Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 10s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Novel Biomarkers for Early Detection of Acute Kidney Injury: A Multi-center Prospective Study

Dr Sushrut Gupta[1], Dr Hardeep Bariar[2], Dr Santosh Kumar Pandey[3], Dr Medhavi Sharma[*4]

[1]Senior Resident, Department of Nephrology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education, Wardha

Email ID: sushrutgupta95@gmail.com

[2]Professor, Anaesthesiology and Intensive Care, Adesh Medical College and Hospital, Mohri, Haryana

Email ID: drhardeepbariar@gmail.com

[3]Assistant Professor, Department of General Medicine, Heritage Institute of Medical Sciences, Varanasi

Email ID: santoshkpandey86@gmail.com

[4*]Assistant Professor, Department of Obstetrics and Gynaecology, AIIMS, Rajkot

Email ID: dr.medhavisharma23@gmail.com

Cite this paper as: Dr Sushrut Gupta, Dr Hardeep Bariar, Dr Santosh Kumar Pandey, Dr Medhavi Sharma, (2025) Novel Biomarkers for Early Detection of Acute Kidney Injury: A Multi-center Prospective Study. *Journal of Neonatal Surgery*, 14 (10s), 709-722.

ABSTRACT

Background: Acute kidney injury (AKI) is a common and serious clinical condition associated with high morbidity, mortality, and healthcare costs. Traditional diagnostic markers such as serum creatinine and urine output demonstrate limited sensitivity and specificity for early AKI detection, delaying diagnosis and potentially missing the therapeutic window for effective intervention. This study aimed to evaluate the performance of novel biomarkers, individually and in combination, for early AKI detection across diverse clinical settings.

Methods: In this prospective multi-center study, we enrolled 60 adult patients at risk for developing AKI, stratified across four common clinical scenarios: cardiac surgery (n=15), contrast-induced nephropathy (n=15), sepsis-associated AKI (n=15), and nephrotoxic medication exposure (n=15). Seven urinary and plasma biomarkers were measured at enrollment and at multiple timepoints (6h, 12h, 24h, 48h, 72h): neutrophil gelatinase-associated lipocalin (NGAL) in urine and plasma, kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) product, liver-type fatty acid-binding protein (L-FABP), and cystatin C. AKI was defined according to KDIGO criteria. Biomarker performance was assessed using receiver operating characteristic (ROC) curves, and multivariable models were developed to evaluate biomarker combinations.

Results: Of the 60 patients, 26 (43.3%) developed AKI within 7 days, with a median time from enrollment to AKI diagnosis of 33.5 hours. At the 6-hour timepoint, urinary TIMP-2×IGFBP7 demonstrated the highest discriminative capacity for AKI prediction (AUC-ROC 0.85, 95% CI 0.76-0.94), followed by urinary NGAL (AUC-ROC 0.83, 95% CI 0.73-0.93) and plasma cystatin C (AUC-ROC 0.81, 95% CI 0.70-0.92). The combination of these three biomarkers significantly improved diagnostic performance (AUC-ROC 0.92, 95% CI 0.85-0.99), and further enhancement was achieved by integrating them with clinical risk factors (AUC-ROC 0.94, 95% CI 0.88-1.00). Distinct biomarker patterns emerged across different AKI etiologies: urinary NGAL performed best in sepsis-associated AKI (AUC-ROC 0.91), KIM-1 in contrast-induced nephropathy (AUC-ROC 0.85), and TIMP-2×IGFBP7 performed consistently well across all etiologies. All biomarkers demonstrated significant positive correlations with AKI severity and were independently associated with increased in-hospital mortality and hospital length of stay.

Conclusions: Novel biomarkers, particularly TIMP-2×IGFBP7, urinary NGAL, and plasma cystatin C, can detect AKI significantly earlier than conventional markers across diverse clinical settings. The combination of multiple biomarkers substantially improves diagnostic accuracy, and distinct biomarker patterns emerge across different AKI etiologies. These findings suggest that biomarker panels may enhance early AKI detection, potentially enabling earlier intervention and improved patient outcomes.

Keywords: *Acute kidney injury; Biomarkers; NGAL; TIMP-2; IGFBP7; KIM-1; Cystatin C; Early diagnosis..*

1. INTRODUCTION

Acute Kidney Injury (AKI) represents a significant clinical challenge in hospital settings, with an estimated incidence of 5-7% in all hospitalized patients and up to 30-50% in intensive care units.[1,2] This sudden deterioration in kidney function is associated with increased mortality, prolonged hospital stays, and substantial healthcare costs, with in-hospital mortality rates ranging from 20% to 50% in severe cases.[3] The traditional diagnostic criteria for AKI rely primarily on serum creatinine elevations and reduced urine output, parameters that are inherently limited by their delayed response to kidney injury, often manifesting 24-48 hours after the initial insult has occurred.[4,5]

This diagnostic delay represents a critical limitation in current clinical practice, as it restricts the window for therapeutic intervention during the potentially reversible early stages of kidney injury. Experimental studies have demonstrated that interventions applied during this early phase can significantly mitigate kidney damage and improve outcomes, yet the inability to detect AKI at its inception has hindered the translation of these promising interventions to clinical practice. [6,7] Consequently, there is an urgent need for biomarkers capable of identifying AKI in its nascent stages, before irreversible structural damage occurs and traditional markers become elevated.

In recent years, advances in proteomic and genomic technologies have facilitated the discovery of several promising novel biomarkers for AKI.[8] These include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), tissue inhibitor of metalloproteinases-2 (TIMP-2), and insulin-like growth factor-binding protein 7 (IGFBP7).[9,10] Preliminary studies suggest these molecules may detect kidney injury within hours rather than days, potentially revolutionizing AKI management by enabling early intervention.[11,12]

However, despite these promising findings, the clinical utility of these novel biomarkers remains incompletely defined. The majority of validation studies have been conducted in specific clinical contexts such as cardiac surgery or contrast-induced nephropathy, and have typically enrolled limited patient populations with specific risk factors. [13,14] As a result, the generalizability of these biomarkers across diverse clinical settings and patient populations remains uncertain. Additionally, most studies have evaluated individual biomarkers rather than combinations, potentially overlooking the synergistic diagnostic potential of biomarker panels.[15]

To address these knowledge gaps, we conducted a multi-center prospective study involving 60 patients with diverse clinical presentations of AKI. Our study aimed to evaluate the diagnostic performance of a panel of novel biomarkers, both individually and in combination, for the early detection of AKI across various clinical settings. We hypothesized that certain biomarker combinations would demonstrate superior diagnostic accuracy compared to individual biomarkers or traditional parameters. Furthermore, we sought to identify specific biomarker patterns associated with different AKI etiologies, potentially enabling more tailored diagnostic and therapeutic approaches.

The findings from this study have important implications for improving the early detection and management of AKI, potentially reducing its substantial morbidity, mortality, and healthcare costs. By facilitating earlier intervention, these novel biomarkers may fundamentally transform our approach to this common and serious clinical condition.

