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Cell cycle arrest biomarkers for predicting renal recovery from acute kidney injury: a prospective validation study

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Abstract

Background: Acute kidney injury (AKI) is a common disease in intensive care unit (ICU). AKI patients with non-recovery of renal function have a markedly increased risk of death compared with recovery patients. The current study aimed to explore and validate the utility of urinary cell cycle arrest biomarkers for predicting non-recovery in patients who developed AKI after ICU admission.

Methods: We prospectively and consecutively enrolled 379 critically ill patients who developed AKI after admission to ICU, which divided into a derivation cohort (194 AKI patients) and a validation cohort (185 AKI patients). The biomarkers of urinary tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) were detected at inclusion (day 0) and 24 hours later (day 1). immediately after AKI diagnosis. The optimal cutoff values of biomarkers for predicting non-recovery was estimated in the derivation cohort, and the predictive accuracy of the biomarkers was assessed in the validation cohort. The primary endpoint was non-recovery from AKI (within 7 days).

Results: 159 of 379 (41.9 %) patients failed to recover from AKI onset, with 79 in the derivation cohort and 80 in the validation cohort. Urinary [TIMP-2]*[IGFBP7] showed a better prediction for non-recovery than TIMP-2 and IGFBP7 alone, with the AUC of 0.751 (95 % CI 0.701 - 0.852, p < 0.001) and an optimal cutoff value of 1.05 $((ng/mL)^2/1000)$. When [TIMP-2]*[IGFBP7] combined with clinical factors of AKI diagnosed by urine output (UO) criteria, AKI stage 2-3 and nonrenal SOFA score for predicting non-recovery, the AUC was significantly improved to 0.852 (95 % CI 0.750 - 0.891, $p < 0.001$), which achieved the sensitivity and specificity of 88.8 % (72.9, 98.7) and 92.6 % (80.8, 100.0), respectively.

Conclusion: Urinary [TIMP-2]*[IGFBP7] represents a sensitive and specific biomarker to predict failure to recover from AKI. The predictive accuracy can be improved when urinary [TIMP-2]*[IGFBP7] combines with clinical factors of AKI diagnosed by UO criteria, AKI stage 2-3 and nonrenal SOFA score.

Background

Acute kidney injury (AKI) is a common disease in intensive care unit (ICU) and carries a significant risk of chronic kidney disease (CKD), short- and long-term mortality [1–3]. Currently specific therapeutic interventions and available preventive measures are limited, so renal recovery after AKI have cumulatively become the focus of research. Moreover, changes in renal functional reserve may substantially affect the clinical outcomes of AKI patients [4–6]. AKI patients with non-recovery of renal function have a markedly increased risk of death compared with recovery patients [7]. Therefore, preventing non-recovery of renal function should be the therapeutic goal of AKI.

Among all AKI biomarkers, cell-cycle arrest of urinary tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) simultaneously, are upregulated early after AKI onset and have been confirmed to be superior in early detection of AKI [8]. However, only few studies have assessed their performance as a prognostic marker for non-renal recovery [5, 9]. If we can predict the patients who failed to recover in early AKI, effectively supportive measures (for example removal of

nephrotoxic agents, optimization of volume management and individualized haemodynamic resuscitation) may be implemented early before irreversible recovery happened [10, 11], which may prevent further progression of AKI and improve clinical prognosis. The current study, measuring urinary TIMP-2 and IGFBP7 when AKI diagnosed, evaluated and validated the utility of urinary [TIMP-2]*[IGFBP7] for predicting non-recovery in patients who developed AKI after ICU admission.

Methods

The study was approved by the Human Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University (Beijing, China), the ethics number was 2018 – 117. Informed consent from patients or their next of kin was wrote before patients participated in this study.

Study setting and population

The present study was performed in two Chinese ICUs of Beijing Chao-yang Hospital and Beijing Lu-he Hospital from July 1, 2018 and December 1, 2020. Study design, performance, and report were complied with the Standards for Reporting of Diagnostic Accuracy guidelines [12]. We screened critically patients who stayed in ICU longer than 24 hours. Patients who developed AKI after ICU admission were prospectively and consecutively enrolled. The exclusion criteria included (1) age < 18 years; (2) developing AKI before ICU admission; (3) acquired insufficient urine samples. All enrolled patients adhere to the following management principles: active treatment of primary disease and comorbidities; the same principles of treatment with antibiotics, nutritional metabolism and organ support.

Biomarker measurements

Urine samples for biomarker assessment were taken from the urinary catheter of eligible patients soon after AKI diagnosed and 24 hours later. The biomarkers of TIMP-2 and IGFBP7 were detected at inclusion (day 0) and 24 hours later (day 1), and measured with NephroCheck™ Test and VITROS 5600 Integrated System (Astute Medical, San Diego, CA, USA). VITROS 5600 Integrated System reports the product of the two protein concentrations ([TIMP-2]•[IGFBP7]) in units of $(ng/mL)^2/1000$. The biomarkers were measured by technicians who were blind to clinical data and physicians in charge were blind to the biomarker test results.

Clinical endpoint and definitions

The primary endpoint was non-recovery from AKI. Renal recovery was defined as the absence of any stage of AKI by either creatinine or urine output (UO) criteria within 7 days (serum creatinine level decreased to less than 150 % of baseline from AKI onset, and be free of periods of oliguria (UO < 0.5 ml/kg/h) longer than 6 hours) [13]. The patients requiring renal replacement therapy (RRT) until the 7th days after AKI were regarded as non-recovery. The secondary endpoints were use of RRT in ICU period, hospital mortality and 30-day mortality. The diagnosis of AKI was dependent on the serum creatinine and UO criteria proposed by Kidney Disease: Improving Global Outcomes (KDIGO) as any of the following: increase in serum creatinine by ≧ 0.3 mg/dl (≧ 26.5 μmol/l) within 48 hours; or increase in serum

creatinine to ≧ 1.5 times baseline; or UO < 0.5 ml/kg/h for > 6 h [14–16]. AKI diagnosed by UO criteria included patients who diagnosed AKI by UO criteria alone or both UO and creatinine criteria. The baseline creatinine was defined as follows: if at least five values were available the median of all values available from six months to seven days prior to enrollment was used. Otherwise, the lowest value in the seven days prior to enrollment was used. If no pre-enrollment creatinine was available or the emergency patient's serum creatinine was abnormal at the time of admission, the baseline creatinine was estimated using the Modification of Diet in Renal Disease (MDRD) equation assuming that baseline eGFR is 75 ml/min per 1.73 m^2. CKD was defined according to the definition of National Kidney Foundation as estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m^2 for at least 3 months irrespective of the cause. GFR was estimated with the Cockcroft-Gault formula [17, 18].

