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Title

Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults: A prospective, observational study

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Abstract

Objective Serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cystatin C (sCysC) are available clinically and beneficial in diagnosing AKI. Our purpose is to identify the performance of their combined diagnosis for AKI in critically ill patients.

Design A prospectively-recruited, observational study was performed.

Setting Adults admitted to the intensive care unit of a grade A tertiary hospital in China.

Participants A total of 1222 critically ill patients were enrolled in the study.

Main outcome measures To identify the performance of the combined diagnosis of serum NT-proBNP and sCysC for AKI in critically ill patients. The area under the receiver operating characteristic curve (AUC-ROC), category-free net reclassification index (NRI), and incremental discrimination improvement (IDI) were utilized for comparing the discriminative powers of NT-proBNP, sCysC and their combination for diagnosing AKI with or without a clinical model.

Results

AKI was detected in 256 out of 1222 included patients (20.9%). AUC-ROC for NT-proBNP plus sCysC panel to detect AKI had a significantly higher accuracy than any individual biomarker ($P < 0.05$). After multivariate adjustment, a level of serum NT-proBNP ≥ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with that below the cutoff value. Similar results were obtained for sCysC levels ($P < 0.001$). To detect AKI, adding NT-proBNP and sCysC to a clinical model further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$). Moreover, the addition of this panel to the clinical model significantly augmented risk

reclassification of AKI beyond that of the clinical model alone or with single biomarker ($P < 0.05$), as measured by NRI and IDI.

Conclusions

In critically ill individuals, serum NT-proBNP, sCysC, and clinical risk factor combination improve the discriminative power for diagnosing AKI.

Keywords

Acute kidney injury, N-terminal pro-B-type natriuretic peptide, Serum cystatin C, Intensive care unit

Strengths and limitations of this study

This is the first study to identify the performance of serum NT-proBNP and sCysC combined diagnosis in detecting AKI.

This prospectively-recruited, observational study was of a long duration and had an adequate sample size.

A limitation of this study was that the relationship between NT-proBNP and sCysC could not be dynamically assessed because we only measured their levels at the time of admission.

Introduction

Acute kidney injury (AKI) is a predominant clinical syndrome affecting more than 50% of patients who underwent treatment at intensive care unit (ICU)[1-3]. Increased morbidity, mortality, hospitalization length, and cost are extremely related to AKI[4-6], so AKI early recognition is critical to guiding management. Urine production and serum creatinine were employed as diagnostic criteria for AKI in accordance with the recommendations of Kidney Disease Improving Global Outcomes (KDIGO) guidelines[7]. Changes in sCr or urine production cannot recognize early renal tubular injury prior to a reduction in glomerular filtration rate [8, 9]. Therefore, early and reliable AKI biomarkers are necessary to promote timely intervention and minimize complications. However, it is impossible for an individual biomarker to adequately evaluate the risk of AKI as a complex multifactorial syndrome [3, 10]. Combining diverse biomarkers in a clinical model evaluation could enhance early detection of AKI in critically ill patients[5, 11, 12].

A hemodynamic marker stress, N-terminal pro-B-type natriuretic peptide (NT-proBNP), has recently received attention as a potential predictor of AKI in a wide diversity of clinical settings[13-17]. High serum NT-proBNP level reflects hemodynamic instability, myocardial wall stress, myocardial ischemia, volume overload, sympathetic nervous system and renin-angiotensin-aldosterone system activation, all of which may contribute to AKI incidence[18-20]. However, there are finite data on clinical use of NT-proBNP for detecting AKI among critically ill patients.

Serum Cystatin C (sCysC), a 122-amino acid low-molecular-weight protein (13 kDa),

is a marker of glomerular filtration[9]. CysC has a half-life of about one-third that of sCr, causing CysC to reach homeostasis three times faster. These properties promote sCysC as an alternative marker of renal function[8]. To date, sCysC has been displayed to be conductive for the early identification of all-cause AKI[21].

Although serum NT-proBNP and sCysC are mainly used for the prognosis assessment of heart disease and the prediction of acute cardiac events with AKI [22-26], the diagnostic accuracy of their combination for AKI in ICU remains unknown. We performed a prospective study in the present research to evaluate the performance of serum NT-proBNP and sCysC at ICU admission, both independently and in combination, for AKI determination among critically ill adults.

Methods

Study design and participants

At Guangdong Provincial People's Hospital in China, a tertiary care hospital, a prospectively recruited observational research was performed in a mixed medical-surgical ICU. From December 2016 to December 2017, we consecutively enrolled patients aged 18 and up in a row. Pregnancy, renal replacement therapy (RRT) prior to ICU admittance, nephrectomy, end-stage renal disease (ESRD), renal transplant, rejection of consent, or missing admission information were all exclusion criteria. The study protocol strengthened the reporting requirements of observational studies in epidemiology[27] and standards for the reporting of diagnostic accuracy[28]. This study protocol was authorized by ethics committee of Guangdong Provincial People's Hospital. Additionally, all procedures were carried out consistent with applicable regulations and guidelines. All patients provided written informed consent.

Data collection

Clinical baseline data were collected prospectively. Within 1 h following ICU admission, blood samples were collected contemporaneously to determine sCr, serum NT-proBNP, and sCysC. Within 24 h after collection, all samples were analyzed in the Guangdong Provincial People's Hospital central laboratory utilizing standard protocol. When a patient is admitted to the ICU, sCr was measured and subsequently done at least once a day until discharge as part of routine clinical care. Throughout admission to ICU, urine production was also been recorded. We evaluated the following clinical variables: age, gender, body mass index (BMI), preexisting clinical conditions, sepsis,

admission type, baseline sCr, baseline estimated glomerular filtration rate (eGFR), Acute Physiology and Chronic Health Evaluation (APACHE) II score, ICU mortality, in-hospital mortality, duration of ICU admittance, length of hospitalization, ICU costs, and total costs. The outcome was the incidence of AKI following ICU enrollment within 1 week. To calculate eGFR, we employed the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [29].

Definitions

The KDIGO classification criteria were utilized to define AKI: as a rise in sCr by ≥ 0.3 mg/dL (26.5μmol/L) within 48 h or a rise in sCr to ≥ 1.5 times the baseline within 1 week, or urine output < 0.5 mL/kg/h for 6 h after ICU admission[7].

Relying on following principles, ranked in descending order of preference, a baseline sCr was affirmed[30]: (1) prior to ICU admittance, the most recent pre-ICU value between 30 and 365 days; (2) a stable pre-ICU value > 365 days prior to ICU admittance for patients < 40 years of age (stable definition is being within 15% of the lowest ICU measurement); (3) pre-ICU value > 365 days prior to ICU admittance and lower than the initial sCr at ICU admission; (4) a pre-ICU value (within 3 and 39 days prior to ICU admittance) lower than or equal to the initial sCr upon ICU admittance not obviously during AKI; (5) the least sCr value obtained at initial ICU admittance, the most recent ICU value, or the lowest value achieved to a 365-day follow up.

Biomarker measurement

The levels of sCysC and sCr were quantified through the UniCel DxC 800 Synchron system usage in compliance with the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). For sCysC, the intra- and inter-assay variation coefficients were 10% and 5%, respectively. Levels of serum NT-proBNP were quantified through an electrochemiluminescence immunoassay employing a Cobas® e602 system usage (Roche Diagnostics, Germany). For NT-proBNP, the uppermost limit of normal for those who seem to be healthy (95th percentage) has been 125 pg/mL. The coefficient of interassay variation for NT-proBNP was < 5%. Each patient's clinical features were blinded to the personnel measuring the biomarkers.

Patient and public involvement

Patients were not involved in the study.

Statistical analysis

For statistical analyses, we utilized SPSS version 21.0 (SPSS, Chicago, IL, USA), MedCalc version 18.2.1 (MedCalc Software, Ostend, Belgium) and R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). The mean ± standard deviation or median had been utilized to express continuous variables (25th to 75th percentage, interquartile range). Numbers (percent) were utilized to represent categorical variables. For nonnormally distributed continuous variables, the Wilcoxon rank-sum test was employed for intergroup difference measurement, and for categorical variables, the chi-square or Fisher's exact test was deployed.

NT-proBNP concentrations were extremely skewed and therefore were log10 transformed before inclusion in the models. Areas under the receiver operating

characteristic curves (AUC-ROCs) were computed. The method exploited by DeLong et al. was used to compare AUC-ROCs between groups[31]. The biomarkers' sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios of the biomarkers were calculated. Youden's index for AKI detection identified the optimal cutoff values for individual biomarkers and their combination[32].

Logistic analysis was utilized to compute the odds ratios (ORs) and 95% confidence intervals for each factor: to identify the independent risk factors of AKI, the clinical variables with $P < 0.10$ in univariate analysis were incorporated into the multivariate logistic model. A forward stepwise method was used to select variables. We categorized NT-proBNP and sCysC levels according to their cutoff values and then performed logistic regression on the created variables. We conducted multiple logistic regression analyses to calculate the adjusted ORs of AKI. The selection of covariates for the adjusted models was based on the clinical risk factors that detect AKI.