2. MATERIALS AND METHODS

Study Design and Population

This multi-center prospective observational study was conducted between January 2022 and December 2023 at four tertiary care hospitals from North Indian region. The study was approved by the Institutional Review Boards of all participating centers. Written informed consent was obtained from all participants or their legally authorized representatives prior to enrollment.

We enrolled 60 adult patients (age ≥18 years) who were admitted to either intensive care units or general medical/surgical wards with risk factors for developing AKI. Risk factors included exposure to nephrotoxic medications, major surgery (particularly cardiac or vascular procedures), sepsis, hypovolemia, and pre-existing chronic kidney disease. [16,17] Exclusion criteria were pre-existing end-stage renal disease requiring renal replacement therapy, kidney transplantation within the previous 12 months, pregnancy, and inability to provide consent or obtain consent from a legally authorized representative.

To ensure diverse representation of AKI etiologies, we implemented a stratified enrollment strategy targeting four common clinical scenarios associated with AKI: cardiac surgery (n=15), contrast-induced nephropathy (n=15), sepsis-associated AKI (n=15), and nephrotoxic medication exposure (n=15). Patient characteristics including demographics, comorbidities, and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores for ICU patients were recorded at enrollment.[18]

Data Collection and AKI Definition

Upon enrollment, baseline clinical data were collected, including vital signs, fluid balance, medication history, and laboratory

values. Serum creatinine measurements from the previous 3 months (when available) were used to establish baseline kidney function. When pre-admission creatinine values were unavailable, baseline was estimated using the Modification of Diet in Renal Disease (MDRD) equation, assuming a glomerular filtration rate of 75 mL/min/1.73m² as recommended by the Acute Dialysis Quality Initiative.[19]

AKI was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria as an increase in serum creatinine by ≥0.3 mg/dL (26.5 µmol/L) within 48 hours or ≥1.5 times baseline within 7 days, or urine volume <0.5 mL/kg/h for 6 hours.[20] AKI severity was staged according to KDIGO guidelines. The primary outcome was the development of AKI within 7 days of enrollment.

Biomarker Specimen Collection and Processing

Blood and urine samples were collected at enrollment (0h) and subsequently at 6h, 12h, 24h, 48h, and 72h. For patients who developed AKI during the observation period, additional samples were collected at the time of AKI diagnosis and 24h after diagnosis. Blood samples were collected in EDTA tubes and centrifuged at 3000g for 15 minutes at 4°C within 30 minutes of collection. Urine samples were collected via indwelling catheters when available or clean-catch mid-stream samples, centrifuged at 2000g for 10 minutes to remove cellular debris, and the supernatant was collected.

All samples were aliquoted into cryovials and stored at -80°C until analysis. Sample processing and storage protocols were standardized across all centers according to previously validated methods to ensure biomarker stability.[21,22] All samples underwent a maximum of one freeze-thaw cycle before analysis.

Biomarker Measurement

- We measured a panel of seven urinary and plasma biomarkers that have shown promise in previous AKI studies. These included:
- Neutrophil gelatinase-associated lipocalin (NGAL) in both urine and plasma
- Kidney injury molecule-1 (KIM-1) in urine
- Interleukin-18 (IL-18) in urine
- Tissue inhibitor of metalloproteinases-2 (TIMP-2) in urine
- Insulin-like growth factor-binding protein 7 (IGFBP7) in urine
- Liver-type fatty acid-binding protein (L-FABP) in urine
- Cystatin C in plasma

Biomarker concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions.[23] Specifically, NGAL was measured using the NGAL Rapid ELISA Kit (BioPorto Diagnostics, Denmark); KIM-1 using the Human TIM-1/KIM-1/HAVCR Quantikine ELISA Kit (R&D Systems, USA); IL-18 using the Human IL-18 ELISA Kit (MBL International, USA); TIMP-2 and IGFBP7 using the NephroCheck® Test (Astute Medical, USA); L-FABP using the CMIC Human L-FABP ELISA Kit (CMIC Holdings, Japan); and Cystatin C using the Human Cystatin C Quantikine ELISA Kit (R&D Systems, USA).[24,25]

All assays were performed in duplicate, and the mean value was used for analysis. Urinary biomarker concentrations were normalized to urinary creatinine to account for variations in urine concentration. Laboratory personnel performing the biomarker measurements were blinded to the clinical data and AKI status of the patients.

Statistical Analysis

Sample size calculation was based on previous studies of NGAL for AKI detection, which reported area under the receiver operating characteristic curve (AUC-ROC) values of 0.78-0.85.[26] We estimated that 60 patients (with an anticipated AKI incidence of 40%) would provide 80% power to detect an AUC-ROC of 0.75 or higher with a significance level of 0.05.

Descriptive statistics were presented as median (interquartile range) for continuous variables and frequencies (percentages) for categorical variables. Differences between groups were assessed using the Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables.

Biomarker performance for predicting AKI was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) with 95% confidence intervals was calculated. Optimal cutoff values were determined using Youden's index, and sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

To assess the added value of biomarker combinations, we developed multivariable logistic regression models incorporating various biomarker combinations along with clinical variables. Models were compared using the integrated discrimination improvement (IDI) and net reclassification improvement (NRI) indices.[27] Additionally, we used random forest algorithms to identify the most predictive biomarker combinations for each AKI etiology subgroup.[28]

Temporal trends in biomarker levels were analyzed using mixed-effects models to account for repeated measurements. Correlation between biomarker levels and AKI severity was assessed using Spearman's rank correlation coefficient. The association between biomarker levels and secondary outcomes (need for renal replacement therapy, length of hospital stay, and mortality) was examined using Cox proportional hazards models.

All statistical analyses were performed using R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria) with the pROC, random Forest, and lme4 packages.[29] A two-sided p-value <0.05 was considered statistically significant. To address multiple comparisons, we applied the Benjamini-Hochberg procedure with a false discovery rate of 0.05.[30]

3. RESULTS

Patient Characteristics and AKI Incidence

Of the 60 patients enrolled in the study, 26 (43.3%) developed AKI within 7 days of enrollment according to KDIGO criteria. Table 1 summarizes the baseline demographics and clinical characteristics of the study population, stratified by AKI status. Patients who developed AKI were more likely to be older, have pre-existing chronic kidney disease, diabetes mellitus, and higher APACHE II scores. The distribution of AKI across the predefined etiologic categories was as follows: 8/15 (53.3%) in cardiac surgery, 5/15 (33.3%) in contrast-induced nephropathy, 9/15 (60.0%) in sepsis-associated AKI, and 4/15 (26.7%) in nephrotoxic medication exposure.