Data collection

All clinical data was prospectively collected on the basis of case report forms (CRF). Clinical patient variables included patient demographic characteristics, prior health history, diagnosis, comorbidities, use of vasopressor, mechanical ventilation. Serum creatinine was detected and recorded at ICU admission and every 12 h thereafter until the 7th day after AKI. UO was measured hourly from the urinary catheter in the ICU period. Acute physiology and chronic health evaluation (APACHE II) and sequential organ failure assessment (SOFA) score were assessed on the day diagnosing AKI. Further, use of RRT in ICU period, duration of ICU stay, hospital stay, death in hospital and 30 days after AKI developed were recorded.

Study phase

The study had 2 phases. Phase I (derivation cohort) was performed from July 1, 2018 and to July 31, 2019. This cohort was conducted to estimate the cutoff value of urinary [TIMP-2]•[IGFBP7] which best distinguished patients who would fail to recover after AKI developed. Phase II (validation cohort) was performed from August 1, 2019 to December 1, 2020. The predictive accuracy of urinary [TIMP-2]•[IGFBP7] for predicting non-recovery was assessed in the validation cohort using the cutoff value previously estimated in the derivation cohort.

Statistical analysis

SPSS statistics 24 (IBM, Chicago, IL) and R 2.1.2 were used for statistical analyses. Continuous variables were presented as mean ± standard deviation (SD) or median values (25th and 75th percentiles), categorical variables were presented as percentiles. Continuous data between two groups (recovery group and non-recovery group) was compared using the repeated measurement analysis of variance or Mann-Whitney U tests, and categorical variables used the Chi square test or Fisher' s exact test. For all analyses, statistical significance was indicated by two-sided $p < 0.05$.

In the derivation cohort, AKI patients were divided into two groups of renal recovery and non-recovery. Clinical parameters were compared between the two groups. Clinical parameters with $p < 0.1$ in univariate analyses were added to the multivariate logistic regression model. Then, variables with $p < 0.05$ in multivariate logistic regression model were independently risk factors for non-recovery. Receiver operating

characteristic (ROC) curve was used for biomarkers to assess the predictive values for non-recovery from AKI, and a combination of ROC curve with multivariate logistic regression analysis was used to assess the predictive value of clinical prediction model, which included biomarker and independently risk factors for non-recovery. The area under the ROC curve (AUC) and their corresponding 95 % confidence intervals (CIs), as well as cutoff biomarker values for predicting non-recovery were recorded. The following values of 0.90-1.0 excellent, 0.80–0.89 good, 0.70–0.79 useful, 0.60–0.69 poor and 0.50–0.59 no useful performance were used to describe AUCs. The optimal cutoff value was determined by the Youden index. The net contribution of the biomarkers to predict non-recovery was validated by Hosmer and Lemeshow' s test, net reclassification improvement (NRI) and integrated discrimination improvement (IDI). Delong test was used to compare the statistical difference of two AUCs.

In the validation cohort, predictive accuracy of the biomarker was assessed by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), which were calculated by the true incidence of non-recovery in the validation cohort.

Results

Total patient characteristics

During the study period, 3154 critically ill patients who stayed longer than 24 hours after ICU admission were screened in two ICUs, among them, 424 (13.4 %) patients developed AKI. After excluding the ineligible patients, 379 were finally enrolled, with 194 in the derivation cohort and 185 in the validation cohort. Baseline characteristics, comorbidities, AKI classification, and short-term prognosis showed no significant difference between the two cohorts. The comparisons are presented in Table 1. The flow diagram is shown in Fig. 1.

Table 1
Patient baseline characteristics in derivation and validation cohorts

Variables	Derivation cohort (*n* = 194)	Validation cohort (*n* = 185)	*p* value
Baseline characteristics			
Age (year)	61 (50, 71)	60 (49, 74)	0.773
Female gender	117 (60.3)	122 (65.9)	0.239
BMI (kg/m^2)	19.9 (17.3, 21.3)	20.0 (17.7, 22.1)	0.803
APACHE II score	15 (10, 18)	14 (10, 18)	0.228
Nonrenal SOFA score	4 (1, 7)	4 (1, 6)	0.543
Baseline serum creatinine (μmol/L)	63.8 (53.5, 73.3)	65.4 (50.4, 73.2)	0.617
Comorbidities			
COPD/asthma	20 (10.3)	20 (10.8)	0.869
Cardiovascular disease	40 (20.6)	43 (23.2)	0.536
Chronic liver disease	42 (21.6)	41 (22.2)	0.902
Diabetes	49 (25.2)	40 (21.6)	0.467
Hypertension	91 (46.9)	79 (42.7)	0.469
CKD	10 (5.1)	7 (3.9)	0.623
Sepsis	89 (45.8)	69 (37.3)	0.102
Mechanical ventilation	160 (82.5)	151 (81.6)	0.894
PaO_2/FiO_2	308.3 (232.5, 405.5)	316.0 (216.0, 403.3)	0.433
Use of vasopressor	68 (35.1)	61 (33.0)	0.658
Use of diuresis	28 (14.4)	23 (12.4)	0.724
Laboratory test on the day AKI diagnosed			
pH	7.41 (7.37, 7.46)	7.41 (7.36, 7.47)	0.821
PaO_2(mmHg)	134 (103, 195)	138 (95, 203)	0.719

Values are median (interquartile range) or n (%), *AKI* acute kidney injury, *BMI* body mass index, *APACHE II* acute physiology and chronic health evaluation, *SOFA* sequential of organ failure assessment, *CKD* chronic kidney disease, *UO* urine output, *RRT* renal replacement therapy, *WBC* white blood cell, *ALT* alanine transaminase, *AST* aspartate transaminase, *TBIL* total bilirubin, *BNP* brain natriuretic peptide.