The performance of AKI detection after adding NT-proBNP and sCysC, or any of them, into the clinical model as categorical variables was assessed by AUC-ROC, category-free net reclassification improvement (NRI) index and integrated discrimination improvement (IDI) index, as described previously[33, 34]. All the tests were 2-tailed, and $P < 0.05$ was regarded statistically significant.

Results

Clinical data and outcomes

A total of 150 (10.9 %) of the 1372 adult patients enrolled in the study were excluded (Figure 1). Therefore, 1222 patients were enrolled and AKI occurred in 256 patients (20.9%). Patient baseline variables and outcomes are exhibited in Table 1. In comparison to patients without AKI, patients with AKI were elderly and were observed more frequently in those with comorbidities, including chronic kidney disease (CKD), hypertension, coronary artery disease (CAD), diabetes mellitus (DM), heart failure (HF), cerebrovascular disease and chronic obstructive pulmonary disease (COPD). Increased sCr, serum NT-proBNP and sCysC levels at admission, as well as increased APACHE II scores, were more prevalent in AKI patients. The baseline sCr and eGFR did not show significant differences between the two groups.

Indeed, AKI patients had a higher risk of adverse outcomes, a higher percentage of ICU and in-hospital mortality, higher expenses, a longer hospitalization duration, and a longer stay in ICU ($P < 0.001$) compared to those without AKI.

Detective abilities of the two biomarkers for AKI

To demonstrate the ability of these biomarkers for AKI detection, we used AUC-ROCs to calculate the two biomarkers, respectively, and in combination. AUC-ROCs for NT-proBNP and sCysC were computed for AKI detection (0.821 and 0.766, respectively). For AKI detection, NT-proBNP had a sensitivity of 78% and a specificity of 75%, while sCysC had high specificity but limited sensitivity. The cutoff values for NT-proBNP and sCysC were 204 pg/mL and 1.02 mg/L, respectively, yielding good sensitivity and specificity. The AUC-ROCs for AKI presented a better performance by NT-proBNP plus sCysC than by any individual biomarker ($P < 0.05$, Table 2 and Figure 2).

Multivariate logistic regression analyses of the two biomarkers for AKI detection

We stratified patients based on the two cutoff values of serum NT-proBNP and sCysC levels into two categories, respectively. Compared with those with serum NT-proBNP < 204 pg/mL, patients with serum NT-proBNP ≥ 204 pg/mL on admission exhibited a higher incidence of AKI (7.2% versus 45.1%, $P < 0.001$). AKI was found to have higher incidence (46.5%) in patients with sCysC ≥ 1.02 mg/L compared to those with sCysC < 1.02 mg/L (10.2%) ($P < 0.001$). Following clinical variables adjustment (including CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores, Table 3), a level of serum NT-proBNP ≥ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with NT-proBNP level below the cutoff value ($P < 0.001$). Similarly, patients with sCysC ≥ 1.02 mg/L were linked to 2.6-fold greater odds of AKI compared to those with sCysC < 1.02 mg/L ($P < 0.001$) (Table 4).

Discrimination and reclassification of the combination of biomarkers and clinical models for AKI

Considering the effect of adding NT-proBNP and sCysC, or any of them, to a clinical model as categorical variables for AKI detection, logistic regression analysis was employed. On ICU admittance for AKI diagnosis, potential available variables including age, sepsis, admission type, sCr, and APACHE II scores as well as comorbidities, including hypertension, DM, CKD, cerebrovascular disease, COPD, CAD, and HF were considered. The clinical model for detecting AKI involved CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores (Table 3).

To evaluate the enhancement of discriminative capacity, a panel of NT-proBNP plus sCysC was introduced to the above-mentioned model. As shown in Table 5, compared with the clinical

model, the addition of NT-proBNP to the clinical model had a higher AUC-ROC ($P < 0.05$), and no statistically significant variation existed when sCyC was added to the clinical model. However, the risk reclassification was markedly improved through the addition of NT-proBNP or sCyC to the clinical model, as measured by category-free NRI and IDI ($P < 0.05$). Adding NT-proBNP and sCysC to a clinical model for AKI detection further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$) (Figure 3). Moreover, this panel addition to the clinical model significantly enhanced the risk reclassification of AKI beyond that of the clinical model with or without any individual biomarkers ($P < 0.05$), with maximum NRI (0.531) and IDI (0.038).

Table 1 Clinical data and outcomes

Characteristics	Total (n=1222)	AKI (n=256)	No AKI (n=966)	*P* value
Age, years	57 (45-67)	63.5 (52-72)	55 (44-65)	<0.001
Male, n (%)	666 (54.5)	151 (59.0)	515 (53.3)	0.105
BMI, kg/m^2	22 (19.6-24.7)	22.3 (19.9-25.0)	21.9 (19.6-24.6)	0.395
Preexisting clinical conditions				
Hypertension, n (%)	252 (20.6)	81 (31.6)	171 (17.7)	<0.001
Diabetes mellitus, n (%)	121 (9.9)	52 (20.3)	69 (7.1)	<0.001
Chronic kidney disease, n (%)	26 (2.1)	21 (8.2)	5 (0.5)	<0.001
Cerebrovascular disease, n (%)	127 (10.4)	50 (19.5)	77 (8.0)	<0.001
Chronic obstructive pulmonary disease, n (%)	35 (2.9)	18 (7.0)	17 (1.8)	<0.001
Coronary artery disease, n (%)	53 (4.3)	24 (9.4)	29 (3.0)	<0.001
Heart failure, n (%)	31 (2.5)	22 (8.6)	9 (0.9)	<0.001
Cancer, n (%)	227 (18.6)	50 (19.5)	177 (18.3)	0.659
Sepsis, n (%)	98 (8.0)	70 (27.3)	28 (2.9)	<0.001
Admission type, n (%)				<0.001
Elective surgical, n (%)	863 (70.6)	84 (32.8)	779 (80.6)	
Emergency surgical, n (%)	83 (6.8)	33 (12.9)	50 (5.2)	
Medical, n (%)	276 (22.6)	139 (54.3)	137 (14.2)	
Baseline serum creatinine, mg/dL	0.78 (0.64-0.96)	0.76 (0.58-1.09)	0.79 (0.65-0.94)	0.949
Baseline eGFR, mL/min/1.73 m^2	119.4 (97.6-145.4)	122.9 (83.3-158.7)	118.7 (100.4-142.9)	0.950
Parameters at ICU admission				
sCr, mg/dl	0.87 (0.72-1.07)	0.97 (0.72-1.55)	0.85 (0.72-1.02)	<0.001
NT-proBNP, pg/mL	97.1 (29.4-464.4)	871.0 (225.8-2919.3)	66.2 (23.7-206.2)	<0.001
sCysC, mg/L	0.78 (0.61-1.06)	1.15 (0.83-1.85)	0.75 (0.58-0.94)	<0.001
APACHE Ⅱ score	7 (4-10)	11 (8-16)	6 (4-9)	<0.001
UP, ml/kg/h	1.67 (1.20-2.26)	1.37 (0.89-2.20)	1.73 (1.26-2.27)	<0.001
Outcomes				
ICU mortality, n (%)	40 (3.3)	33 (12.9)	7 (0.7)	<0.001
In-hospital mortality, n (%)	53 (4.3)	40 (15.6)	13 (1.3)	<0.001
ICU stay, days	2 (2-4)	6 (3-13.3)	2 (2-3)	<0.001
Hospital stay, days	16 (12-23)	22 (13-33.3)	15 (11-21)	<0.001
ICU costs, CNY	39461.7 (28966.2-59437.2)	66140.1 (36526.0-131843.8)	37667.5 (27881.5-48238.4)	<0.001
Total costs, CNY	58295.3 (44201.6-98602.8)	128945.6 (71506.8-199685.4)	52773.8 (40856.2-78718.9)	<0.001

Abbreviations: AKI, acute kidney injury; BMI, body mass index; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; sCr, serum creatinine, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score; UP, Urine production first 24 h after admission; CNY, Chinese yuan.

Continuous variables are expressed as mean ± standard deviation or median (25th to 75th percentile, interquartile range). Categorical variables are expressed as a n (%).

