatric risk of mortality score. Crit Care Med. 1996;24:743–52.
13. Fang F, Hu X, Dai X, Wang S, Bai Z, Chen J, Pan J, Li X, Wang J, Li Y. Subclinical acute kidney injury is associated with adverse outcomes in critically ill neonates and children. Crit Care. 2018;22:256.
14. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin Pract. 2012;120:c179–184.
15. Chen J, Sun Y, Wang S, Dai X, Huang H, Bai Z, Li X, Wang J, Li Y. The effectiveness of urinary TIMP-2 and IGFBP-7 in predicting acute kidney injury in critically ill neonates. Pediatr Res. 2020;87:1052–9.
16. Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, Bihorac A, Birkhahn R, Cely CM, Chawla LS, et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care. 2013;17:R25.
17. Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, Edipidis K, Forni LG, Gomersall CD, Govil D, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive Care Med. 2015;41:1411–23.
18. Zappitelli M, Washburn KK, Arikan AA, Loftis L, Ma Q, Devarajan P, Parikh CR, Goldstein SL. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. Crit Care. 2007;11:R84.
19. Mishra OP, Rai AK, Srivastava P, Pandey K, Abhinay A, Prasad R, Mishra RN, Schaefer F. Predictive ability of urinary biomarkers for outcome in children with acute kidney injury. Pediatr Nephrol. 2017;32:521–7.
20. Zwiers AJ, de Wildt SN, van Rosmalen J, de Rijke YB, Buijs EA, Tibboel D, Cransberg K. Urinary neutrophil gelatinase-associated lipocalin identifies critically ill young children with acute kidney injury following intensive care admission: a prospective cohort study. Crit Care. 2015;19:181.
21. Westhoff JH, Tonshoff B, Waldherr S, Poschl J, Teufel U, Westhoff TH, Fichtner A. Urinary tissue inhibitor of metalloproteinase-2 (TIMP-2) * insulin-like growth factor-binding protein 7 (IGFBP7) predicts adverse outcome in pediatric acute kidney injury. PLoS One. 2015;10:e0143628.
22. Ivanisevic I, Peco-Antic A, Vulicevic I, Hercog D, Milovanovic V, Kotur-Stevuljevic J, Stefanovic A, Kocev N. L-FABP can be an early marker of acute kidney injury in children. Pediatr Nephrol. 2013;28:963–9.

23. Bai Z, Fang F, Xu Z, Lu C, Wang X, Chen J, Pan J, Wang J, Li Y. Serum and urine FGF23 and IGFBP-7 for the prediction of acute kidney injury in critically ill children. BMC Pediatr. 2018;18:192.
24. Kuai Y, Huang H, Dai X, Zhang Z, Bai Z, Chen J, Fang F, Pan J, Li X, Wang J, et al. In PICU acute kidney injury stage 3 or mortality is associated with early excretion of urinary renin. Pediatr Res. 2021.
25. Erez DL, Denburg MR, Afolayan S, Jodele S, Wallace G, Davies SM, Seif AE, Bunin N, Laskin BL, Sullivan KE. Acute kidney injury in children after hematopoietic cell transplantation is associated with elevated urine CXCL10 and CXCL9. Biol Blood Marrow Transplant. 2020;26:1266–72.
26. Jia HM, Huang LF, Zheng Y, Li WX. Diagnostic value of urinary tissue inhibitor of metalloproteinase-2 and insulin-like growth factor binding protein 7 for acute kidney injury: a meta-analysis. Crit Care. 2017;21:77.
27. George B, Wen X, Mercke N, Gomez M, O'Bryant C, Bowles DW, Hu Y, Hogan SL, Joy MS, Aleksunes LM. Profiling of kidney injury biomarkers in patients receiving cisplatin: time-dependent changes in the absence of clinical nephrotoxicity. Clin Pharmacol Ther. 2017;101:510–8.
28. O'Seaghdha CM, Hwang SJ, Larson MG, Meigs JB, Vasan RS, Fox CS. Analysis of a urinary biomarker panel for incident kidney disease and clinical outcomes. J Am Soc Nephrol. 2013;24:1880–8.
29. Woolley DE, Roberts DR, Evanson JM. Inhibition of human collagenase activity by a small molecular weight serum protein. Biochem Biophys Res Commun. 1975;66:747–54.
30. Ries C. Cytokine functions of TIMP-1. Cell Mol Life Sci. 2014;71:659–72.
31. Hoffmann D, Fuchs TC, Henzler T, Matheis KA, Herget T, Dekant W, Hewitt P, Mally A. Evaluation of a urinary kidney biomarker panel in rat models of acute and subchronic nephrotoxicity. Toxicology. 2010;277:49–58.
32. Won AJ, Kim S, Kim YG, Kim KB, Choi WS, Kacew S, Kim KS, Jung JH, Lee BM, Kim S, et al. Discovery of urinary metabolomic biomarkers for early detection of acute kidney injury. Mol Biosyst. 2016;12:133–44.
33. Kim KS, Yang HY, Song H, Kang YR, Kwon J, An J, Son JY, Kwack SJ, Kim YM, Bae ON, et al. Identification of a sensitive urinary biomarker, selenium-binding protein 1, for early detection of acute kidney injury. J Toxicol Environ Health A. 2017;80:453–64.
34. Bojic S, Kotur-Stevuljevic J, Kalezic N, Stevanovic P, Jelic-Ivanovic Z, Bilanovic D, Memon L, Damnjanovic M, Kalaba Z, Simic-Ogrizovic S. Diagnostic value of matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in sepsis-associated acute kidney injury. Tohoku J Exp Med. 2015;237:103–9.
35. Lorente L, Martin MM, Labarta L, Diaz C, Sole-Violan J, Blanquer J, Orbe J, Rodriguez JA, Jimenez A, Borreguero-Leon JM, et al. Matrix metalloproteinase-9, -10, and tissue inhibitor of matrix metalloproteinases-1 blood levels as biomarkers of severity and mortality in sepsis. Crit Care. 2009;13:R158.