Variables	Derivation cohort (*n* = 194)	Validation cohort (*n* = 185)	*p* value
HCO_3^- (mmol/L)	25.0 (22.9, 27.0)	24.5 (23.0, 26.2)	0.517
WBC (* 10^6)	8.9 (5.5, 11.8)	8.8 (5.8, 12.0)	0.190
Hemoglobin (g/L)	91 (83, 107)	92 (82, 106)	0.385
ALT (U/L)	65 (21, 216)	46 (16, 269)	0.459
AST (U/L)	106 (28, 505)	120 (24, 567)	0.353
TBIL (μmol/L)	36.1 (14.0, 90.4)	28.7 (13.0, 103.7)	0.619
BNP (pg/mL)	86.0 (45.0, 168.5)	74.0 (42.0, 155.0)	0.573
AKI diagnosed by UO criteria	65 (33.5)	64 (34.6)	0.822
AKI classification			
Stage 1	104 (53.6)	94 (38.7)	0.588
2	58 (29.9)	58 (44.0)	0.526
3	32 (16.5)	33 (17.8)	0.713
Outcomes			
Renal recovery in 7 days	115 (60.9)	105 (62.3)	0.677
Need of RRT in ICU	35 (18.1)	30 (19.3)	0.583
Hospital mortality	33 (9.2)	33 (8.6)	0.893
30-day mortality	34 (13.3)	42 (12.9)	0.393

Values are median (interquartile range) or n (%), *AKI* acute kidney injury, *BMI* body mass index, *APACHE II* acute physiology and chronic health evaluation, *SOFA* sequential of organ failure assessment, *CKD* chronic kidney disease, *UO* urine output, *RRT* renal replacement therapy, *WBC* white blood cell, *ALT* alanine transaminase, *AST* aspartate transaminase, *TBIL* total bilirubin, *BNP* brain natriuretic peptide.

Characteristics and outcomes of AKI patients with and without renal recovery in derivation cohort

In the derivation cohort, 115 (59.3 %) patients had renal recovery from AKI onset and 79 (40.7 %) patients suffered from non-recovery. There were no significant differences of demographic characteristics and the comorbidities showed in patients with and without renal recovery. However, the APACHE II score and nonrenal SOFA score were observed remarkably higher in patients who failed to recover compared with those recovery patients. Moreover, PaO_2/FiO_2, use of vasopressor, AKI diagnosed by UO criteria, persistent

AKI and AKI stage 2–3 showed significant statistical difference between patients with and without renal recovery. AKI diagnosed by UO criteria, AKI stage 2–3, APACHE II score and nonrenal SOFA score were independently risk factors for non-recovery of renal function in sequentially multivariate logistic regression. Significant difference of the biomarker concentrations of urinary [TIMP-2]*[IGFBP7], TIMP-2 and IGFBP7 on day 0 were observed. Recovery patients showed the concentrations as 0.3 (0.1, 0.6) $((ng/mL)^2/1000)$, 3.3 (2.2, 6.3) ng/ml, and 35.2 (20.0, 90.0) ng/ml, respectively. Whereas, patients failing to recover showed higher concentrations of 1.1 (0.2, 5.5) $((ng/mL)^2/1000)$, 8.5 (3.5, 21.5) ng/ml and 100.9 (41.2, 329.1) ng/ml, respectively. Table 2 summarizes these characteristic comparisons of patients with and without renal recovery.

Table 2
Baseline characteristics between AKI patients with and without renal recovery in the derivation cohort

Variables	Recovery (*n* = 115)	Non-recovery (*n* = 79)	*p* value
Baseline characteristics			
Age (year)	62 (49, 76)	62 (50, 71)	0.483
Female gender	71 (61.7)	46 (58.2)	0.656
BMI (kg/m^2)	23.3 (20.5, 24.8)	22.6 (19.5, 23.9)	0.354
APACHE II score	14.0 (12.0, 16.0)	16.0 (14.0, 18.0)	< 0.001
Nonrenal SOFA score	4 (2, 7)	4.5 (1, 8)	< 0.001
Comorbidities	85.0 (73.0, 91.5)	89.0 (83.0, 100.0)	0.199
COPD/asthma	9 (7.8)	11 (13.9)	0.229
Cardiovascular disease	26 (22.6)	14 (17.7)	0.472
Chronic liver disease	26 (22.6)	16 (20.3)	0.726
Diabetes	28 (24.3)	21 (26.6)	0.739
Hypertension	50 (43.5)	41 (51.9)	0.305
CKD	6 (5.2)	4 (5.1)	1.000
Sepsis	51 (44.3)	38 (48.1)	0.661
Mechanical ventilation	96 (83.5)	64 (81.0)	0.703
PaO_2/FiO_2	316.0 (223.7, 404.0)	284.2 (210.8, 359.15)	0.026
Use of vasopressor	34 (29.6)	34 (43.0)	0.065
Use of diuresis	13 (11.3)	15 (19.0)	0.216
AKI diagnosed by UO criteria	30 (26.1)	35 (44.3)	0.002
AKI stage 2–3	39 (33.9)	49 (62.0)	< 0.001
Persistent AKI	38 (33.0)	53 (67.1)	< 0.001
[TIMP-2]*[IGFBP7] day 0 ($(ng/mL)^2/1000$)	0.3 (0.1, 0.6)	1.1 (0.2, 5.5)	< 0.001
TIMP-2 day 0 (ng/mL)	3.3 (2.2, 6.3)	8.5 (3.5, 21.5)	< 0.001

Values are median (interquartile range) or *n* (%), *AKI* acute kidney injury, *BMI* body mass index, *APACHE II* acute physiology and chronic health evaluation, *SOFA* sequential organ failure assessment, *COPD* chronic obstructive pulmonary disease, *CKD* chronic kidney disease, *UO* urine output, *TIMP-2* tissue inhibitor of metalloproteinases-2, *IGFBP-7* insulin-like growth factor-binding protein 7.