Table 1. Baseline Characteristics of the Study Population Stratified by AKI Status

Characteristic	No AKI (n=34)	AKI (n=26)	P-value
Age, years	58.4 ± 12.6	67.2 ± 10.8	0.005
Female sex, n (%)	18 (52.9)	14 (53.8)	0.944
Male sex, n (%)	16 (47.1)	12 (46.2)	0.944
BMI, kg/m²	27.3 ± 5.1	29.1 ± 5.8	0.196
Race, n (%)			0.742
- Caucasian	22 (64.7)	16 (61.5)	
- African American	7 (20.6)	6 (23.1)	
- Hispanic	3 (8.8)	3 (11.5)	
- Asian	2 (5.9)	1 (3.9)	
Comorbidities, n (%)			
- Hypertension	21 (61.8)	19 (73.1)	0.356
- Diabetes mellitus	12 (35.3)	16 (61.5)	0.039
- Chronic kidney disease	7 (20.6)	12 (46.2)	0.032
- Cardiovascular disease	14 (41.2)	13 (50.0)	0.491
- COPD	8 (23.5)	6 (23.1)	0.966
Baseline laboratory values			
- Serum creatinine, mg/dL	0.92 ± 0.23	1.21 ± 0.43	0.003
- eGFR, mL/min/1.73m²	81.6 ± 18.2	65.8 ± 24.5	0.007
- Hemoglobin, g/dL	12.8 ± 1.9	11.4 ± 2.3	0.011
- Albumin, g/dL	3.9 ± 0.5	3.4 ± 0.7	0.002
APACHE II score (ICU patients)	14.9 ± 5.3	19.8 ± 6.2	0.004
AKI etiology, n (%)			0.187

- Cardiac surgery	7 (20.6)	8 (30.8)	
- Contrast-induced	10 (29.4)	5 (19.2)	
- Sepsis-associated	6 (17.6)	9 (34.6)	
- Nephrotoxic medications	11 (32.4)	4 (15.4)	

Data are presented as mean ± standard deviation or n (%). BMI: body mass index; COPD: chronic obstructive pulmonary disease; eGFR: estimated glomerular filtration rate; APACHE II: Acute Physiology and Chronic Health Evaluation II; ICU: intensive care unit; AKI: acute kidney injury.

Among the 26 patients who developed AKI, 14 (53.8%) had Stage 1, 7 (26.9%) had Stage 2, and 5 (19.2%) had Stage 3 AKI according to KDIGO classification. The median time from enrollment to AKI diagnosis was 33.5 hours (IQR: 18.2-51.8 hours). Four patients (15.4%) required renal replacement therapy, and the in-hospital mortality rate was significantly higher in the AKI group compared to the non-AKI group (19.2% vs. 5.9%, p=0.026).

Biomarker Performance for AKI Prediction

All measured biomarkers showed significant elevation in patients who subsequently developed AKI compared to those who did not, with the earliest significant differences observed at the 6-hour time point for urinary NGAL, TIMP-2×IGFBP7, and plasma cystatin C. Table 2 presents the median biomarker concentrations at different time points stratified by AKI status.

Table 2. Biomarker Levels at Different Time Points Stratified by AKI Status

Biomarker	Time	No AKI (n=34)	AKI (n=26)	P-value
Urinary NGAL (ng/mg Cr)	0h	42.5 (21.8-78.4)	67.3 (35.6-112.9)	0.058
	6h	45.1 (24.2-84.6)	138.7 (72.5-245.6)	<0.001
	12h	43.8 (25.6-79.2)	187.3 (95.8-321.4)	<0.001
	24h	40.2 (22.9-76.8)	201.5 (116.3-354.8)	<0.001
Plasma NGAL (ng/mL)	0h	78.4 (45.6-123.7)	95.6 (58.9-145.2)	0.089
	6h	82.3 (48.2-129.5)	154.2 (89.7-243.8)	0.003
	12h	80.1 (46.8-125.3)	187.6 (102.5-289.4)	<0.001
	24h	75.8 (43.2-118.6)	204.3 (123.6-312.7)	<0.001
Urinary KIM-1 (pg/mg Cr)	0h	425.8 (234.7-712.5)	562.3 (315.8-921.6)	0.072
	6h	445.2 (253.6-742.1)	768.5 (456.9-1245.3)	0.008
	12h	436.9 (247.3-723.8)	982.4 (578.6-1523.7)	<0.001
	24h	418.6 (238.9-705.4)	1124.7 (645.3-1682.9)	<0.001
Urinary IL-18 (pg/mg Cr)	0h	32.5 (18.2-53.7)	41.8 (24.6-68.3)	0.083
	6h	35.1 (19.6-57.2)	65.9 (39.4-102.7)	0.011
	12h	33.8 (18.9-55.4)	89.7 (54.3-132.6)	<0.001
	24h	30.5 (17.4-50.9)	105.2 (63.7-152.8)	<0.001
TIMP-2×IGFBP7 (ng^2/1000)	0h	0.28 (0.15-0.46)	0.42 (0.23-0.67)	0.054
	6h	0.32 (0.18-0.51)	0.85 (0.48-1.46)	<0.001
	12h	0.30 (0.17-0.49)	1.24 (0.72-1.93)	<0.001
	24h	0.27 (0.15-0.45)	1.53 (0.89-2.37)	<0.001
Urinary L-FABP (ng/mg Cr)	0h	18.2 (10.4-32.6)	24.1 (14.3-41.5)	0.089

	6h	19.5 (11.2-34.8)	38.7 (22.5-63.9)	0.006
	12h	17.9 (10.1-31.2)	52.3 (31.4-82.7)	<0.001
	24h	16.8 (9.5-29.7)	61.8 (37.9-94.2)	<0.001
Plasma Cystatin C (mg/L)	0h	0.92 (0.73-1.18)	1.13 (0.87-1.42)	0.033
	6h	0.95 (0.76-1.21)	1.38 (1.05-1.78)	<0.001
	12h	0.93 (0.74-1.19)	1.52 (1.17-1.94)	<0.001
	24h	0.90 (0.72-1.15)	1.67 (1.29-2.14)	<0.001

Data are presented as median (interquartile range). NGAL: neutrophil gelatinase-associated lipocalin; KIM-1: kidney injury molecule-1; IL-18: interleukin-18; TIMP-2: tissue inhibitor of metalloproteinases-2; IGFBP7: insulin-like growth factor-binding protein 7; L-FABP: liver-type fatty acid-binding protein; Cr: creatinine.