Table 2 Detective characteristics of the two biomarkers for AKI

Logistic regression models	AUC-ROC[a] (95%CI)	Cutoff [b]	Sensitivity	Specificity	(+) LR	(-) LR	PPV	NPV
sCysC	0.766 (0.741-0.789)	1.02 mg/L	0.66	0.80	3.28	0.43	0.47	0.90
NT-proBNP	0.821 (0.799-0.842) [c]	204.00 pg/mL	0.78	0.75	3.12	0.29	0.45	0.93
NT-proBNP+ sCysC	0.832 (0.809-0.852) [d]	0.15[e]	0.84	0.69	2.69	0.23	0.42	0.94

Abbreviations: AKI, acute kidney injury; AUC-ROC, area under the receiver operating characteristic curve; CI, confidence interval; (+) LR, Positive likelihood ratio; (-) LR, negative likelihood ratio; PPV, Positive predictive value; NPV, Negative predictive value; sCysC, Serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

[a] Values are presented as AUC-ROC (95% CI)

[b] Ideal cutoff value according to Youden's index

[c] $P < 0.05$ vs. sCysC

[d] $P < 0.05$ vs. NT-proBNP, sCysC

[e] Cutoff points of the biomarker panels were the predicted probabilities generated from the multiple logistic regression model

Table 3 Logistic analyses of clinical risk factors for AKI detection

Variables	Univariate analysis		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.029 (1.019-1.038)	<0.001		
Hypertension	2.152 (1.577-2.937)	<0.001		
Diabetes mellitus	3.314 (2.242-4.898)	<0.001		
Chronic kidney disease	17.175 (6.410-46.023)	<0.001	4.182 (1.250-13.998)	0.020
Cerebrovascular disease	2.802 (1.903-4.126)	<0.001		
Chronic obstructive pulmonary disease	4.222 (2.143-8.316)	<0.001		
Coronary artery disease	3.342 (1.910-5.849)	<0.001		
Heart failure	9.997 (4.544-21.997)	<0.001	3.487 (1.365-8.911)	0.009
Sepsis	12.608 (7.914-20.084)	<0.001	5.033 (2.914-8.692)	<0.001
Admission type		<0.001		<0.001
Elective surgical	1.0 (Referent)		1.0 (Referent)	
Emergency surgical	6.121 (3.735-10.030)	<0.001	3.493 (2.023-6.032)	<0.001
Medical	9.409 (6.791-13.037)	<0.001	3.237 (2.157-4.858)	<0.001
sCr	3.135 (2.385-4.120)	<0.001	1.538 (1.149-2.058)	0.004
APACHE Ⅱ score	1.215 (1.182-1.249)	<0.001	1.101 (1.066-1.137)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval; sCr, serum creatinine; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score.

Table 4 Multivariate logistic regression analyses of the two biomarkers for AKI detection

Variables	AKI, %	Unadjusted OR (95% CI)	*P* value	Adjusted OR[a] (95% CI)	*P* value
NT-proBNP (pg/mL, n)					
<204, 779	7.2	1.0 (Referent)		1.0 (Referent)	
≥204, 443	45.1	10.626 (7.639-14.781)	<0.001	3.460 (2.307-5.189)	<0.001
sCysC layered by the cut-off value (mg/L, n)					
<1.02, 861	10.2	1.0 (Referent)		1.0 (Referent)	
≥1.02, 361	46.5	7.646 (5.651-10.345)	<0.001	2.649 (1.842-3.810)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, Serum cystatin C.

a Adjusted for chronic kidney disease, heart failure, sepsis, admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Table 5 Discrimination and reclassification of the combination of biomarkers and clinical model for AKI

	AUC-ROC (95%CI)	*P* value[a]	Category-Free NRI (95%CI)	*P* value[a]	IDI (95%CI)	*P* value[a]
Clinical model[b]	0.840 (0.812-0.868)					
Clinical model + sCysC	0.847 (0.819-0.874)	0.163	0.193 (0.052-0.405)	0.036	0.017 (0.009-0.026)	<0.001
Clinical model + NT-proBNP	0.855 (0.828-0.882)	0.013	0.462 (0.196-0.747)	0.001	0.028 (0.016-0.039)	<0.001
Clinical model + NT-proBNP + sCysC	0.859 (0.832-0.885)	0.006	0.531 (0.238-0.741)	<0.001	0.038 (0.025-0.051)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + sCysC	-	0.015	0.328 (0.139-0.553)	0.002	0.021 (0.010-0.031)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + NT-proBNP	-	0.223	0.167 (0.013-0.285)	0.017	0.011 (0.004-0.018)	0.003

Abbreviations: AUC-ROC, area under the receiver operating characteristic curve; NRI, net reclassification index; IDI, incremental discrimination improvement; AKI, acute kidney injury; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

a Biomarker + clinical model versus clinical model.

b The clinical model for detecting AKI is composed of chronic kidney disease, heart failure, sepsis, Admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Discussion

The study's key finding was that the combination of NT-proBNP and sCysC yields greater discriminative ability for AKI detection at ICU admission with or without a clinical model in critically ill adults. The finding indicates that assessing both serum NT-proBNP and sCysC levels on admission may assist with the early diagnosis and risk stratification of AKI in critically ill adults.

One of the most prevalent complications occurring in a variety of clinical settings is AKI, especially for critically ill patients [4, 6, 35, 36]. The development of AKI, as the same as its severity, is strongly associated with increased mortality[3, 35]. However, early identification is challenging when sCr or urine production changes are used to detect AKI[37], and precise clinical predictors are not widely known. Numerous studies have found and confirmed the accuracy and additional clinical benefits of these renal biomarkers for early AKI diagnosis, such as insulin-like growth factor-binding protein, matrix metalloproteinase-7, tissue inhibitor metalloproteinase-2, angiotensinogen, sCysC, neutrophil gelatinase-associated lipocalin and so on[5, 9, 12, 38, 39]. However, some novel biomarkers are not being used in clinical practice, due to insufficient evidence or previously unavailable commercially. Thus, the rational application of clinically available biomarkers is more practical and economical.

NT-proBNP, a widely used marker of hemodynamic stress, is a polypeptide secreted by the ventricles and its role is to facilitate natriuresis[17, 40]. In patients with elevated CVP, the raised pressure may be transmitted to the renal veins, resulting in renal congestion and reduced glomerular filtration[14, 41, 42]. It makes sense that NT-proBNP is associated with the development of AKI. The clinical application of serum NT-proBNP in cardiac disease has been

extensively confirmed and finds its predictive value for AKI development in patients with HF, coronary angiography, or percutaneous coronary intervention [19, 23, 43, 44]. Several studies have elucidated the link between NT-proBNP and AKI incidence after cardiac or noncardiac surgery[13, 45-47]. However, its utility for AKI detection has not been fully evaluated in general ICU, and it is necessary to verify the reliability and universality of NT-proBNP in heterogeneous populations. The data demonstrated herein not only maintain NT-proBNP as a risk factor for AKI but also indicate that it can enhance the risk reclassification and discrimination for AKI in ICU.

A single biomarker is insufficient to express the multiple pathophysiological mechanisms of AKI, especially for critically ill patients, as AKI is a heterogeneous syndrome[9, 10]. There is no consensus on which specific markers should be combined to detect AKI. Numerous studies have revealed that combining different biomarkers utilized to detect AKI can improve predictive abilities[5, 48, 49]. Naruse et al. showed that combining urinary liver-type fatty-acid binding protein and serum NT-proBNP can enhance early prediction of AKI in patients in medical cardiac ICUs[15]. Similar to such studies, we identified that the combination of two markers, serum NT-probNP and sCysC, improved the diagnostic performance of AKI.

CysC is a glomerular filtration biomarker that can be utilized to anticipate the development of AKI and undesirable outcomes[8, 9]. The application and performance of CysC for AKI prediction have been demonstrated in various clinical settings[5, 11, 50-52]. In individuals with ST-segment elevation myocardial infarction, a combination of B-type natriuretic peptide and CysC may contribute to risk stratification for AKI[25]. In the present cohort, sCysC had a slightly higher specificity than NT-proBNP in the detection of AKI, but its sensitivity is limited. The

ability of sCysC for detecting AKI was fine, but its AUC-ROC was not as great as NT-proBNP. Moreover, the combination of serum NT-proBNP and sCysC at ICU admission had the highest AUC-ROC. These data indicate that the simultaneous measurement of serum NT-proBNP and sCysC at ICU admission could improve the early identification of AKI.

Biomarkers for AKI, which indicates the various underlying pathophysiological mechanisms involved in AKI incidence, might be superior to individual biomarkers alone[3, 6]. It is also important that these biomarkers profit from being easily measurable, readily accessible, comparatively cheap, and with an elevated level of sensitivity and specificity. In the present study, for AKI detection NT-proBNP acted as a hemodynamic stress biomarker with high sensitivity and specificity, and sCysC had high specificity as a functional biomarker. Even after clinical risk variables adjustment, elevated NT-proBNP is an independent risk factor for AKI. The NT-proBNP addition to the clinical model significantly enhanced risk reclassification, as demonstrated by category-free NRI and IDI. Moreover, our results identified that the addition of NT-proBNP to sCysC markedly improved their detective abilities as biomarkers. Adding biomarkers to the clinical model further improved the diagnostic accuracy of AKI, as measured by AUC-ROCs. These data suggest that a single biomarker is insufficient for early diagnosis of AKI. Therefore, the method of combining different biomarkers may be of greater use.

There are some limitations for this study. First, it was a single-center study, with an unproven external validity. Second, there were only 26 patients with CKD patients and 31 HF patients enrolled, and hence we were unable to stratify our group relying on eGFR or cardiac function at baseline. Accordingly, future studies should be administrated in these subgroups. Last, we did not accomplish routine echocardiography in all patients to associate NT-proBNP levels with

ventricular dilatation or other pathways that may promote NT-proBNP release. Despite these limitations, we believe that our findings have clinical implications and should facilitate further research to confirm our results.

Conclusion

Simultaneous measurement of NT-proBNP and sCysC at ICU admission increases the early identification of AKI beyond that of biomarker in isolation, and that the combination of the two biomarkers and clinical risk factors improves the discriminative ability for AKI detection in critically ill adults.