Variables	Recovery (*n* = 115)	Non-recovery (*n* = 79)	*p* value
IGFBP7 day 0 (ng/mL)	35.2 (20.0, 90.0)	100.9 (41.2, 329.1)	< 0.001
[TIMP-2]*[IGFBP7] day 1 ((ng/mL)^2/1000)	0.3 (0.1, 0.8)	0.4 (0.2, 1.8)	0.104
TIMP-2 day 1 (ng/mL)	3.6 (2.5, 6.8)	5.9 (2.8, 13.5)	0.591
IGFBP7 day 1 (ng/mL)	70.0 (40.6, 120.4)	73.2 (30.7, 179.5)	0.019

Values are median (interquartile range) or *n* (%), *AKI* acute kidney injury, *BMI* body mass index, *APACHE II* acute physiology and chronic health evaluation, *SOFA* sequential organ failure assessment, *COPD* chronic obstructive pulmonary disease, *CKD* chronic kidney disease, *UO* urine output, *TIMP-2* tissue inhibitor of metalloproteinases-2, *IGFBP-7* insulin-like growth factor-binding protein 7.

RRT was used in 4 (3.4) and 31 (39.2) patients in recovery and non-recovery patients, respectively. Duration of hospital stay was 24 (11.5–33.0) days in non-recovery patients, which was longer than recovery patients (18 [11.5–25.0] days, $p = 0.026$). Moreover, 30-day mortality was higher in non-recovery patients than recovery patients (24 [30.3 %] vs. 19 [16.5 %], $p = 0.018$). Table 3 shows the outcome comparisons.

Table 3
Outcomes between AKI patients with and without renal recovery in the derivation cohort

Variables	Recovery (*n* = 115)	Non-recovery (*n* = 79)	*p* value
RRT	4 (3.4)	31 (39.2)	< 0.001
ICU stay (day)	6 (4, 12)	7.5 (4, 14)	0.214
Hospital stay (day)	18 (11.5, 25)	24 (11.5, 33)	0.026
Hospital mortality	17 (14.8)	20 (25.3)	0.049
30-day mortality	19 (16.5)	24 (30.3)	0.018

Values are median (interquartile range) or *n* (%), *AKI* acute kidney injury, *ICU* intensive care unit, *RRT* renal replacement therapy.

Predicting non-recovery from AKI in the derivation cohort

AKI diagnosed by UO criteria, AKI stage 2–3, APACHE II score and nonrenal SOFA score were independently risk factors for non-recovery. There was positive linear correlation between APACHE II and nonrenal SOFA score (r = 0.567, $p < 0.001$). Therefore, nonrenal SOFA score with the better predictive value was included in the clinical risk prediction model. Four clinical risk prediction models were assessed. Model 1 consisted of AKI diagnosed by UO criteria, AKI stage 2–3 and nonrenal SOFA score; model 2 consisted of AKI diagnosed by UO criteria, AKI stage 2–3; model 3 consisted of AKI stage 2–3 and

nonrenal SOFA score; model 4 consisted of AKI diagnosed by UO criteria and nonrenal SOFA score. Among them, clinical risk prediction model 1 achieved the best AUC of 0.722 (95% CI 0.640–0.802, $p < 0.001$) for predicting non-recovery from AKI.

Urinary [TIMP-2]*[IGFBP7] on day 0 showed the AUC of 0.751 (95 % CI 0.701–0.852, $p < 0.001$) for predicting non-recovery from AKI with the optimal cutoff value of 1.05 $((ng/mL)^2/1000)$. Moreover, TIMP-2 and IGFBP7 on day 0 alone also showed useful predictive value for non-recovery, with the AUC of 0.744 (95 % CI 0.688–0.850, $p < 0.001$) and 0.721 (95 % CI 0.537–0.806, $p = 0.037$), respectively. However, the biomarkers of urinary [TIMP-2]*[IGFBP7], TIMP-2 and IGFBP7 on day 1 performed poorly for predicting non-recovery, respectively. When [TIMP-2]*[IGFBP7] on day 0 combined with clinical risk prediction model 1 to predict non-recovery, the power was significantly improved. It yielded the best predictive AUC of 0.852 (95 % CI 0.750–0.891, $p < 0.001$), confirmed by Hosmer and Lemeshow' s test ($p > 0.05$). The AUCs of (TIMP-2 day 0) - and (IGFBP7 day 0) - clinical risk prediction model 1 were 0.822 (95 % CI 0.744–0.900, $p < 0.001$) and 0.805 (95 % CI 0.725–0.886, $p < 0.001$), respectively. The predictive value of ([TIMP-2]*[IGFBP7] day 0) - clinical risk prediction model 1 was superior to (TIMP-2 day 0) - and (IGFBP7 day 0) - clinical risk prediction model 1 in predicting non-recovery from AKI, which was supported by Delong test ([TIMP-2]*[IGFBP7] vs. TIMP-2 $p = 0.032$, [TIMP-2]*[IGFBP7] vs. IGFBP7 $p = 0.026$, respectively), NRI ([TIMP-2]*[IGFBP7] vs. TIMP-2 $p = 0.041$, [TIMP-2]*[IGFBP7] vs. IGFBP7 $p = 0.027$) and IDI analysis ([TIMP-2]*[IGFBP7] vs. TIMP-2 $p = 0.019$, [TIMP-2]*[IGFBP7] vs. IGFBP7 $p = 0.002$). Multivariate logistic regression analysis calculated the probability for non-recovery basing on ([TIMP-2]*[IGFBP7] day 0) - clinical risk prediction model 1: the probability for non-recovery = $1/(1 + e^{-z})$, z = -2.451 + 0.397 * ([TIMP-2]*[IGFBP7] day 0) + 0.060 * nonrenal SOFA score + 1.043 * AKI diagnosed by UO criteria + 0.978 * AKI stage 2–3. The optimal cutoff probability value was 0.290. AKI patients who had a probability value more than 0.290 may fail to recover. Table 4 shows the predictive performances of the biomarkers and combination models, their ROC curves are presented in Fig. 2.