36. Bojic S, Kotur-Stevuljevic J, Aleksic A, Gacic J, Memon L, Simic-Ogrizovic S. Matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in sepsis after major abdominal surgery. Dis Markers. 2018;2018:5064684.
37. Alkandari O, Eddington KA, Hyder A, Gauvin F, Ducruet T, Gottesman R, Phan V, Zappitelli M. Acute kidney injury is an independent risk factor for pediatric intensive care unit mortality, longer length of stay and prolonged mechanical ventilation in critically ill children: a two-center retrospective cohort study. Crit Care. 2011;15:R146.

Figures

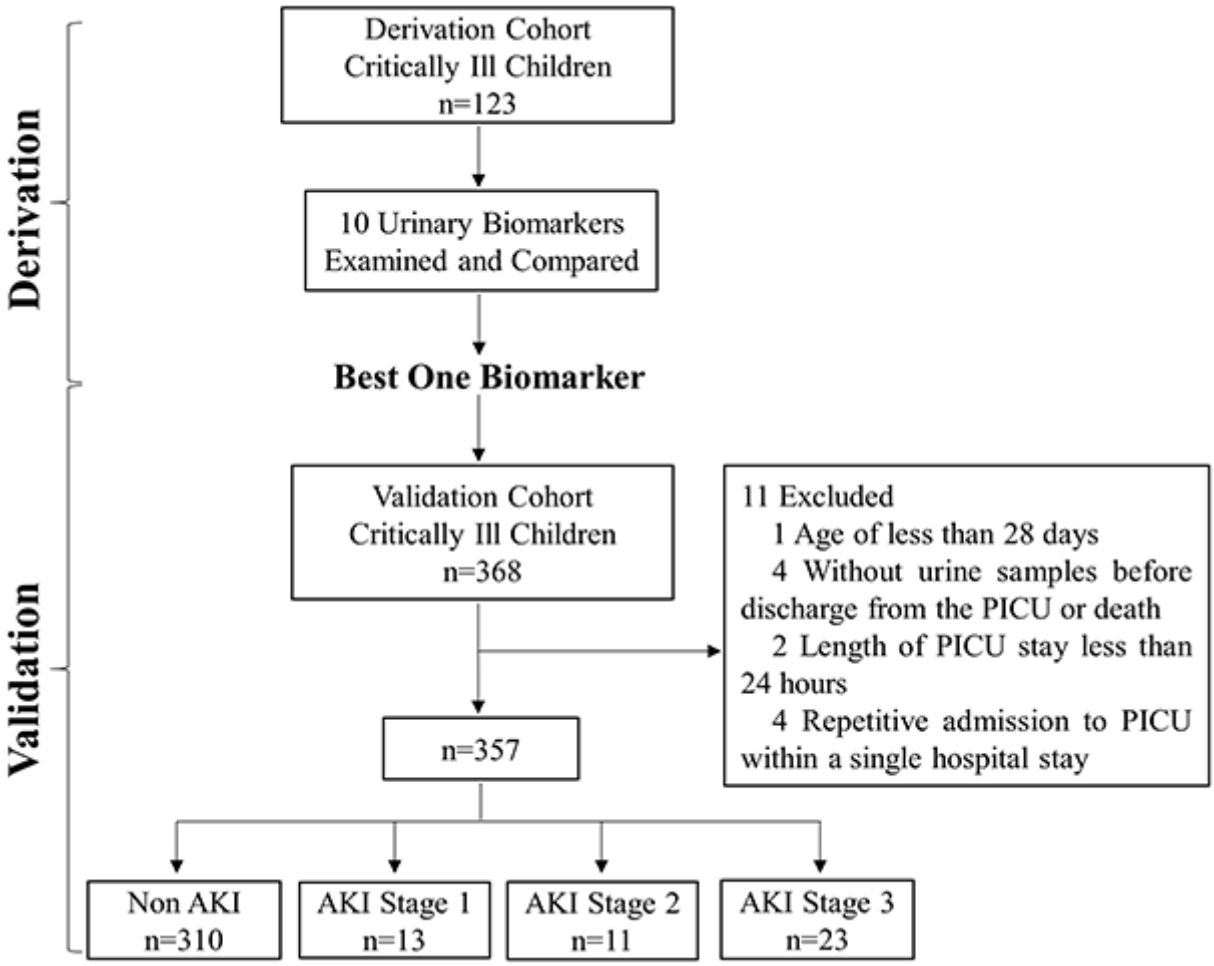

Figure 1

A flow chart representing study design. AKI acute kidney injury, PICU pediatric intensive care unit.