Abbreviations

(−) LR, negative likelihood ratios

(+) LR, positive likelihood ratios

AKI, Acute kidney injury

APACHE II score, Acute Physiology and Chronic Health Evaluation II score

AUC-ROC, area under the receiver operating characteristic curve

BMI, body mass index

CAD, coronary artery disease

CI, confidence interval

CNY, Chinese yuan

CKD, chronic kidney disease

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration

COPD, chronic obstructive pulmonary disease

DM, diabetes mellitus

eGFR, estimated glomerular filtration rate

ESRD, end-stage renal disease

HF, heart failure

ICU, intensive care unit

IDI, incremental discrimination improvement

KDIGO, Kidney Disease Improving Global Outcomes

NPV, negative predictive values

NRI net reclassification index

NT-proBNP, N-terminal pro-B-type natriuretic peptide

ORs, odds ratios

PPV, positive predictive values

RRT, renal replacement therapy

sCr, serum creatinine

sCysC, serum cystatin C

UP, Urine production first 24 h after admission

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Authors' contributions

JD, LLH, YFL and LHH equally contributed to the design of the research, analysis and interpretation of the data. CBC and JD contributed to the conception and design of the research as well as interpretation of the data, and critically revised the manuscript. JD, LLH, YFL, LHH, JX, HF and YL performed the research and collected data. All authors contributed to the acquisition and analysis of the data, drafted the manuscript, and agree to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during this study are not publicly available, owing to currently ongoing research studies, but the data are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethics committee of the Guangdong Provincial People's Hospital approved the study design, protocol, ethical issue, and data and sample collection (No. GDREC2015396H(R1)). Written informed consent was obtained from each patient or from the appropriate surrogates.

Patient consent for publication

Not required.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Consent for publication

Not applicable. No individual personal data are included in the study. All patients provided necessary consent to participate in the present study.

Competing interests

The authors declare that they have no competing interests.

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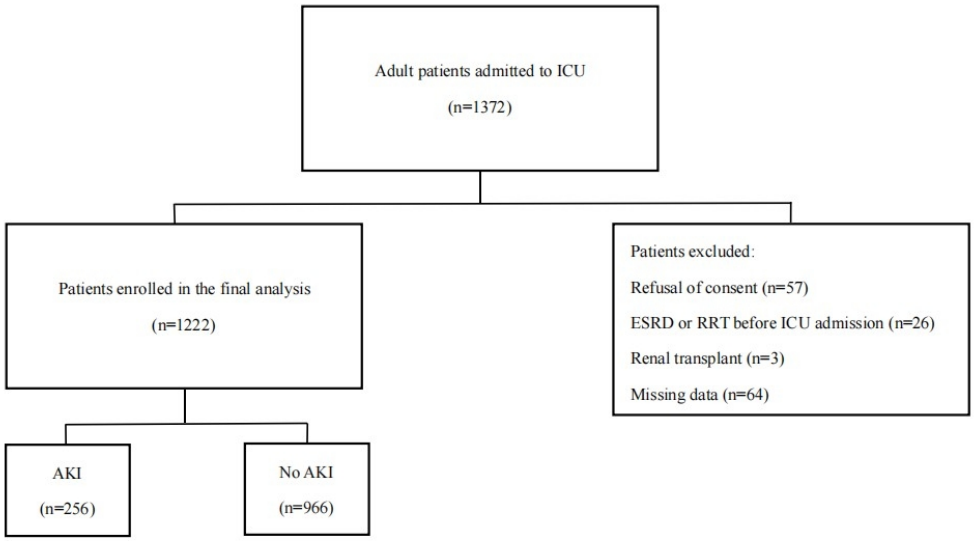
Figure legends

Figure 1. Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

Figure 2. ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

Figure 3. ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.



Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

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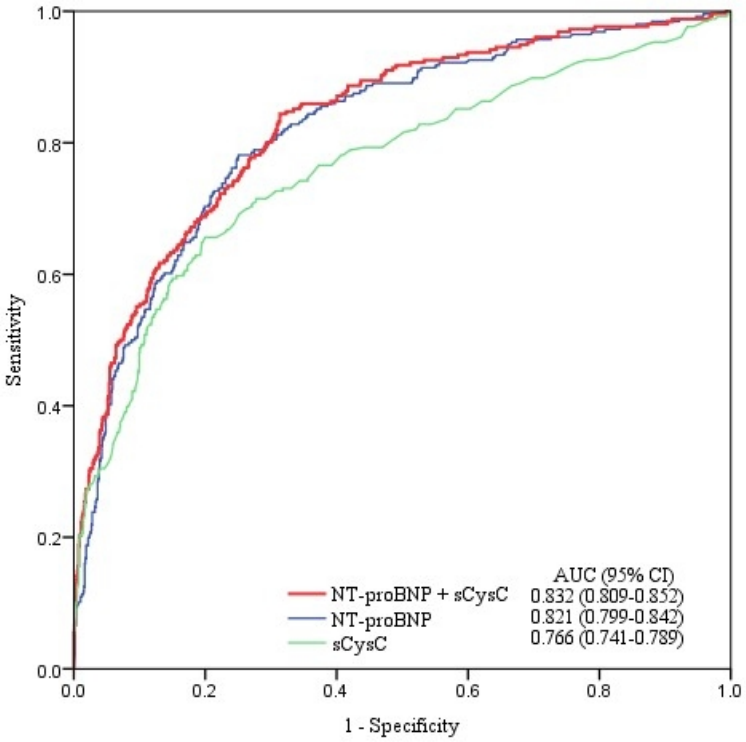

ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

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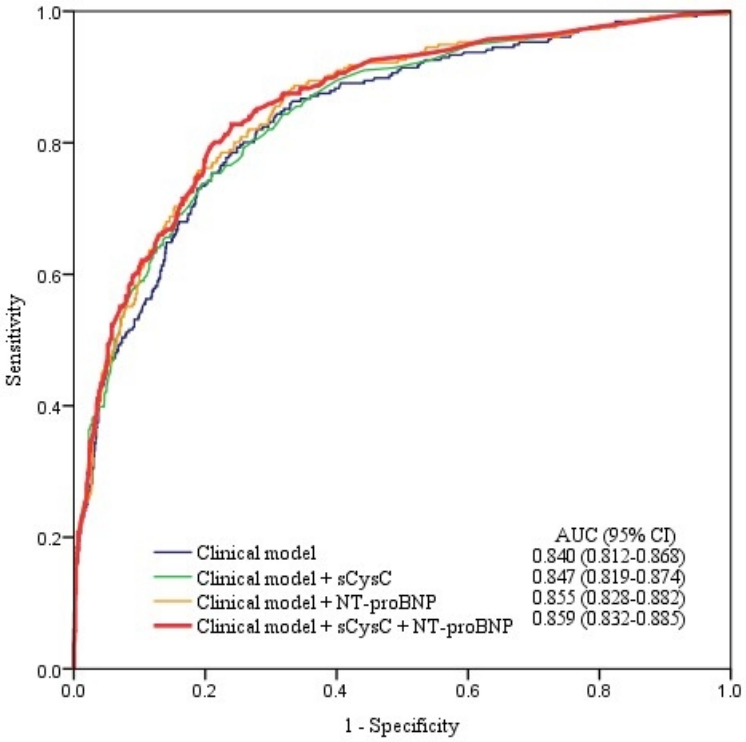

ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

165x132mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P7
Objectives	3	State specific objectives, including any prespecified hypotheses	P8
Methods			
Study design	4	Present key elements of study design early in the paper	P9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	P9
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	P9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P10-11
Bias	9	Describe any efforts to address potential sources of bias	P11
Study size	10	Explain how the study size was arrived at	P9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P11
		(*b*) Describe any methods used to examine subgroups and interactions	P11
		(*c*) Explain how missing data were addressed	P9
		(*d*) If applicable, explain how loss to follow-up was addressed	P11
		(*e*) Describe any sensitivity analyses	P11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P13
		(b) Give reasons for non-participation at each stage	P13
		(c) Consider use of a flow diagram	P13
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P13
		(b) Indicate number of participants with missing data for each variable of interest	Not Applicable
		(c) Summarise follow-up time (eg, average and total amount)	Not Applicable
Outcome data	15*	Report numbers of outcome events or summary measures over time	P13-15

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	P13-15 P14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	P15
Discussion			
Key results	18	Summarise key results with reference to study objectives	P21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P23
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P21-24
Generalisability	21	Discuss the generalisability (external validity) of the study results	P22-23
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P27

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Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults: A prospective, observational study

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Title

Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults: A prospective, observational study

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Abstract

Objective Serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cystatin C (sCysC) are available clinically and beneficial in diagnosing AKI. Our purpose is to identify the performance of their combined diagnosis for AKI in critically ill patients.

Design A prospectively-recruited, observational study was performed.

Setting Adults admitted to the intensive care unit of a tertiary hospital in China.

Participants A total of 1222 critically ill patients were enrolled in the study.

Main outcome measures To identify the performance of the combined diagnosis of serum NT-proBNP and sCysC for AKI in critically ill patients. The area under the receiver operating characteristic curve (AUC-ROC), category-free net reclassification index (NRI), and incremental discrimination improvement (IDI) were utilized for comparing the discriminative powers of a combined and single biomarker adjusted model of clinical variables enriched with NTproBNP and Cystatin C for AKI.