Table 4
Biomarkers and combination models for predicting non-recovery from AKI

	AUC (95 % CI)	Cutoff value	*p* value
[TIMP-2]*[IGFBP7] day 0 ((ng/mL)^2/1000)	0.751 (0.701, 0.852)	1.05	< 0.001
TIMP-2 day 0 (ng/mL)	0.744 (0.688, 0.850)	8.50	< 0.001
IGFBP7 day 0 (ng/mL)	0.721 (0.623, 0.820)	117.60	< 0.001
[TIMP-2]*[IGFBP7] day 1 ((ng/mL)^2/1000)	0.668 (0.551, 0.785)	0.89	0.028
TIMP-2 day 1 (ng/mL)	0.653 (0.499, 0.726)	7.50	0.050
IGFBP7 day 1 (ng/mL)	0.603 (0.482, 0.725)	144.90	0.163
Clinical risk prediction model 1	0.722 (0.640, 0.802)	0.436	< 0.001
Clinical risk prediction model 2	0.679 (0.603, 0.754)	0.256	< 0.001
Clinical risk prediction model 3	0.695 (0.619, 0.771)	0.231	< 0.001
Clinical risk prediction model 4	0.675 (0.598, 0.751)	0.254	< 0.001
([TIMP-2]*[IGFBP7] day 0) -clinical risk prediction model 1	0.852 (0.750, 0.891)	0.290	< 0.001
(TIMP-2 day 0) -clinical risk prediction model 1	0.822 (0.744, 0.900)	0.224	< 0.001
(IGFBP7 day 0) -clinical risk prediction model 1	0.805 (0.725, 0.886)	0.180	< 0.001
([TIMP-2]*[IGFBP7] day 0) -clinical risk prediction model 2	0.826 (0.770, 0.883)	0.198	< 0.001
(TIMP-2 day 0) -clinical risk prediction model 2	0.818 (0.760, 0.877)	0.214	< 0.001

Clinical risk prediction model 1 consisting of AKI diagnosed by UO criteria, AKI stage 2–3 and nonrenal SOFA score; Clinical risk prediction model 2 consisting of AKI diagnosed by UO criteria, AKI stage 2–3; Clinical risk prediction model 3 consisting of AKI stage 2–3 and nonrenal SOFA score; Clinical risk prediction model 4 consisting of AKI diagnosed by UO criteria and nonrenal SOFA score. *AUC* area under the receiver operating characteristic, *CI* confidence interval, *AKI* acute kidney injury, *TIMP-2* tissue inhibitor of metalloproteinases-2, *IGFBP-7* insulin-like growth factor-binding protein 7.

	AUC (95 % CI)	Cutoff value	*p* value
(IGFBP7 day 0) -clinical risk prediction model 2	0.803 (0.742, 0.864)	0.190	< 0.001
([TIMP-2]*[IGFBP7] day 0) -clinical risk prediction model 3	0.818 (0.759, 0.878)	0.188	< 0.001
(TIMP-2 day 0) -clinical risk prediction model 3	0.806 (0.744, 0.868)	0.178	< 0.001
(IGFBP7 day 0) -clinical risk prediction model 3	0.788 (0.722, 0.853)	0.196	< 0.001
([TIMP-2]*[IGFBP7] day 0) -clinical risk prediction model 4	0.792 (0.728, 0.857)	0.215	< 0.001
(TIMP-2 day 0) -clinical risk prediction model 4	0.790 (0.725, 0.854)	0.224	< 0.001
(IGFBP7 day 0) -clinical risk prediction model 4	0.776 (0.710, 0.842)	0.192	< 0.001

Clinical risk prediction model 1 consisting of AKI diagnosed by UO criteria, AKI stage 2–3 and nonrenal SOFA score; Clinical risk prediction model 2 consisting of AKI diagnosed by UO criteria, AKI stage 2–3; Clinical risk prediction model 3 consisting of AKI stage 2–3 and nonrenal SOFA score; Clinical risk prediction model 4 consisting of AKI diagnosed by UO criteria and nonrenal SOFA score. *AUC* area under the receiver operating characteristic, *CI* confidence interval, *AKI* acute kidney injury, *TIMP-2* tissue inhibitor of metalloproteinases-2, *IGFBP-7* insulin-like growth factor-binding protein 7.

Predictive accuracy of urinary [TIMP-2]*[IGFBP7] for predicting non-recovery from AKI in the validation cohort

In the validation cohort, 79/194 (40.7 %) patients failed to recover from AKI. The predictive accuracy was assessed in the validation cohort using cutoff values acquired in the derivation cohort. The urinary [TIMP-2]*[IGFBP7] on day 0 showed the best predictive accuracy for non-recovery than urinary TIMP-2 and IGFBP7 alone, with the sensitivity, specificity, PPV, NPV and their 95 % CIs of 82.3 % (67.4, 93.8), 76.9 % (72.4, 88.5), 65.0 % (43.2, 78.6) and 88.5 % (76.3, 95.8), respectively. When urinary [TIMP-2]*[IGFBP7] on day 0 combined with clinical risk prediction model 1, the predictive accuracy was improved. The sensitivity, specificity, PPV and NPV increased to 88.8 % (72.9, 98.7), 86.2 % (70.4, 97.3), 80.0 % (65.9, 92.5) and 92.6 % (80.8, 100.0), respectively. The assessment of predictive accuracy for non-recovery is shown in Table 5.