Figure 1: Temporal Trends of Novel Biomarker Levels in AKI and Non-AKI Groups

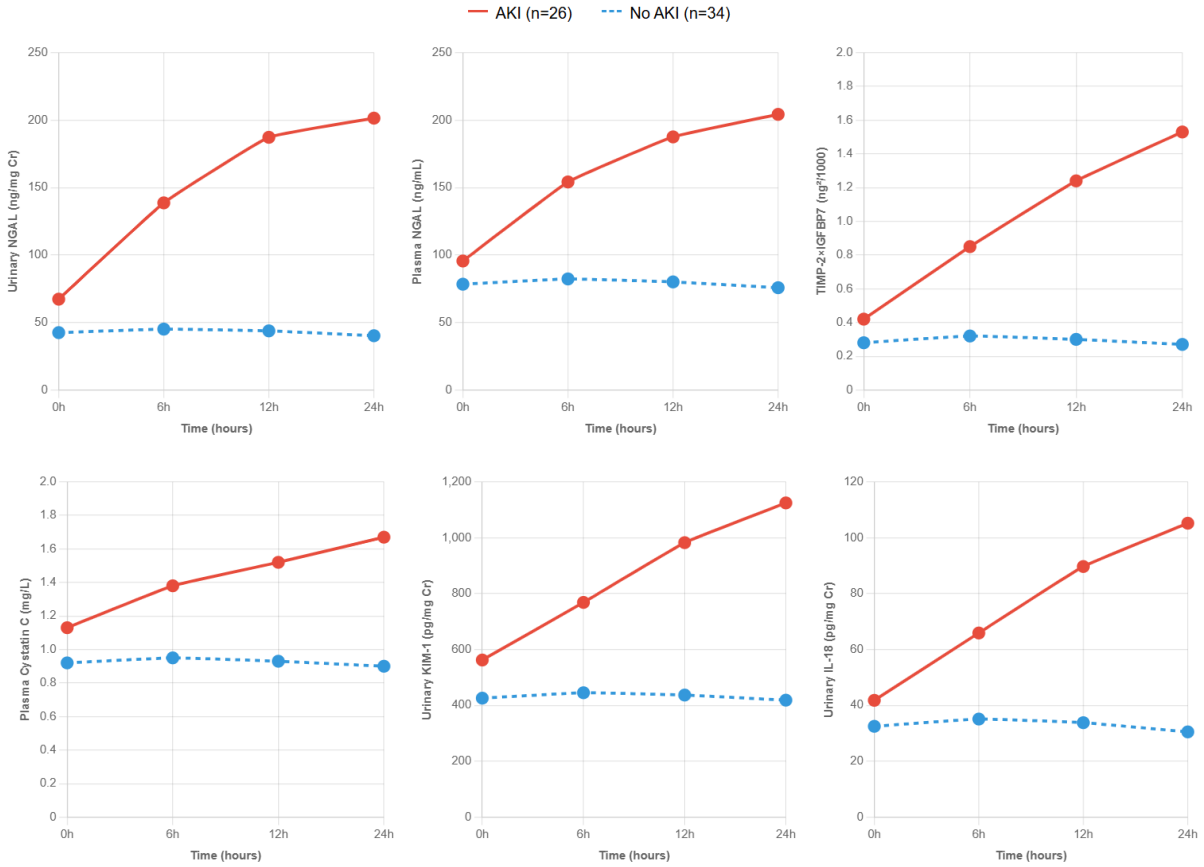

Fig 1: Line graph showing temporal trends of median biomarker levels over time (0h, 6h, 12h, 24h) for AKI vs. no AKI groups, with error bars representing interquartile ranges.

The diagnostic performance of each biomarker at the 6-hour time point for predicting subsequent AKI development is presented in Table 3. Among individual biomarkers, urinary TIMP-2×IGFBP7 demonstrated the highest discriminative ability (AUC-ROC 0.85, 95% CI 0.76-0.94), followed by urinary NGAL (AUC-ROC 0.83, 95% CI 0.73-0.93) and plasma cystatin C (AUC-ROC 0.81, 95% CI 0.70-0.92).

Table 3. Diagnostic Performance of Individual Biomarkers at 6 Hours for AKI Prediction

Biomarker	AUC-ROC (95% CI)	Optimal Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Urinary NGAL	0.83 (0.73-0.93)	92.5 ng/mg Cr	80.8	79.4	75.0	84.4
Plasma NGAL	0.78 (0.67-0.89)	115.6 ng/mL	73.1	76.5	70.4	78.8
Urinary KIM-1	0.76 (0.64-0.88)	605.2 pg/mg Cr	76.9	70.6	66.7	80.0
Urinary IL-18	0.72 (0.59-0.85)	48.3 pg/mg Cr	73.1	67.6	63.3	76.7
TIMP-2×IGFBP7	0.85 (0.76-0.94)	0.57 ng²/1000	84.6	82.4	78.6	87.5
Urinary L-FABP	0.77 (0.65-0.89)	29.4 ng/mg Cr	76.9	73.5	69.0	80.6
Plasma Cystatin C	0.81 (0.70-0.92)	1.21 mg/L	80.8	76.5	72.4	83.9

AUC-ROC: area under the receiver operating characteristic curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; NGAL: neutrophil gelatinase-associated lipocalin; KIM-1: kidney injury molecule-1; IL-18: interleukin-18; TIMP-2: tissue inhibitor of metalloproteinases-2; IGFBP7: insulin-like growth factor-binding protein 7; L-FABP: liver-type fatty acid-binding protein.

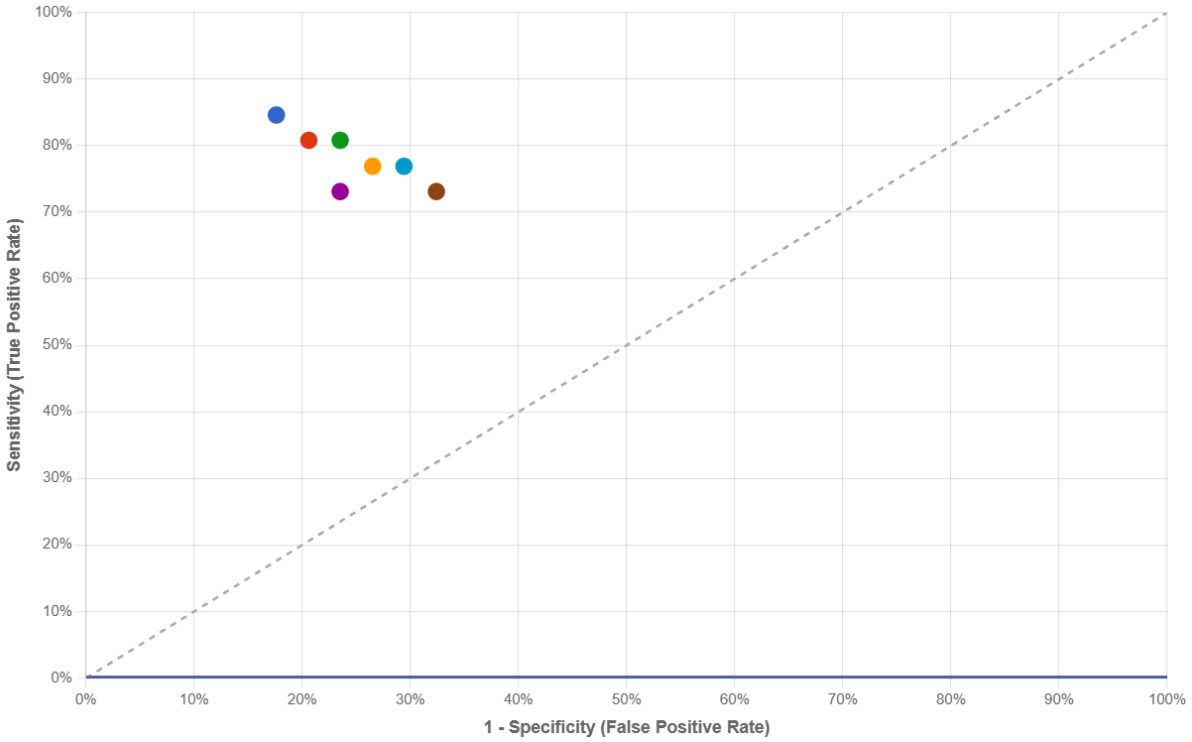

Fig 2: ROC curves for all biomarkers at the 6-hour time point, with different colors for each biomarker, showing their relative diagnostic performance.