Results

AKI was detected in 256 out of 1222 included patients (20.9%). AUC-ROC for NT-proBNP and sCysC to detect AKI had a significantly higher accuracy than any individual biomarker ($P < 0.05$). After multivariate adjustment, a level of serum NT-proBNP $\geq$ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with those below the cutoff value. Similar results were obtained for sCysC levels ($P < 0.001$). To detect AKI, adding NT-proBNP and sCysC to a clinical model further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$). Moreover, the addition of these two to the clinical model significantly improved risk

reclassification of AKI beyond that of the clinical model alone or with single biomarker ($P < 0.05$), as measured by NRI and IDI.

Conclusions

In critically ill individuals, serum NT-proBNP, sCysC, and clinical risk factors combination improve the discriminative power for diagnosing AKI.

Keywords

Acute kidney injury, N-terminal pro-B-type natriuretic peptide, Serum cystatin C, Intensive care unit

Strengths and limitations of this study

This is the first study to identify the performance of serum NT-proBNP and sCysC combined diagnosis in detecting AKI.

This prospectively-recruited, observational study was of a long duration and had an adequate sample size.

We evaluated the ability of NT-proBNP and sCysC to diagnose AKI primarily by calculating area under the curve and category-free net reclassification index, and incremental discrimination improvement.

A limitation of this study was that the relationship between NT-proBNP and sCysC could not be dynamically assessed because we only measured their levels at the time of admission.

Introduction

Acute kidney injury (AKI) is a predominant clinical syndrome affecting more than 50% of patients who underwent treatment at intensive care unit (ICU)[1-3]. Increased morbidity, mortality, hospitalization length, and cost are extremely related to AKI[4-6], so early recognition of AKI is critical to guiding management. Urine production and serum creatinine were employed as diagnostic criteria for AKI in accordance with the recommendations of Kidney Disease Improving Global Outcomes (KDIGO) guidelines[7]. Changes in sCr or urine production cannot recognize early renal tubular injury prior to a reduction in glomerular filtration rate [8, 9]. Therefore, early and reliable AKI biomarkers are necessary to promote timely intervention and minimize complications. However, it is impossible for an individual biomarker to adequately evaluate the risk of AKI as a complex multifactorial syndrome [3, 10]. Combining diverse biomarkers in a clinical model evaluation could enhance early detection of AKI in critically ill patients[5, 11, 12].

A hemodynamic marker stress, N-terminal pro-B-type natriuretic peptide (NT-proBNP), has recently received attention as a potential predictor of AKI in a wide diversity of clinical settings[13-17]. High serum NT-proBNP level reflects hemodynamic instability, myocardial wall stress, myocardial ischemia, volume overload, sympathetic nervous system and renin-angiotensin-aldosterone system activation, all of which may contribute to AKI incidence[18-20]. However, there are finite data on clinical use of NT-proBNP for detecting AKI among critically ill patients.

Serum Cystatin C (sCysC), a 122-amino acid low-molecular-weight protein (13 kDa),

is a marker of glomerular filtration[9]. CysC has a half-life of about one-third that of sCr, causing CysC to reach homeostasis three times faster. These properties promote sCysC as an alternative marker of renal function[8]. To date, sCysC has been displayed to be conductive for the early identification of all-cause AKI[21].

Although serum NT-proBNP and sCysC are mainly used for the prognosis assessment of heart disease and the prediction of acute cardiac events with AKI [22-26], the diagnostic accuracy of their combination for AKI in ICU remains unknown. We performed a prospective study in the present research to evaluate the performance of serum NT-proBNP and sCysC at ICU admission, both independently and in combination, for AKI determination among critically ill adults.

Methods

Study design and participants

At Guangdong Provincial People's Hospital in China, a tertiary care hospital, a prospectively recruited observational research was performed in a mixed medical-surgical ICU. From December 2016 to December 2017, we consecutively enrolled patients aged 18 and up in a row. Pregnancy, renal replacement therapy (RRT) prior to ICU admittance, nephrectomy, end-stage renal disease (ESRD), renal transplant, rejection of consent, or missing admission information were all exclusion criteria. The study protocol strengthened the reporting requirements of observational studies in epidemiology[27] and standards for the reporting of diagnostic accuracy[28]. This study protocol was authorized by ethics committee of Guangdong Provincial People's Hospital. Additionally, all procedures were carried out consistent with applicable regulations and guidelines. All patients provided written informed consent.

Data collection

Clinical baseline data were collected prospectively. Within 1 h following ICU admission, blood samples were collected contemporaneously to determine sCr, serum NT-proBNP, and sCysC. Within 24 h after collection, all samples were analyzed in the Guangdong Provincial People's Hospital central laboratory utilizing standard protocol. When a patient is admitted to the ICU, sCr was measured and subsequently done at least once a day until discharge as part of routine clinical care. Throughout admission to ICU, urine production was also been recorded. We evaluated the following clinical variables: age, gender, body mass index (BMI), preexisting clinical conditions, sepsis,

admission type, baseline sCr, baseline estimated glomerular filtration rate (eGFR), Acute Physiology and Chronic Health Evaluation (APACHE) II score, ICU mortality, in-hospital mortality, duration of ICU admittance, length of hospitalization, ICU costs, and total costs. The outcome was the incidence of AKI following ICU enrollment within 1 week. To calculate eGFR, we employed the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [29].

Definitions

The KDIGO classification criteria were utilized to define AKI: as a rise in sCr by ≥ 0.3 mg/dL (26.5μmol/L) within 48 h or a rise in sCr to ≥ 1.5 times the baseline within 1 week, or urine output < 0.5 mL/kg/h for 6 h after ICU admission[7].

Relying on following principles, ranked in descending order of preference, a baseline sCr was affirmed[30]: (1) prior to ICU admittance, the most recent pre-ICU value between 30 and 365 days; (2) a stable pre-ICU value > 365 days prior to ICU admittance for patients < 40 years of age (stable definition is being within 15% of the lowest ICU measurement); (3) pre-ICU value > 365 days prior to ICU admittance and lower than the initial sCr at ICU admission; (4) a pre-ICU value (within 3 and 39 days prior to ICU admittance) lower than or equal to the initial sCr upon ICU admittance not obviously during AKI; (5) the least sCr value obtained at initial ICU admittance, the most recent ICU value, or the lowest value achieved to a 365-day follow up.

Biomarker measurement

The levels of sCysC and sCr were quantified through the UniCel DxC 800 Synchron system usage in compliance with the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). For sCysC, the intra- and inter-assay variation coefficients were 10% and 5%, respectively. Levels of serum NT-proBNP were quantified through an electrochemiluminescence immunoassay employing a Cobas® e602 system usage (Roche Diagnostics, Germany). For NT-proBNP, the uppermost limit of normal for those who seem to be healthy (95th percentage) has been 125 pg/mL. The coefficient of interassay variation for NT-proBNP was < 5%. Each patient's clinical features were blinded to the personnel measuring the biomarkers.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Statistical analysis

For statistical analyses, we utilized SPSS version 21.0 (SPSS, Chicago, IL, USA), MedCalc version 18.2.1 (MedCalc Software, Ostend, Belgium) and R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). The mean ± standard deviation or median (25th to 75th percentage, interquartile range) had been utilized to express continuous variables. Numbers (percent) were utilized to represent categorical variables. For nonnormally distributed continuous variables, the Wilcoxon rank-sum test was employed for intergroup difference measurement, and for categorical variables, the chi-square or Fisher's exact test was deployed.

NT-proBNP concentrations were extremely skewed and therefore were log10

transformed before inclusion in the models. Areas under the receiver operating characteristic curves (AUC-ROCs) were computed. The method exploited by DeLong et al. was used to compare AUC-ROCs between groups[31]. The biomarkers' sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios of the biomarkers were calculated. Youden's index for AKI detection identified the optimal cutoff values for individual biomarkers and their combination[32].

Logistic analysis was utilized to compute the odds ratios (ORs) and 95% confidence intervals for each factor: to identify the independent risk factors of AKI, with a forward stepwise method, in which the clinical variables with $P < 0.10$ in univariate analysis were incorporated into the multivariate logistic model. We categorized NT-proBNP and sCysC levels according to their cutoff values and then performed logistic regression on the created variables. We conducted multiple logistic regression analyses to calculate the adjusted ORs of AKI, which was based on the clinical risk factors for AKI.

The performance of AKI detection after adding NT-proBNP and sCysC, or any of them, into the clinical model as categorical variables was assessed by AUC-ROC, category-free net reclassification improvement (NRI) index and integrated discrimination improvement (IDI) index, as described previously[33, 34]. To better quantify how accurately the reference and reclassification model would perform with independent data, we adopted a 10-fold cross-validation[35]. All the tests were 2-tailed, and $P < 0.05$ was regarded statistically significant.

Results

Clinical data and outcomes

A total of 150 (10.9 %) of the 1372 adult patients enrolled in the study were excluded (Figure 1). Therefore, 1222 patients were enrolled and AKI occurred in 256 patients (20.9%). Patient baseline variables and outcomes are exhibited in Table 1. In comparison to patients without AKI, patients with AKI were elderly and were observed more frequently in those with comorbidities, including chronic kidney disease (CKD), hypertension, coronary artery disease (CAD), diabetes mellitus (DM), heart failure (HF), cerebrovascular disease and chronic obstructive pulmonary disease (COPD). Increased sCr, serum NT-proBNP and sCysC levels at admission, as well as increased APACHE II scores, were more prevalent in AKI patients. The baseline sCr and eGFR did not show significant differences between the two groups.