Table 5
Predictive accuracy of the biomarkers for non-recovery

	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
[TIMP-2]*[IGFBP7] day 0 ((ng/mL)^2/1000)	1.05	82.3 (67.4, 93.8)	76.9 (72.4, 88.5)	65.0 (43.2, 78.6)	88.5 (76.3, 95.8)
Clinical risk prediction model 1	0.436	77.1 (62.5, 92.3)	76.4 (60.2, 91.0)	67.5 (50.3, 81.4)	84.8 (65.3, 94.3)
([TIMP-2]*[IGFBP7] day 0) -clinical risk prediction model 1	0.290	88.8 (72.9, 98.7)	86.2 (70.4, 97.3)	80.0 (65.9, 92.5)	92.6 (80.8, 100.0)

TIMP-2 tissue inhibitor of metalloproteinases-2, *IGFBP-7* insulin-like growth factor-binding protein 7, *PPV* positive predictive value, *NPV* negative predictive value.

Discussion

AKI remains a common and serious clinical syndrome in critically ill patients. It is well recognized an episode of AKI may have persistent impairment in renal function, with the potential to progress to CKD, use of RRT and end-stage kidney disease (ESKD) with dialysis dependence, which is in turn strongly associated with increased short- and long-term mortality [3, 4]. Therefore, renal recovery after an episode of AKI is necessary. Urinary [TIMP-2]*[IGFBP7] was identified to be used for risk stratification in high-risk patients for AKI [8]. The current study evaluated the ability of urinary [TIMP-2]*[IGFBP7] for predicting failure to recover after AKI development. The main findings were: 1) all of urinary [TIMP-2]*[IGFBP7], TIMP-2 and IGFBP7 on day 0 showed useful values for predict non-recovery from AKI, but urinary [TIMP-2]*[IGFBP7] was superior to TIMP-2 and IGFBP7 alone; 2) urinary [TIMP-2]*[IGFBP7], TIMP-2 and IGFBP7 on day 1 performed poorly for predicting AKI recovery; 3) urinary [TIMP-2]*[IGFBP7] was validated to be able to help clinicians recognize the patients who failed to recover early at the time diagnosing AKI, with the sensitivity and specificity of 82.3 % and 76.9 %, respectively; 4) when adding urinary [TIMP-2]*[IGFBP7] on day 0 to clinical risk prediction model 1, the predictive value was greatly improved to 0.852. The utility of ([TIMP-2]*[IGFBP7] day 0) - clinical risk prediction model 1 was confirmed in the diverse critically ill patients, with the sensitivity and specificity of 88.8 % and 92.6 %, respectively. 5) non-recovery patients had worse short-term prognosis compared with recovery patients.

Two novel biomarkers, urine TIMP-2 and IGFBP7, are inducers of the G1 cell cycle arrest found in renal tubular cells. Study by Kashani K, et al [8] firstly identified their ability to predict the development of KDIGO stage 2 or 3 AKI within 12 hours in high-risk patients. Many other studies subsequently confirmed the effectively predictive value for detection of AKI. And our meta-analysis showed the same conclusion [19]. AKI is associated with the mechanisms of inflammation, oxidative stress, and apoptosis in cellular

and molecular pathways [20, 21], and AKI may occur following ischemic or toxic insults. TIMP-2 and IGFBP7 participate in these mechanisms and reflect early damage of the kidney [22]. Most studies evaluated the prediction for AKI development, only few estimated the prognostic value in AKI patients.

In study by Pilarczyk K, et al [18] patients with AKI 2 or 3 showed significantly higher values for [TIMP-2]* [IGFBP7] than patients with AKI 0–1. And another study showed higher median values of [TIMP-2]* [IGFBP7] were associated with an increased degree of renal injury, and patients requiring RRT had the highest median [TIMP-2]*[IGFBP7] test results, which illustrated that the degree of early cellular damage was associated with the severity of the functional impairment [23]. The study further assessed the value of [TIMP-2]*[IGFBP7] for RRT and 28-day death, and the result showed the AUC for use of RRT was 0.83 and for 28-day mortality was 0.77 [23]. Study by Dewitte A, et al [24] enrolled 57 consecutive patients presenting with AKI within the first 24 hours after admission. They found urinary [TIMP-2]*[IGFBP7] had a useful prediction for renal recovery within 48 hours after AKI. Recently, Cho WY, et al [7] conducted a single-center study prospectively enrolling 124 patients diagnosed with AKI. The results showed urine TIMP-2/IGFBP7 could serve as a biomarker for predicting renal recovery. We enlarged population including consecutive AKI patients in two Chinese ICUs and conducted two cohorts to derivate and validate the utility of urinary [TIMP-2]*[IGFBP7] for predicting patients who failed to recover within 7 days. Urinary [TIMP-2]*[IGFBP7] on day 0 showed a useful predictive value for non-recovery. When it was added to clinical risk prediction model 1 consisting of AKI diagnosed by UO criteria, AKI stage 2–3 and nonrenal SOFA score, the performance for predicting nonrecovery improved to be good.

In previous study, many risk factors could contribute to non-recovery after AKI, such as age,

comorbidity, more severe AKI, The severity of extrarenal organ dysfunction and so on [5, 13, 24]. Our study did not show the difference of age and comorbidity between patients with and without recovery, but more severe AKI and higher nonrenal SOFA score were observed in non-recovery patients.