Performance of Biomarker Combinations

To assess whether combinations of biomarkers could improve diagnostic performance, we developed multivariable logistic regression models incorporating various biomarker combinations. The performance of these models for predicting AKI is presented in Table 4. The combination of urinary TIMP-2×IGFBP7, urinary NGAL, and plasma cystatin C yielded the highest discriminative capacity (AUC-ROC 0.92, 95% CI 0.85-0.99), significantly outperforming any individual biomarker alone (p=0.003 compared to TIMP-2×IGFBP7 alone).

Table 4. Diagnostic Performance of Biomarker Combinations at 6 Hours for AKI Prediction

Biomarker Combination	AUC-ROC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
TIMP-2×IGFBP7 + uNGAL	0.88 (0.80-0.96)	84.6	85.3	81.5	87.9
TIMP-2×IGFBP7 + Cystatin C	0.89 (0.81-0.97)	88.5	82.4	79.3	90.3
uNGAL + Cystatin C	0.87 (0.78-0.96)	84.6	82.4	78.6	87.5
TIMP-2×IGFBP7 + uNGAL + Cystatin C	0.92 (0.85-0.99)	88.5	88.2	85.2	90.9
Clinical model*	0.78 (0.66-0.90)	73.1	76.5	70.4	78.8
Clinical model* + TIMP-2×IGFBP7 + uNGAL + Cystatin C	0.94 (0.88-1.00)	92.3	88.2	85.7	93.8

*Clinical model includes age, diabetes mellitus, baseline estimated glomerular filtration rate, and APACHE II score. uNGAL: urinary neutrophil gelatinase-associated lipocalin; TIMP-2: tissue inhibitor of metalloproteinases-2; IGFBP7: insulin-like growth factor-binding protein 7; AUC-ROC: area under the receiver operating characteristic curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

The addition of the biomarker panel (TIMP-2×IGFBP7, urinary NGAL, and plasma cystatin C) to a clinical model that included established risk factors (age, diabetes mellitus, baseline eGFR, and APACHE II score) further improved predictive performance (AUC-ROC 0.94, 95% CI 0.88-1.00). This combined model showed significant improvement in both the integrated discrimination improvement (IDI 0.18, 95% CI 0.12-0.24, $p<0.001$) and net reclassification improvement (NRI 0.72, 95% CI 0.48-0.96, $p<0.001$) compared to the clinical model alone.

Biomarker Performance Across Different AKI Etiologies

We further analyzed the performance of individual biomarkers across the four predefined AKI etiologic categories. Table 5 presents the AUC-ROC values for each biomarker at 6 hours stratified by AKI etiology.

Table 5. AUC-ROC Values (95% CI) for Individual Biomarkers at 6 Hours Stratified by AKI Etiology

Biomarker	Cardiac Surgery (n=15)	Contrast-Induced (n=15)	Sepsis-Associated (n=15)	Nephrotoxic Medications (n=15)
Urinary NGAL	0.87 (0.69-1.00)	0.78 (0.53-1.00)	0.91 (0.78-1.00)	0.73 (0.45-1.00)
Plasma NGAL	0.81 (0.60-1.00)	0.72 (0.46-0.98)	0.89 (0.75-1.00)	0.70 (0.42-0.98)
Urinary KIM-1	0.83 (0.63-1.00)	0.85 (0.65-1.00)	0.69 (0.43-0.95)	0.79 (0.54-1.00)
Urinary IL-18	0.75 (0.52-0.98)	0.70 (0.44-0.96)	0.78 (0.56-1.00)	0.68 (0.40-0.96)
TIMP-2×IGFBP7	0.89 (0.74-1.00)	0.83 (0.63-1.00)	0.87 (0.70-1.00)	0.82 (0.58-1.00)
Urinary L-FABP	0.86 (0.68-1.00)	0.76 (0.51-1.00)	0.75 (0.52-0.98)	0.71 (0.43-0.99)
Plasma Cystatin C	0.84 (0.65-1.00)	0.80 (0.58-1.00)	0.82 (0.62-1.00)	0.77 (0.51-1.00)

AUC-ROC: area under the receiver operating characteristic curve; CI: confidence interval; NGAL: neutrophil gelatinase-associated lipocalin; KIM-1: kidney injury molecule-1; IL-18: interleukin-18; TIMP-2: tissue inhibitor of metalloproteinases-2; IGFBP7: insulin-like growth factor-binding protein 7; L-FABP: liver-type fatty acid-binding protein.

Notable patterns emerged across different AKI etiologies. Urinary NGAL showed the highest discriminative ability in sepsis-associated AKI (AUC-ROC 0.91, 95% CI 0.78-1.00), while urinary KIM-1 performed best in contrast-induced nephropathy (AUC-ROC 0.85, 95% CI 0.65-1.00). TIMP-2×IGFBP7 demonstrated consistently strong performance across all etiologic categories, with the highest AUC-ROC in cardiac surgery (0.89, 95% CI 0.74-1.00).

Using random forest algorithms, we identified the most predictive biomarker combinations for each AKI etiology: TIMP-

2×IGFBP7 and urinary NGAL for cardiac surgery-associated AKI; TIMP-2×IGFBP7 and urinary KIM-1 for contrast-induced nephropathy; urinary NGAL and plasma cystatin C for sepsis-associated AKI; and TIMP-2×IGFBP7 and plasma cystatin C for nephrotoxic medication-associated AKI.

Correlation Between Biomarkers and AKI Severity

All biomarkers demonstrated significant positive correlations with AKI severity. Table 6 presents the Spearman's correlation coefficients between peak biomarker concentrations within the first 24 hours and AKI stage.

Table 6. Correlation Between Peak Biomarker Concentrations and AKI Severity

Biomarker	Spearman's ρ	P-value
Urinary NGAL	0.73	<0.001
Plasma NGAL	0.68	<0.001
Urinary KIM-1	0.65	<0.001
Urinary IL-18	0.59	<0.001
TIMP-2×IGFBP7	0.76	<0.001
Urinary L-FABP	0.62	<0.001
Plasma Cystatin C	0.71	<0.001

AKI: acute kidney injury; NGAL: neutrophil gelatinase-associated lipocalin; KIM-1: kidney injury molecule-1; IL-18: interleukin-18; TIMP-2: tissue inhibitor of metalloproteinases-2; IGFBP7: insulin-like growth factor-binding protein 7; L-FABP: liver-type fatty acid-binding protein.