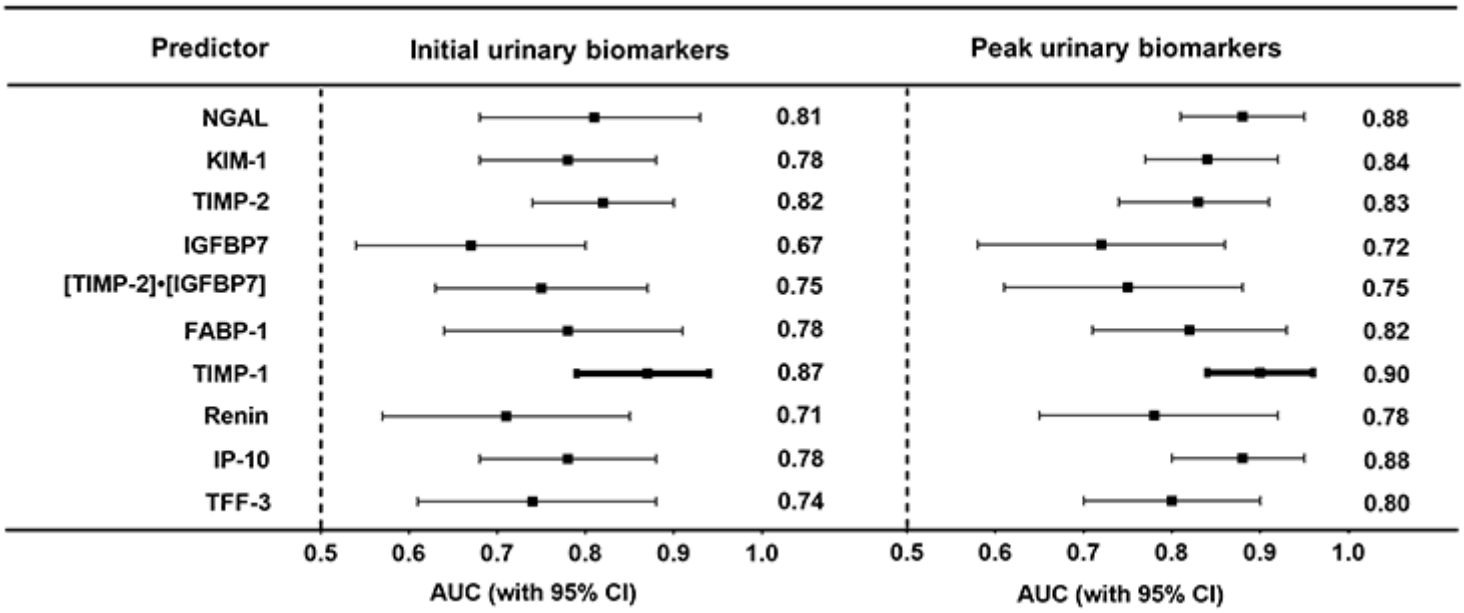

Figure 2

Predictive characteristics of urinary biomarkers for AKI stage 3 or death in derivation cohort. AKI acute kidney injury, AUC the area under the ROC curve, CI confidence interval, FABP-1 fatty acid binding protein 1, IGFBP7 insulin-like growth factor-binding protein 7, IP-10 interferon inducible protein-10, KIM-1 kidney injury molecule-1, NGAL neutrophil gelatinase-associated lipocalin, TFF-3 trefoil factor-3, TIMP-1 tissue inhibitor of metalloproteinases-1, TIMP-2 tissue inhibitor of metalloproteinases-2.