Notably, the variable of AKI diagnosed by UO criteria played an important role in the prediction model for non-recovery of AKI. In this study, 129 (34.0 %) patients showed oliguria (reaching UO criteria for AKI diagnosis) and were diagnosed AKI by UO. Oliguria was of the oldest “biomarkers” of AKI, which may occur following a normal physiological response or reflecting an underlying pathological process [25]. Many different pathophysiological pathways may cause oliguria, such as the neuro-hormonal pathway, absolute (hypovolemia), and relative (hemodynamic perturbations) reductions in effective blood volume [26]. Moreover, renal blood flow (RBF) may be preserved or even be increased in sepsis-associated AKI. In this situation, abnormal distribution of intra-renal blood flow may be more influential than global RBF [27]. Besides circulatory changes, immunologic and inflammatory mechanisms may participate in renal endothelial injury and microvascular dysfunction, which may lead to oliguria [27]. In study by Federspiel CK, et al [28], a UO < 0.5 ml/kg/h was associated with lower rates of resolving AKI (Hazard Ratio 0.31; 95% CI 0.20–0.47). This was also found in another study that enrolled 264 patients with severe cardiac surgery-associated AKI (CS-AKI) requiring RRT. The result showed significantly fewer patients with oliguria recovered renal function [29]. Therefore, including the clinical factor of AKI diagnosed by UO

criteria for prediction of renal recovery was reasonable. In general, urinary [TIMP-2]*[IGFBP7] combining with the easily available clinical factors of AKI diagnosed by UO criteria, AKI stage 2–3 and nonrenal SOFA score performed well to distinguish the patients who would fail to recover from AKI at an early time, which would be feasible and helpful in clinic.

Despite several meaningful findings, our study has several limitations. We did not distinguish AKI causes before detecting urinary [TIMP-2]*[IGFBP7] and predicting non-recovery in this study. AKI with different causes has different mechanisms for renal injury. It would be more accurate if the predictive value of urinary [TIMP-2]*[IGFBP7] was tested in AKI following the same mechanism of development. Furthermore, we assessed the short-term prognosis, but did not explore long-term prognosis. It would also be helpful for clinic to explore the association between urinary [TIMP-2]*[IGFBP7] with long-term prognosis of AKI.

Conclusion

Urinary [TIMP-2]*[IGFBP7] represents a sensitive and specific biomarker to predict failure to recover from AKI. The predictive accuracy can be improved when urinary [TIMP-2]*[IGFBP7] combines with clinical factors of AKI diagnosed by UO criteria, AKI stage 2-3 and nonrenal SOFA score.

Declarations

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Availability of data and materials

All data generated and/or analyzed during this study are included in this published article.

Authors' contributions9

H-MJ contributed to urine collection, data interpretation, drafting of the manuscript and critical revision of the manuscript. LC, Y-BE, XZ, J-YW and Y-JJ contributed to urine collection, data interpretation and performed statistical analysis. XX, S-YG, C-DC, F-XG and Y-ZH contributed to data collection and data interpretation. W-XL chaired the group, conceived and designed the study, performed statistical analysis and contributed to data collection, data interpretation, and critical revision of the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Human Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University (Beijing, China), the ethics number was 2018-117. Informed consent from patients or their next of kin was wrote before patients participated in this study.

Consent for publication

The manuscript has been read and its submission approved by all co-authors.

Competing interests

The authors declare that they have no competing interests.