Indeed, AKI patients had a higher risk of adverse outcomes, a higher percentage of ICU and in-hospital mortality, higher expenses, a longer hospitalization duration, and a longer stay in ICU ($P < 0.001$) compared to those without AKI.

Detective abilities of the two biomarkers for AKI

To demonstrate the ability of these biomarkers for AKI detection, we used AUC-ROCs to calculate the two biomarkers, respectively, and in combination. AUC-ROCs for NT-proBNP and sCysC were computed for AKI detection (0.821 and 0.766, respectively). For AKI detection, NT-proBNP had a sensitivity of 78% and a specificity of 75%, while sCysC had high specificity but limited sensitivity. The cutoff values for NT-proBNP and sCysC were 204 pg/mL and 1.02 mg/L, respectively, yielding good sensitivity and specificity. We included NT-proBNP and sCysC in a multivariate logistic regression model to derived their combined AUC for comparison with the single biomarker's. The AUC-ROCs for AKI presented a better

performance by NT-proBNP and sCysC (0.832) than any individual biomarker ($P < 0.05$, Table 2 and Figure 2).

Multivariate logistic regression analyses of the two biomarkers for AKI detection

We stratified patients based on the two cutoff values of serum NT-proBNP and sCysC levels into two categories, respectively. Compared to those with serum NT-proBNP < 204 pg/mL, patients with serum NT-proBNP ≥ 204 pg/mL on admission exhibited a higher incidence of AKI (7.2% versus 45.1%, $P < 0.001$). AKI was found to have higher incidence (46.5%) in patients with sCysC ≥ 1.02 mg/L compared to those with sCysC < 1.02 mg/L (10.2%) ($P < 0.001$). Following clinical variables adjustment (including CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores, Table 3), a level of serum NT-proBNP ≥ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with NT-proBNP level below the cutoff value ($P < 0.001$). Similarly, patients with sCysC ≥ 1.02 mg/L were linked to 2.6-fold greater odds for AKI compared to those with sCysC < 1.02 mg/L ($P < 0.001$) (Table 4).

Discrimination and reclassification of the combination of biomarkers and clinical models for AKI

Considering the effect of adding NT-proBNP and sCysC, or any of them, to a clinical model as categorical variables for AKI detection, logistic regression analysis was employed. On ICU admittance for AKI diagnosis, potential available variables including age, sepsis, admission type, sCr, and APACHE II scores as well as comorbidities, including hypertension, DM, CKD, cerebrovascular disease, COPD, CAD, and HF were considered. The clinical model for detecting AKI involved CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores (Table 3). The cross-validated baseline performance characterized by accuracy and

Kappa for the clinical model was 0.844 and 0.448.

To evaluate the enhancement of discriminative capacity, a panel of NT-proBNP and sCysC was introduced to the above-mentioned model. As shown in Table 5, compared with the clinical model, the addition of NT-proBNP to the clinical model had a higher AUC-ROC ($P < 0.05$), and no statistically significant variation existed when sCyC was added to the clinical model. However, the risk reclassification was markedly improved through the addition of NT-proBNP or sCyC to the clinical model, as measured by category-free NRI and IDI ($P < 0.05$). Adding NT-proBNP and sCysC to a clinical model for AKI detection further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$) (Figure 3). The cross-validated baseline performance characterized by accuracy and Kappa for the clinical model enriched with NTproBNP and Cystatin C was 0.848 and 0.475. Moreover, this panel addition to the clinical model significantly enhanced the risk reclassification of AKI beyond that of the clinical model with or without any individual biomarkers ($P < 0.05$), with maximum NRI (0.531) and IDI (0.038).

Table 1 Clinical data and outcomes

Characteristics	Total (n=1222)	AKI (n=256)	No AKI (n=966)	*P* value
Age, years	57 (45-67)	63.5 (52-72)	55 (44-65)	<0.001
Male, n (%)	666 (54.5)	151 (59.0)	515 (53.3)	0.105
BMI, kg/m^2	22 (19.6-24.7)	22.3 (19.9-25.0)	21.9 (19.6-24.6)	0.395
Preexisting clinical conditions				
Hypertension, n (%)	252 (20.6)	81 (31.6)	171 (17.7)	<0.001
Diabetes mellitus, n (%)	121 (9.9)	52 (20.3)	69 (7.1)	<0.001
Chronic kidney disease, n (%)	26 (2.1)	21 (8.2)	5 (0.5)	<0.001
Cerebrovascular disease, n (%)	127 (10.4)	50 (19.5)	77 (8.0)	<0.001
Chronic obstructive pulmonary disease, n (%)	35 (2.9)	18 (7.0)	17 (1.8)	<0.001
Coronary artery disease, n (%)	53 (4.3)	24 (9.4)	29 (3.0)	<0.001
Heart failure, n (%)	31 (2.5)	22 (8.6)	9 (0.9)	<0.001
Cancer, n (%)	227 (18.6)	50 (19.5)	177 (18.3)	0.659
Sepsis, n (%)	98 (8.0)	70 (27.3)	28 (2.9)	<0.001
Admission type, n (%)				<0.001
Elective surgical, n (%)	863 (70.6)	84 (32.8)	779 (80.6)	
Emergency surgical, n (%)	83 (6.8)	33 (12.9)	50 (5.2)	
Medical, n (%)	276 (22.6)	139 (54.3)	137 (14.2)	
Baseline serum creatinine, mg/dL	0.78 (0.64-0.96)	0.76 (0.58-1.09)	0.79 (0.65-0.94)	0.949
Baseline eGFR, mL/min/1.73 m^2	119.4 (97.6-145.4)	122.9 (83.3-158.7)	118.7 (100.4-142.9)	0.950
Parameters at ICU admission				
sCr, mg/dl	0.87 (0.72-1.07)	0.97 (0.72-1.55)	0.85 (0.72-1.02)	<0.001
NT-proBNP, pg/mL	97.1 (29.4-464.4)	871.0 (225.8-2919.3)	66.2 (23.7-206.2)	<0.001
sCysC, mg/L	0.78 (0.61-1.06)	1.15 (0.83-1.85)	0.75 (0.58-0.94)	<0.001
APACHE Ⅱ score	7 (4-10)	11 (8-16)	6 (4-9)	<0.001
UP, ml/kg/h	1.67 (1.20-2.26)	1.37 (0.89-2.20)	1.73 (1.26-2.27)	<0.001
Outcomes				
ICU mortality, n (%)	40 (3.3)	33 (12.9)	7 (0.7)	<0.001
In-hospital mortality, n (%)	53 (4.3)	40 (15.6)	13 (1.3)	<0.001
ICU stay, days	2 (2-4)	6 (3-13.3)	2 (2-3)	<0.001
Hospital stay, days	16 (12-23)	22 (13-33.3)	15 (11-21)	<0.001
ICU costs, CNY	39461.7 (28966.2-59437.2)	66140.1 (36526.0-131843.8)	37667.5 (27881.5-48238.4)	<0.001
Total costs, CNY	58295.3 (44201.6-98602.8)	128945.6 (71506.8-199685.4)	52773.8 (40856.2-78718.9)	<0.001

Abbreviations: AKI, acute kidney injury; BMI, body mass index; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; sCr, serum creatinine, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score; UP, Urine production first 24 h after admission; CNY, Chinese yuan.

Continuous variables are expressed as mean ± standard deviation or median (25th to 75th percentile, interquartile range). Categorical variables are expressed as a n (%).

Table 2 Detective characteristics of the two biomarkers for AKI

Logistic regression models	AUC-ROC[a] (95%CI)	Cutoff [b]	Sensitivity	Specificity	(+) LR	(-) LR	PPV	NPV
sCysC	0.766 (0.741-0.789)	1.02 mg/L	0.66	0.80	3.28	0.43	0.47	0.90
NT-proBNP	0.821 (0.799-0.842) [c]	204.00 pg/mL	0.78	0.75	3.12	0.29	0.45	0.93
NT-proBNP+ sCysC	0.832 (0.809-0.852) [d]	0.15[e]	0.84	0.69	2.69	0.23	0.42	0.94

Abbreviations: AKI, acute kidney injury; AUC-ROC, area under the receiver operating characteristic curve; CI, confidence interval; (+) LR, Positive likelihood ratio; (-) LR, negative likelihood ratio; PPV, Positive predictive value; NPV, Negative predictive value; sCysC, Serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

[a] Values are presented as AUC-ROC (95% CI)

[b] Ideal cutoff value according to Youden's index

[c] $P < 0.05$ vs. sCysC (P=0.0011)

[d] $P < 0.05$ vs. NT-proBNP (P=0.0145), sCysC(P<0.0001)

[e] Cutoff points of the biomarker panels were the predicted probabilities generated from the multiple logistic regression model

Table 3 Logistic analyses of clinical risk factors for AKI detection

Variables	Univariate analysis		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.029 (1.019-1.038)	<0.001		
Hypertension	2.152 (1.577-2.937)	<0.001		
Diabetes mellitus	3.314 (2.242-4.898)	<0.001		
Chronic kidney disease	17.175 (6.410-46.023)	<0.001	4.182 (1.250-13.998)	0.020
Cerebrovascular disease	2.802 (1.903-4.126)	<0.001		
Chronic obstructive pulmonary disease	4.222 (2.143-8.316)	<0.001		
Coronary artery disease	3.342 (1.910-5.849)	<0.001		
Heart failure	9.997 (4.544-21.997)	<0.001	3.487 (1.365-8.911)	0.009
Sepsis	12.608 (7.914-20.084)	<0.001	5.033 (2.914-8.692)	<0.001
Admission type		<0.001		<0.001
Elective surgical	1.0 (Referent)		1.0 (Referent)	
Emergency surgical	6.121 (3.735-10.030)	<0.001	3.493 (2.023-6.032)	<0.001
Medical	9.409 (6.791-13.037)	<0.001	3.237 (2.157-4.858)	<0.001
sCr	3.135 (2.385-4.120)	<0.001	1.538 (1.149-2.058)	0.004
APACHE Ⅱ score	1.215 (1.182-1.249)	<0.001	1.101 (1.066-1.137)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval; sCr, serum creatinine; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score.