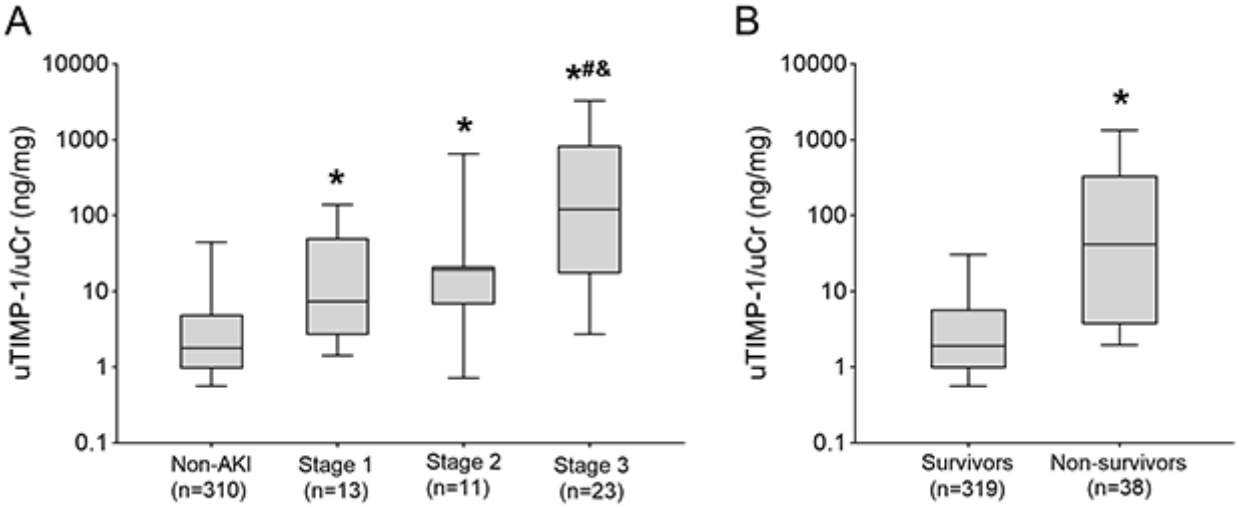

Figure 3

Urinary TIMP-1 levels among non-AKI and AKI status (A), and survivors and non-survivors (B) in validation cohort. AKI acute kidney injury, TIMP-1 tissue inhibitor of metalloproteinases-1. Lines denote median values, boxes represent 25th to 75th percentiles and whiskers indicate the range. Numbers of samples are indicated in parentheses. *P<0.05 vs. non-AKI (A) or survivors (B), #P<0.05 vs. AKI stage 1, &P<0.05 vs. AKI stage 2.