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Figures

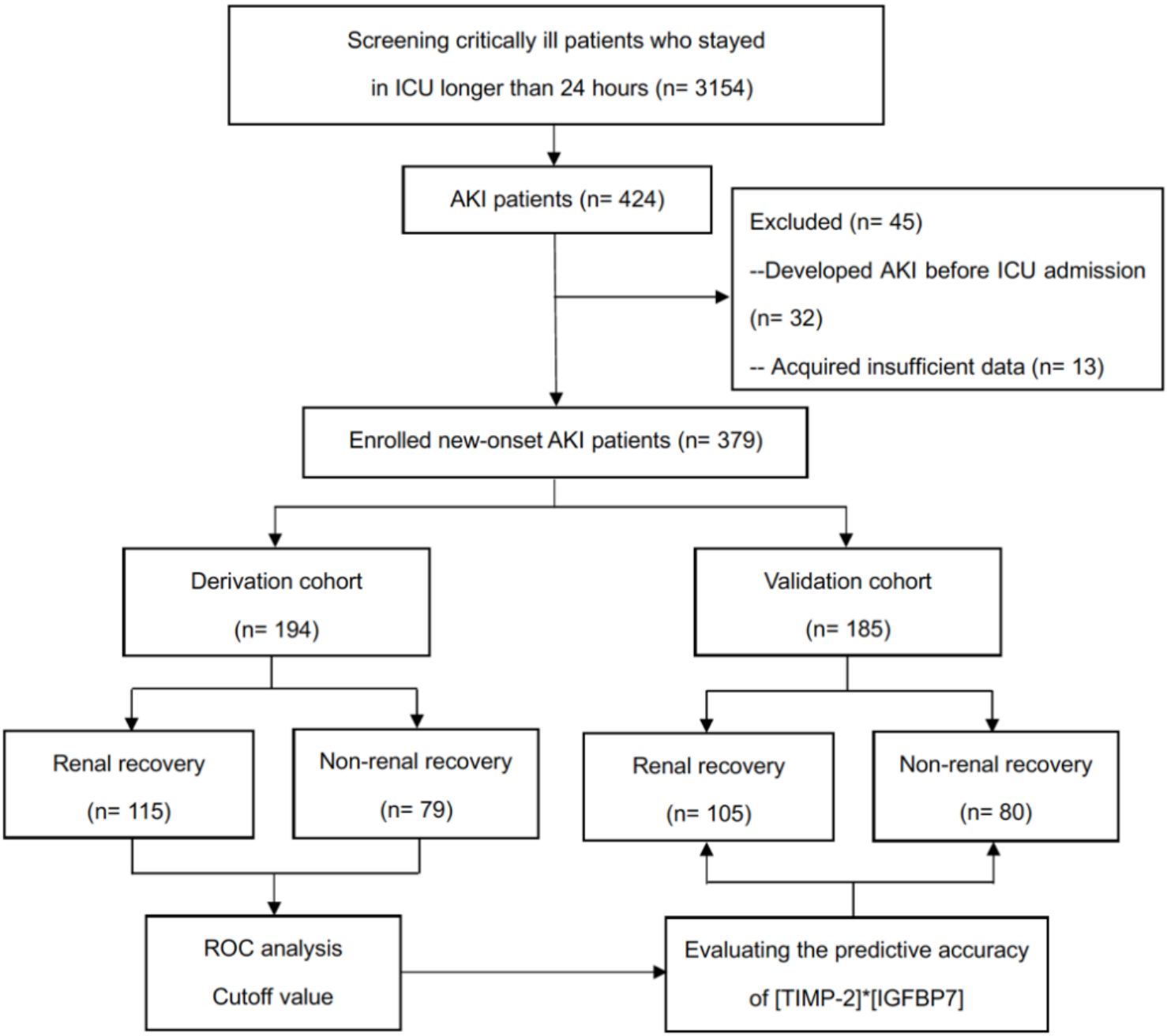

Figure 1

Study flow diagram. AKI acute kidney injury, ICU intensive care unit, ROC receiver operating characteristic.

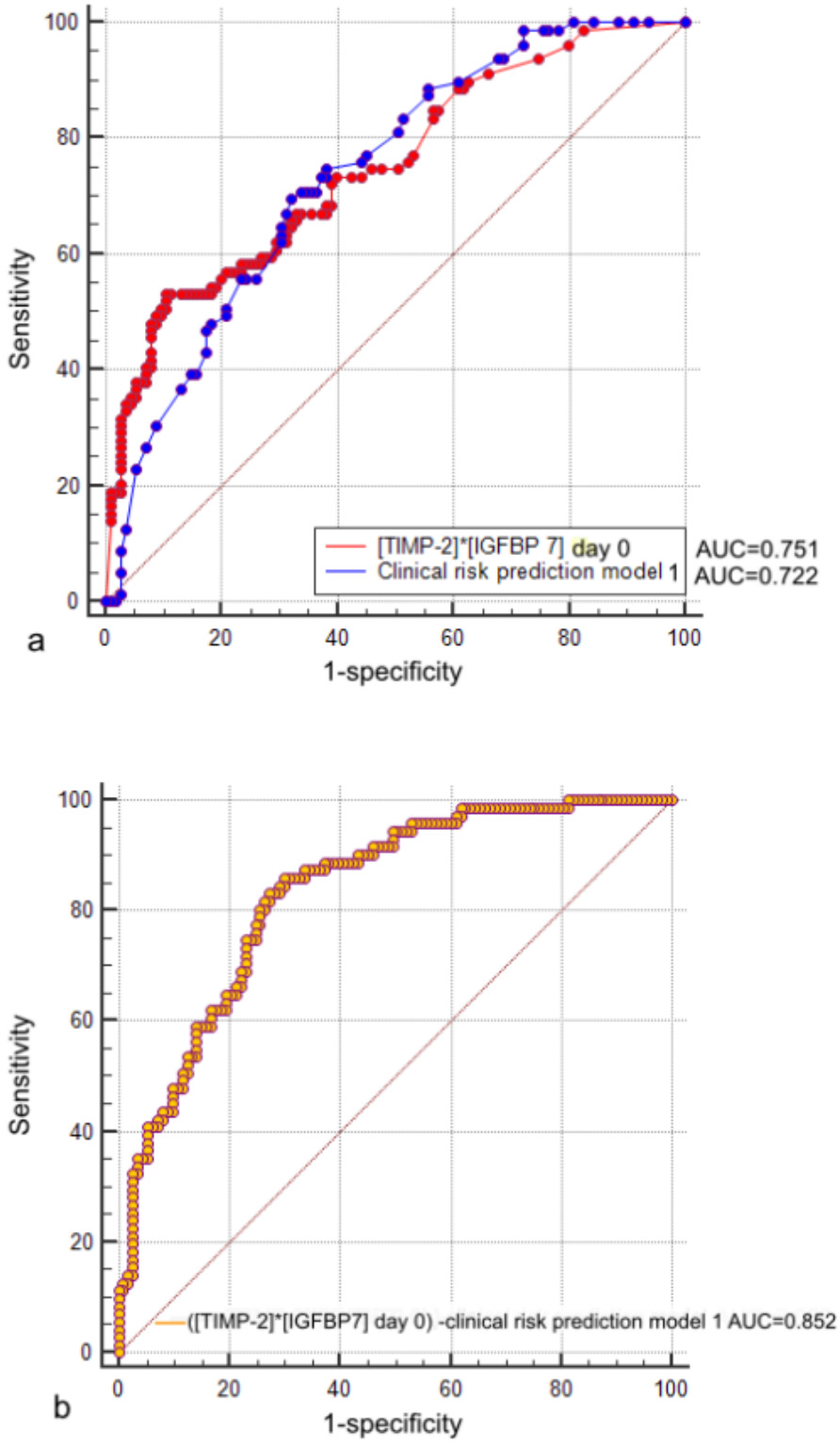

Figure 2

The predictive value of biomarker and the corresponding model. The ROC curves of urinary [TIMP-2]*[IGFBP7] on day 0 and the corresponding model for predicting failure to recover from AKI in the derivation cohort. (a) The AUC of urinary [TIMP-2]*[IGFBP7] on day 0 and clinical risk prediction model 1. (b) The AUC of ([TIMP-2]*[IGFBP7] day 0) - clinical risk prediction model 1. ROC receiver operating characteristic, AUC area under the ROC.