Table 4 Multivariate logistic regression analyses of the two biomarkers for AKI detection

Variables	AKI, %	Unadjusted OR (95% CI)	*P* value	Adjusted OR[a] (95% CI)	*P* value
NT-proBNP (pg/mL, n)					
<204, 779	7.2	1.0 (Referent)		1.0 (Referent)	
≥204, 443	45.1	10.626 (7.639-14.781)	<0.001	3.460 (2.307-5.189)	<0.001
sCysC layered by the cut-off value (mg/L, n)					
<1.02, 861	10.2	1.0 (Referent)		1.0 (Referent)	
≥1.02, 361	46.5	7.646 (5.651-10.345)	<0.001	2.649 (1.842-3.810)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, Serum cystatin C.

a Adjusted for chronic kidney disease, heart failure, sepsis, admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Table 5 Discrimination and reclassification of the combination of biomarkers and clinical model for AKI

	AUC-ROC (95%CI)	*P* value[a]	Category-Free NRI (95%CI)	*P* value[a]	IDI (95%CI)	*P* value[a]
Clinical model[b]	0.840 (0.812-0.868)					
Clinical model + sCysC	0.847 (0.819-0.874)	0.163	0.193 (0.052-0.405)	0.036	0.017 (0.009-0.026)	<0.001
Clinical model + NT-proBNP	0.855 (0.828-0.882)	0.013	0.462 (0.196-0.747)	0.001	0.028 (0.016-0.039)	<0.001
Clinical model + NT-proBNP + sCysC	0.859 (0.832-0.885)	0.006	0.531 (0.238-0.741)	<0.001	0.038 (0.025-0.051)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + sCysC	-	0.015	0.328 (0.139-0.553)	0.002	0.021 (0.010-0.031)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + NT-proBNP	-	0.223	0.167 (0.013-0.285)	0.017	0.011 (0.004-0.018)	0.003

Abbreviations: AUC-ROC, area under the receiver operating characteristic curve; NRI, net reclassification index; IDI, incremental discrimination improvement; AKI, acute kidney injury; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

a Biomarker + clinical model versus clinical model.

b The clinical model for detecting AKI is composed of chronic kidney disease, heart failure, sepsis, Admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Hosmer–Lemeshow goodness-of-fit test: for clinical model, chi-squared value=14.249(P=0.075); for clinical model + sCysC, chi-squared value=6.971 (P=0.54); for clinical model + NT-proBNP, chi-squared value=9.362 (P=0.313); or clinical model + NT-proBNP +sCysC, chi-squared value=4.245 (P=0.834).

Discussion

The study's key finding was that the combination of NT-proBNP and sCysC yields greater discriminative ability for AKI detection at ICU admission with or without a clinical model in critically ill adults. The finding indicates that assessing both serum NT-proBNP and sCysC levels on admission may assist with the early diagnosis and risk stratification of AKI in critically ill adults.

One of the most prevalent complications occurring in a variety of clinical settings is AKI, especially for critically ill patients [4, 6, 36, 37]. The development of AKI, as the same as its severity, is strongly associated with increased mortality[3, 36]. However, early identification is challenging when sCr or urine production changes are used to detect AKI[38], and precise clinical predictors are not widely known. Numerous studies have found and confirmed the accuracy and additional clinical benefits of these renal biomarkers for early AKI diagnosis, such as insulin-like growth factor-binding protein, matrix metalloproteinase-7, tissue inhibitor metalloproteinase-2, angiotensinogen, sCysC, neutrophil gelatinase-associated lipocalin and so on[5, 9, 12, 39, 40]. However, some novel biomarkers are not being used in clinical practice, due to insufficient evidence or previously unavailable commercially. Thus, the rational application of clinically available biomarkers is more practical and economical.

NT-proBNP, a widely used marker of hemodynamic stress, is a polypeptide secreted by the ventricles and its role is to facilitate natriuresis[17, 41]. In patients with elevated CVP, the raised pressure may be transmitted to the renal veins, resulting in renal congestion and reduced glomerular filtration[14, 42, 43]. It makes sense that NT-proBNP is associated with the development of AKI. The clinical application of serum NT-proBNP in cardiac disease has been

extensively confirmed and finds its predictive value for AKI development in patients with HF, coronary angiography, or percutaneous coronary intervention [19, 23, 44, 45]. Several studies have elucidated the link between NT-proBNP and AKI incidence after cardiac or noncardiac surgery[13, 46-48]. However, its utility for AKI detection has not been fully evaluated in general ICU, and it is necessary to verify the reliability and universality of NT-proBNP in heterogeneous populations. The data demonstrated herein not only maintain NT-proBNP as a risk factor for AKI but also indicate that it can enhance the risk reclassification and discrimination for AKI in ICU.

A single biomarker is insufficient to express the multiple pathophysiological mechanisms of AKI, especially for critically ill patients, as AKI is a heterogeneous syndrome[9, 10]. There is no consensus on which specific markers should be combined to detect AKI. Numerous studies have revealed that combining different biomarkers utilized to detect AKI can improve predictive abilities[5, 49, 50]. Naruse et al. showed that combining urinary liver-type fatty-acid binding protein and serum NT-proBNP can enhance early prediction of AKI in patients in medical cardiac ICUs[15]. Similar to such studies, we identified that the combination of two markers, serum NT-probNP and sCysC, improved the diagnostic performance of AKI.

CysC is a glomerular filtration biomarker that can be utilized to anticipate the development of AKI and undesirable outcomes[8, 9]. The application and performance of CysC for AKI prediction have been demonstrated in various clinical settings[5, 11, 51-53]. In individuals with ST-segment elevation myocardial infarction, a combination of B-type natriuretic peptide and CysC may contribute to risk stratification for AKI[25]. In the present cohort, sCysC had a slightly higher specificity than NT-proBNP in the detection of AKI, but its sensitivity is limited. The

ability of sCysC for detecting AKI was fine, but its AUC-ROC was not as great as NT-proBNP. Moreover, the combination of serum NT-proBNP and sCysC at ICU admission had the highest AUC-ROC. These data indicate that the simultaneous measurement of serum NT-proBNP and sCysC at ICU admission could improve the early identification of AKI.

Biomarkers for AKI, which indicates the various underlying pathophysiological mechanisms involved in AKI incidence, might be superior to individual biomarkers alone[3, 6]. It is also important that these biomarkers profit from being easily measurable, readily accessible, comparatively cheap, and with an elevated level of sensitivity and specificity. In the present study, for AKI detection NT-proBNP acted as a hemodynamic stress biomarker with high sensitivity and specificity, and sCysC had high specificity as a functional biomarker. Even after clinical risk variables adjustment, elevated NT-proBNP is an independent risk factor for AKI. The NT-proBNP addition to the clinical model significantly enhanced risk reclassification, as demonstrated by category-free NRI and IDI. Moreover, our results identified that the addition of NT-proBNP to sCysC markedly improved their detective abilities as biomarkers. Adding biomarkers to the clinical model further improved the diagnostic accuracy of AKI, as measured by AUC-ROCs. These data suggest that a single biomarker is insufficient for early diagnosis of AKI. Therefore, the method of combining different biomarkers may be of greater use.

There are some limitations for this study. First, it was a single-center study, with an unproven external validity. Second, there were only 26 patients with CKD patients and 31 HF patients enrolled, and hence we were unable to stratify our group relying on eGFR or cardiac function at baseline. Accordingly, future studies should be administrated in these subgroups. Last, we did not accomplish routine echocardiography in all patients to associate NT-proBNP levels with

ventricular dilatation or other pathways that may promote NT-proBNP release. Despite these limitations, we believe that our findings have clinical implications and should facilitate further research to confirm our results.

Conclusion

In the present cohort, simultaneous measurement of NT-proBNP and sCysC at ICU admission increases the early identification of AKI beyond that of biomarker in isolation, and that the combination of the two biomarkers and clinical risk factors improves the discriminative ability for AKI detection in critically ill adults.