TIMP-2×IGFBP7 showed the strongest correlation with AKI severity (ρ=0.76, p<0.001), followed by urinary NGAL (ρ=0.73, p<0.001) and plasma cystatin C (ρ=0.71, p<0.001). Notably, patients who required renal replacement therapy had significantly higher early (6-hour) biomarker levels compared to those who did not require RRT, with the largest differences observed for TIMP-2×IGFBP7 (median 2.34 vs. 0.53 ng²/1000, p<0.001) and urinary NGAL (median 286.5 vs. 78.9 ng/mg Cr, p<0.001).

Prognostic Value of Biomarkers for Clinical Outcomes

In multivariate Cox proportional hazards models adjusting for age, baseline kidney function, and comorbidities, elevated concentrations of TIMP-2×IGFBP7 (HR 1.89 per doubling, 95% CI 1.42-2.51, p<0.001), urinary NGAL (HR 1.74 per doubling, 95% CI 1.35-2.24, p<0.001), and plasma cystatin C (HR 2.15 per doubling, 95% CI 1.58-2.93, p<0.001) at the 6-hour time point were independently associated with increased in-hospital mortality.

Similarly, these biomarkers were significantly associated with increased hospital length of stay. In multivariate linear regression models, elevated levels of TIMP-2×IGFBP7, urinary NGAL, and plasma cystatin C at 6 hours were associated with 2.8 (95% CI 1.6-4.0), 2.5 (95% CI 1.4-3.6), and 3.1 (95% CI 1.9-4.3) additional hospital days, respectively, after adjusting for potential confounders.

4. DISCUSSION

This prospective multi-center study evaluated the performance of seven novel biomarkers, both individually and in combination, for the early detection of AKI across diverse clinical settings. Our findings demonstrate that several biomarkers, particularly urinary TIMP-2×IGFBP7, urinary NGAL, and plasma cystatin C, can detect AKI significantly earlier than conventional markers. Furthermore, specific biomarker combinations substantially improved diagnostic accuracy, and distinct biomarker patterns emerged across different AKI etiologies. These findings have important implications for the early detection and management of AKI in clinical practice.

Early Detection of AKI Using Novel Biomarkers

Our results demonstrate that several novel biomarkers were significantly elevated at the 6-hour time point in patients who subsequently developed AKI, well before changes in serum creatinine became apparent (median time to AKI diagnosis: 33.5 hours). This early diagnostic capability addresses a critical limitation of conventional AKI markers. As Siew et al. noted in their comprehensive review, the delayed rise in serum creatinine frequently results in missed therapeutic windows, during which interventions might prevent or mitigate kidney injury.[31]

Among individual biomarkers, urinary TIMP-2×IGFBP7 demonstrated the highest discriminative capacity for early AKI

detection (AUC-ROC 0.85), consistent with findings from the Sapphire study by Kashani et al., which reported an AUC-ROC of 0.80 for predicting moderate-to-severe AKI.[32] Our results extend these findings by demonstrating the effectiveness of TIMP-2×IGFBP7 across diverse AKI etiologies, whereas the Sapphire study primarily focused on critically ill patients.

The strong performance of urinary NGAL in our study (AUC-ROC 0.83) aligns with previous investigations in various clinical settings. In their meta-analysis of 19 studies comprising 2,538 patients, Haase et al. reported a pooled AUC-ROC of 0.82 for urinary NGAL in predicting AKI.[33] Similarly, Mishra et al. demonstrated in their pioneering study that urinary NGAL could predict AKI in children undergoing cardiac surgery with an AUC-ROC of 0.99, though subsequent studies in adults have generally reported more modest diagnostic performance, likely reflecting the greater heterogeneity of adult populations.[34]

Plasma cystatin C, with an AUC-ROC of 0.81 in our study, has shown variable performance in previous investigations. Zhang et al. performed a meta-analysis of 15 studies involving 2,467 patients and reported a pooled AUC-ROC of 0.83 for plasma cystatin C in predicting AKI, comparable to our findings.[35] The relatively rapid elevation of plasma cystatin C compared to serum creatinine likely reflects its independence from muscle mass and its nearly constant production rate, as noted by Dharnidharka et al.[36]

The relatively modest performance of urinary IL-18 (AUC-ROC 0.72) in our study contrasts with some earlier investigations. Parikh et al. reported an AUC-ROC of 0.90 for urinary IL-18 in predicting AKI after cardiopulmonary bypass in children.[37] However, a subsequent multi-center study by Parikh et al. in adults demonstrated more moderate performance (AUC-ROC 0.74), closer to our findings.[38] This discrepancy may reflect differences in AKI pathophysiology between children and adults or varying performance across different clinical settings.

Synergistic Value of Biomarker Combinations

A particularly significant finding of our study is the superior diagnostic performance achieved by combining multiple biomarkers. The combination of TIMP-2×IGFBP7, urinary NGAL, and plasma cystatin C yielded an AUC-ROC of 0.92, significantly outperforming any individual biomarker. This synergistic effect likely reflects the complementary pathophysiological processes captured by these different biomarkers: TIMP-2 and IGFBP7 are associated with G1 cell cycle arrest in tubular epithelial cells,[39] NGAL is upregulated in response to tubular injury,[40] and cystatin C primarily reflects glomerular filtration rate.[41]

The concept of biomarker combinations for enhanced AKI detection has gained traction in recent years. Koyner et al. demonstrated in the Sapphire study that combining TIMP-2×IGFBP7 with urinary KIM-1 improved AKI prediction (AUC-ROC 0.85) compared to either biomarker alone.[42] Similarly, Arthur et al. reported that a combination of urinary NGAL, IL-18, and KIM-1 improved AKI prediction following cardiac surgery (AUC-ROC 0.88).[43] Our findings extend this concept by identifying the most effective biomarker combinations across different AKI etiologies.

The significant improvement in both IDI (0.18) and NRI (0.72) observed when adding biomarkers to clinical predictors underscores the incremental value of these novel markers. This aligns with findings from Luo et al., who demonstrated that combining biomarkers with clinical risk factors substantially improved AKI prediction following cardiac surgery (NRI 0.69).[44] These results suggest that the optimal approach to early AKI detection may involve integrating novel biomarkers with established clinical risk factors.