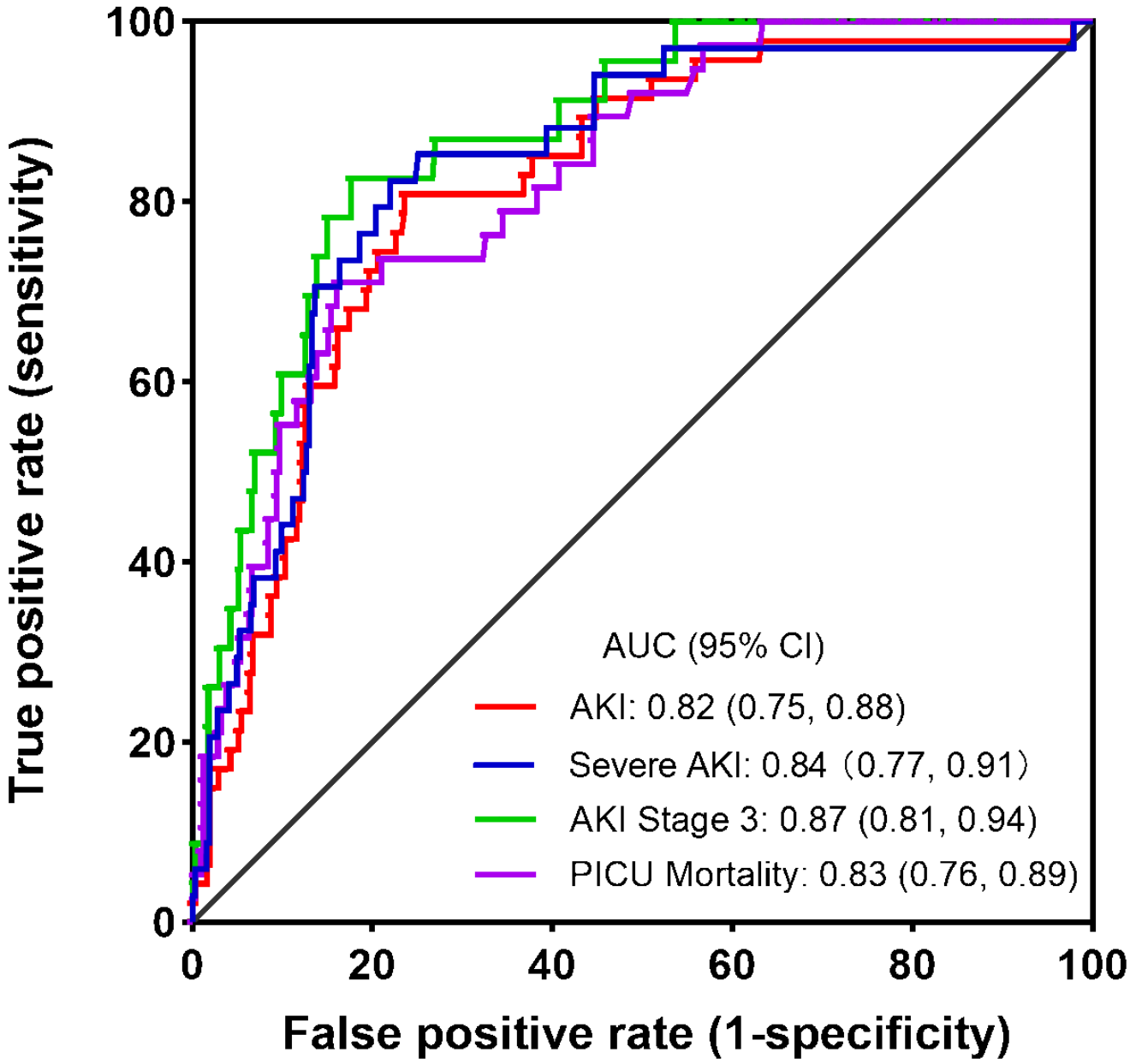

Figure 4

ROCs of urinary TIMP-1 to predict AKI, severe AKI, AKI stage 3 and PICU mortality in validation cohort. AKI acute kidney injury, AUC the area under the ROC curve, CI confidence interval, PICU pediatric intensive care unit, ROC receiver operating characteristic, TIMP-1 tissue inhibitor of metalloproteinases-1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx

- figureS1A.tif
- figureS1B.tif