Etiology-Specific Biomarker Patterns

Our study uniquely explores biomarker performance across different AKI etiologies, revealing distinct patterns that reflect varying pathophysiological mechanisms. Urinary NGAL showed the highest performance in sepsis-associated AKI (AUC-ROC 0.91), consistent with findings from Bagshaw et al., who reported substantial NGAL elevation in septic AKI compared to non-septic AKI.[45] This may reflect NGAL's dual role as both a kidney injury marker and an acute phase reactant induced during systemic inflammation, as described by Schmidt-Ott et al.[46]

Urinary KIM-1 demonstrated particularly strong performance in contrast-induced nephropathy (AUC-ROC 0.85), which aligns with experimental studies by Ichimura et al. showing that KIM-1 is markedly upregulated in proximal tubule cells following ischemic and nephrotoxic injury.[47] The relative underperformance of KIM-1 in sepsis-associated AKI (AUC-ROC 0.69) may reflect the more complex pathophysiology of septic AKI, which involves both hemodynamic and inflammatory components, as elucidated by Bellomo et al.[48]

TIMP-2×IGFBP7 showed consistently strong performance across all AKI etiologies, with the highest AUC-ROC in cardiac surgery (0.89). This broad applicability likely reflects the fundamental role of G1 cell cycle arrest as a protective mechanism in response to various kidney stressors, as demonstrated by Yang et al. in experimental models.[49] Our findings extend the work of Gocze et al., who showed that TIMP-2×IGFBP7 effectively predicted AKI following major surgery, by demonstrating its utility across multiple clinical contexts.[50]

The etiology-specific biomarker patterns identified in our study could potentially guide the selection of the most appropriate

biomarkers based on the clinical context. For instance, prioritizing NGAL measurement in patients with sepsis or KIM-1 in those receiving contrast agents might optimize diagnostic efficiency. The random forest algorithm's identification of the most predictive biomarker combinations for each etiology (e.g., TIMP-2×IGFBP7 and KIM-1 for contrast-induced nephropathy) further refines this approach.

Clinical Implications

The strong correlation between early biomarker elevations and AKI severity, as well as their significant association with clinical outcomes including mortality and hospital length of stay, underscores the potential prognostic value of these biomarkers. These findings are consistent with those of Pike et al., who demonstrated that elevated urinary NGAL was independently associated with increased mortality and prolonged hospitalization, even after adjusting for AKI severity.[51]

The early predictive capacity of these biomarkers could potentially transform AKI management by enabling preventive interventions during a critical window of opportunity. As highlighted by Ronco et al. in their conceptual framework for AKI, interventions applied during the early phases of kidney injury may prevent progression to established AKI.[52] The substantial lead time provided by biomarkers in our study (median 27.5 hours before creatinine elevation) could facilitate timely implementation of kidney-protective strategies, such as optimizing hemodynamics, avoiding nephrotoxins, and adjusting medication dosing.

The significant improvement in diagnostic performance achieved by biomarker combinations suggests that a panel approach, rather than reliance on a single biomarker, may be optimal for early AKI detection. This aligns with the recommendation by Kellum et al. for incorporating multiple biomarkers that capture different aspects of AKI pathophysiology.[53] The consistently strong performance of TIMP-2×IGFBP7 across various clinical settings positions it as a core component of such panels, potentially supplemented by context-specific biomarkers depending on the clinical scenario.

However, the translation of these findings into clinical practice requires consideration of practical factors including cost, assay availability, and standardization. As noted by Prowle et al., widespread implementation of novel biomarkers necessitates accessible point-of-care testing platforms and standardized reference ranges.[54] Additionally, as emphasized by Coca et al., the ultimate value of biomarker-guided strategies depends on the availability of effective interventions that can modify AKI outcomes when initiated early.[55]

Limitations and Future Directions

Several limitations of our study warrant consideration. First, despite stratified enrollment across different AKI etiologies, the sample size within each etiologic category was relatively small, potentially limiting the precision of our estimates for biomarker performance in specific contexts. Larger studies focused on individual AKI etiologies are needed to validate our findings.

Second, our study did not include a validation cohort to confirm the identified biomarker cutoff values and combinations. External validation in independent cohorts would strengthen the generalizability of our results, as emphasized by Moons et al. in their guidelines for biomarker studies.[56]

Third, while our study assessed a comprehensive panel of seven biomarkers, emerging markers such as urinary chitinase 3-like protein 1 (CHI3L1),[57] microRNAs,[58] and exosome-derived markers[59] were not evaluated. Future studies incorporating these novel markers could potentially identify even more effective biomarker combinations.

Fourth, our study focused primarily on diagnostic performance rather than assessing whether biomarker-guided interventions improve clinical outcomes. Randomized controlled trials evaluating the impact of biomarker-guided management strategies on patient-centered outcomes are necessary to definitively establish their clinical utility, as highlighted by Pickkers et al.[60]

Finally, our study did not comprehensively assess the cost-effectiveness of biomarker implementation. Future studies should evaluate the economic implications of biomarker-guided strategies, considering both direct assay costs and potential savings from prevented AKI complications, as suggested by Lewington et al.[61]

5. CONCLUSION

In conclusion, our multi-center prospective study demonstrates that novel biomarkers, particularly TIMP-2×IGFBP7, urinary NGAL, and plasma cystatin C, can detect AKI significantly earlier than conventional markers across diverse clinical settings. Specific biomarker combinations substantially improved diagnostic accuracy, and distinct biomarker patterns emerged across different AKI etiologies. These findings have important implications for the early detection and management of AKI in clinical practice and provide a foundation for future studies investigating whether biomarker-guided interventions can improve patient outcomes in this common and serious condition.

REFERENCES

[1] Hoste EA, Kellum JA, Selby NM, et al. Global epidemiology and outcomes of acute kidney injury. Nat Rev Nephrol. 2018;14(10):607-625.

[2] Case J, Khan S, Khalid R, Khan A. Epidemiology of acute kidney injury in the intensive care unit. Crit Care Res Pract. 2013;2013:479730.

[3] Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol. 2005;16(11):3365-3370.

[4] Star RA. Treatment of acute renal failure. Kidney Int. 1998;54(6):1817-1831.

[5] Waikar SS, Bonventre JV. Creatinine kinetics and the definition of acute kidney injury. J Am Soc Nephrol. 2009;20(3):672-679.

[6] Molitoris BA, Sutton TA. Endothelial injury and dysfunction: role in the extension phase of acute renal failure. Kidney Int. 2004;66(2):496-499.

[7] Kellum JA, Prowle JR. Paradigms of acute kidney injury in the intensive care setting. Nat Rev Nephrol. 2018;14(4):217-230.

[8] Beker BM, Corleto MG, Fieiras C, Musso CG. Novel acute kidney injury biomarkers: their characteristics, utility and concerns. Int Urol Nephrol. 2018;50(4):705-713.

[9] Devarajan P. Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. Biomark Med. 2010;4(2):265-280.

[10] Vijayan A, Faubel S, Askenazi DJ, et al. Clinical use of the urine biomarker [TIMP-2] × [IGFBP7] for acute kidney injury risk assessment. Am J Kidney Dis. 2016;68(1):19-28.

[11] Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol. 2008;48:463-493.

[12] Murray PT, Mehta RL, Shaw A, 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(3):513-521.

[13] Ho J, Tangri N, Komenda P, et al. Urinary, plasma, and serum biomarkers' utility for predicting acute kidney injury associated with cardiac surgery in adults: a meta-analysis. Am J Kidney Dis. 2015;66(6):993-1005.

[14] Briguori C, Visconti G, Rivera NV, et al. Cystatin C and contrast-induced acute kidney injury. Circulation. 2010;121(19):2117-2122.

[15] McCullough PA, Shaw AD, Haase M, et al. Diagnosis of acute kidney injury using functional and injury biomarkers: workgroup statements from the tenth Acute Dialysis Quality Initiative Consensus Conference. Contrib Nephrol. 2013;182:13-29.

[16] Bellomo R, Kellum JA, Ronco C. Acute kidney injury. Lancet. 2012;380(9843):756-766.

[17] Mehta RL, Pascual MT, Soroko S, et al. Spectrum of acute renal failure in the intensive care unit: the PICARD experience. Kidney Int. 2004;66(4):1613-1621.

[18] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13(10):818-829.

[19] Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative workgroup. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care. 2004;8(4):R204-R212.

[20] Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney Int Suppl. 2012;2:1-138.

[21] Mårtensson J, Martling CR, Bell M. Novel biomarkers of acute kidney injury and failure: clinical applicability. Br J Anaesth. 2012;109(6):843-850.

[22] Moore E, Bellomo R, Nichol A. Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice. Minerva Anestesiol. 2010;76(6):425-440.

[23] Vaidya VS, Waikar SS, Ferguson MA, et al. Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. Clin Transl Sci. 2008;1(3):200-208.

[24] Bihorac A, Chawla LS, Shaw AD, et al. Validation of cell-cycle arrest biomarkers for acute kidney injury using

clinical adjudication. Am J Respir Crit Care Med. 2014;189(8):932-939.

[25] Comper WD, Hilliard LM, Nikolic-Paterson DJ, Russo LM. Disease-dependent mechanisms of albuminuria. Am J Physiol Renal Physiol. 2008;295(6):F1589-F1600.

[26] Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A; NGAL Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis. 2009;54(6):1012-1024.

[27] Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(2):157-172.

[28] Breiman L. Random forests. Machine Learning. 2001;45(1):5-32.

[29] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2021. URL https://www.R-project.org/.

[30] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol. 1995;57(1):289-300.

[31] Siew ED, Ware LB, Ikizler TA. Biological markers of acute kidney injury. J Am Soc Nephrol. 2011;22(5):810-820.

[32] Kashani K, Al-Khafaji A, Ardiles T, et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care. 2013;17(1):R25.

[33] Haase M, Bellomo R, Devarajan P, et al. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis. 2009;54(6):1012-1024.

[34] Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. 2005;365(9466):1231-1238.

[35] Zhang Z, Lu B, Sheng X, Jin N. Cystatin C in prediction of acute kidney injury: a systemic review and meta-analysis. Am J Kidney Dis. 2011;58(3):356-365.

[36] Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis. 2002;40(2):221-226.

[37] Parikh CR, Mishra J, Thiessen-Philbrook H, et al. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. Kidney Int. 2006;70(1):199-203.

[38] Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. J Am Soc Nephrol. 2005;16(10):3046-3052.

[39] Emlet DR, Pastor-Soler N, Marciszyn A, et al. Insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2: differential expression and secretion in human kidney tubule cells. Am J Physiol Renal Physiol. 2017;312(2):F284-F296.

[40] Mori K, Lee HT, Rapoport D, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest. 2005;115(3):610-621.

[41] Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem. 2002;48(5):699-707.

[42] Koyner JL, Shaw AD, Chawla LS, et al. Tissue inhibitor metalloproteinase-2 (TIMP-2)·IGF-binding protein-7 (IGFBP7) levels are associated with adverse long-term outcomes in patients with AKI. J Am Soc Nephrol. 2015;26(7):1747-1754.

[43] Arthur JM, Hill EG, Alge JL, et al. Evaluation of 32 urine biomarkers to predict the progression of acute kidney injury after cardiac surgery. Kidney Int. 2014;85(2):431-438.

[44] Luo Q, Zhou F, Dong H, et al. Implication of combined urinary biomarkers in early diagnosis of acute kidney injury following percutaneous coronary intervention. Clin Nephrol. 2013;79(2):85-92.

[45] Bagshaw SM, Bennett M, Haase M, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. Intensive Care Med. 2010;36(3):452-461.

[46] Schmidt-Ott KM, Mori K, Li JY, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. 2007;18(2):407-413.

[47] Ichimura T, Bonventre JV, Bailly V, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem. 1998;273(7):4135-4142.

[48] Bellomo R, Wan L, Langenberg C, May C. Septic acute kidney injury: new concepts. Nephron Exp Nephrol. 2008;109(4):e95-e100.

[49] Yang L, Besschetnova TY, Brooks CR, Shah JV, Bonventre JV. Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. Nat Med. 2010;16(5):535-543.

[50] Gocze I, Koch M, Renner P, et al. Urinary biomarkers TIMP-2 and IGFBP7 early predict acute kidney injury after major surgery. PLoS One. 2015;10(3):e0120863.

[51] Pike F, Murugan R, Keener C, et al. Biomarker enhanced risk prediction for adverse outcomes in critically ill patients receiving RRT. Clin J Am Soc Nephrol. 2015;10(8):1332-1339.

[52] Ronco C, Bellomo R, Kellum JA. Acute kidney injury. Lancet. 2019;394(10212):1949-1964.

[53] Kellum JA, Devarajan P. What can we expect from biomarkers for acute kidney injury? Biomark Med. 2014;8(10):1239-1245.

[54] Prowle JR, Liu YL, Licari E, et al. Oliguria as predictive biomarker of acute kidney injury in critically ill patients. Crit Care. 2011;15(4):R172.

[55] Coca SG, Yalavarthy R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. Kidney Int. 2008;73(9):1008-1016.

[56] Moons KG, Altman DG, Reitsma JB, et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. Ann Intern Med. 2015;162(1):W1-W73.

[57] De Loor J, Decruyenaere J, Demeyere K, et al. Urinary chitinase 3-like protein 1 for early diagnosis of acute kidney injury: a prospective cohort study in adult critically ill patients. Crit Care. 2016;20(1):38.

[58] Lorenzen JM, Kielstein JT, Hafer C, et al. Circulating miR-210 predicts survival in critically ill patients with acute kidney injury. Clin J Am Soc Nephrol. 2011;6(7):1540-1546.

[59] Zhou H, Pisitkun T, Aponte A, et al. Exosomal Fetuin-A identified by proteomics: a novel urinary biomarker for detecting acute kidney injury. Kidney Int. 2006;70(10):1847-1857.

[60] Pickkers P, Ostermann M, Joannidis M, et al. The intensive care medicine agenda on acute kidney injury. Intensive Care Med. 2017;43(9):1198-1209.

[61] Lewington AJ, Cerdá J, Mehta RL. Raising awareness of acute kidney injury: a global perspective of a silent killer. Kidney Int. 2013;84(3):457-467.