Abbreviations

(−) LR, negative likelihood ratios

(+) LR, positive likelihood ratios

AKI, Acute kidney injury

APACHE II score, Acute Physiology and Chronic Health Evaluation II score

AUC-ROC, area under the receiver operating characteristic curve

BMI, body mass index

CAD, coronary artery disease

CI, confidence interval

CNY, Chinese yuan

CKD, chronic kidney disease

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration

COPD, chronic obstructive pulmonary disease

DM, diabetes mellitus

eGFR, estimated glomerular filtration rate

ESRD, end-stage renal disease

HF, heart failure

ICU, intensive care unit

IDI, incremental discrimination improvement

KDIGO, Kidney Disease Improving Global Outcomes

NPV, negative predictive values

NRI net reclassification index

NT-proBNP, N-terminal pro-B-type natriuretic peptide

ORs, odds ratios

PPV, positive predictive values

RRT, renal replacement therapy

sCr, serum creatinine

sCysC, serum cystatin C

UP, Urine production first 24 h after admission

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Authors' contributions

JD, LLH, YFL and LHH equally contributed to the design of the research, analysis and interpretation of the data. CBC and JD contributed to the conception and design of the research as well as interpretation of the data, and critically revised the manuscript. JD, LLH, YFL, LHH, JX, HF and YL performed the research and collected data. All authors contributed to the acquisition and analysis of the data, drafted the manuscript, and agree to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during this study are not publicly available, owing to currently ongoing research studies, but the data are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethics committee of the Guangdong Provincial People's Hospital approved the study design, protocol, ethical issue, and data and sample collection (No. GDREC2015396H(R1)). Written informed consent was obtained from each patient or from the appropriate surrogates.

Patient consent for publication

Not required.

Consent for publication

Not applicable. No individual personal data are included in the study. All patients provided necessary consent to participate in the present study.

Competing interests

The authors declare that they have no competing interests.

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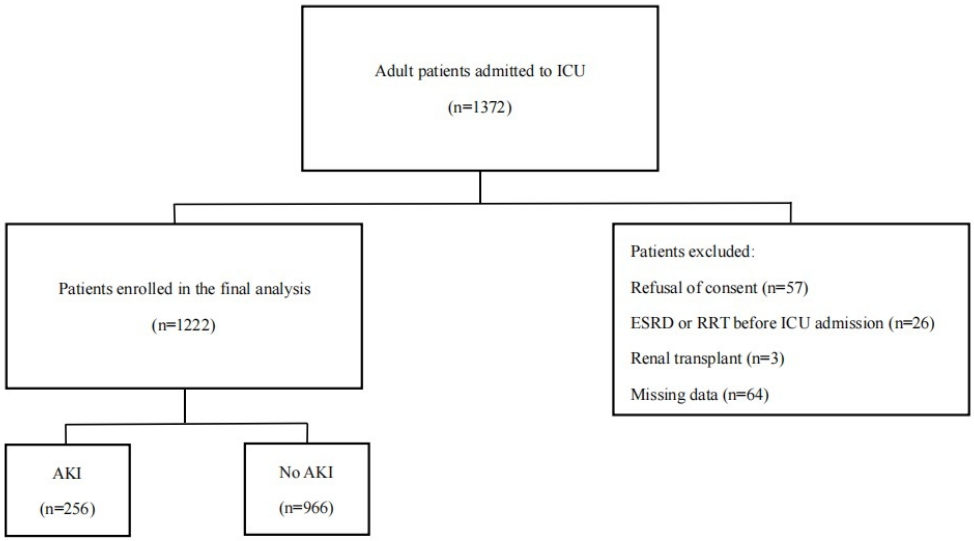
Figure legends

Figure 1. Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

Figure 2. ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

Figure 3. ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.



Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

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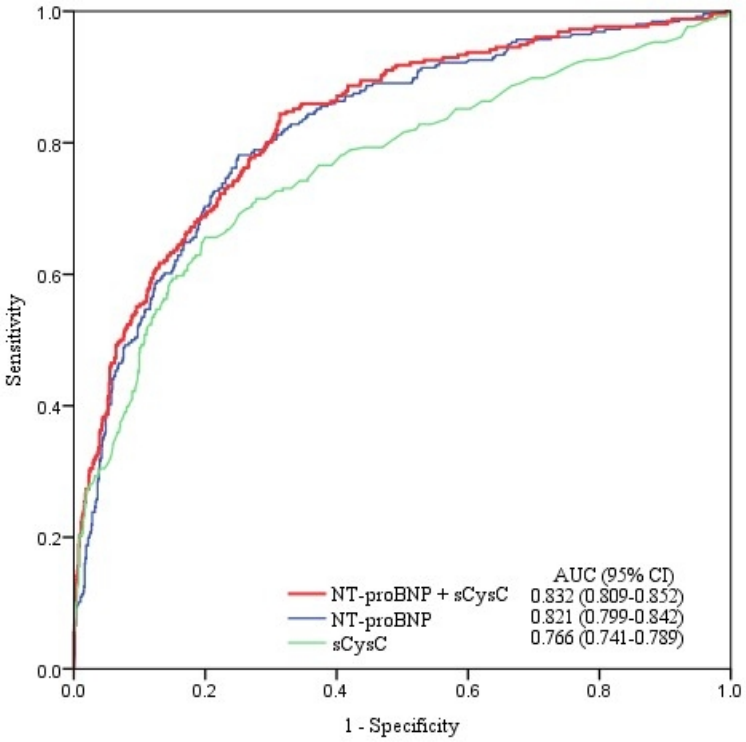

ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

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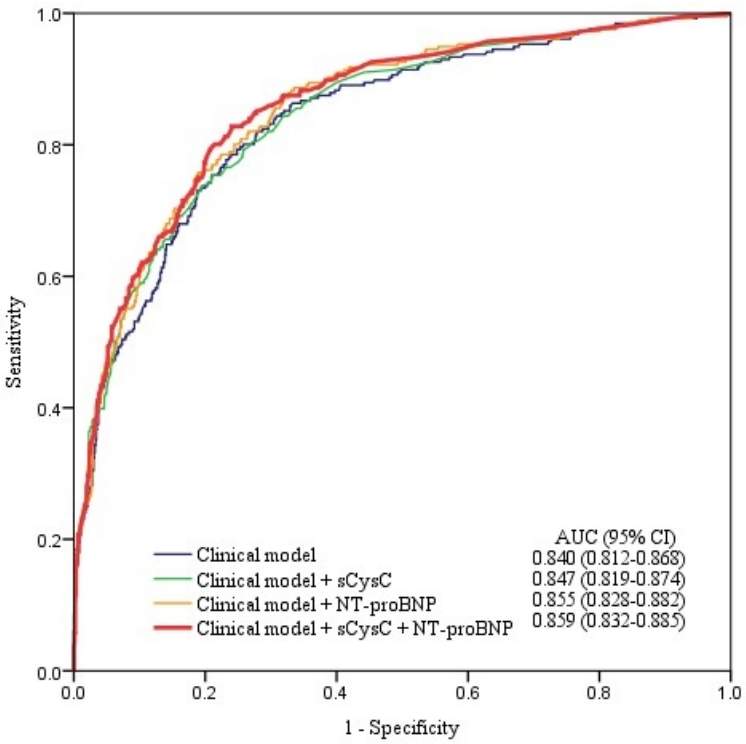

ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

165x132mm (96 x 96 DPI)

TRIPOD

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	4-5
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	7-8
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	8
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	9-10
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	9-10
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	9
	5b	Describe eligibility criteria for participants.	9
	5c	Give details of treatments received, if relevant.	NA
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	10
	6b	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	9-10
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	Explain how the study size was arrived at.	9
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	9
Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	9
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	11-12
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	12
Risk groups	11	Provide details on how risk groups were created, if done.	11-12
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	13, figure1
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	13
Model development	14a	Specify the number of participants and outcome events in each analysis.	14-15
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	14
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	13-15
	15b	Explain how to the use the prediction model.	14
Model performance	16	Report performance measures (with CIs) for the prediction model.	14-15
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	23-24
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	21-23
Implications	20	Discuss the potential clinical use of the model and implications for future research.	21-24
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	25-32
Funding	22	Give the source of funding and the role of the funders for the present study.	27

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

BMJ Open

Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults: A prospective, observational study

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Title

Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults: A prospective, observational study

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Abstract

Objective Serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cystatin C (sCysC) are available clinically and beneficial in diagnosing AKI. Our purpose is to identify the performance of their combined diagnosis for AKI in critically ill patients.

Design A prospectively-recruited, observational study was performed.

Setting Adults admitted to the intensive care unit of a tertiary hospital in China.

Participants A total of 1222 critically ill patients were enrolled in the study.

Main outcome measures To identify the performance of the combined diagnosis of serum NT-proBNP and sCysC for AKI in critically ill patients. The area under the receiver operating characteristic curve (AUC-ROC), category-free net reclassification index (NRI), and incremental discrimination improvement (IDI) were utilized for comparing the discriminative powers of a combined and single biomarker adjusted model of clinical variables enriched with NTproBNP and Cystatin C for AKI.

Results

AKI was detected in 256 out of 1222 included patients (20.9%). AUC-ROC for NT-proBNP and sCysC to detect AKI had a significantly higher accuracy than any individual biomarker ($P < 0.05$). After multivariate adjustment, a level of serum NT-proBNP $\geq$ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with those below the cutoff value. Similar results were obtained for sCysC levels ($P < 0.001$). To detect AKI, adding NT-proBNP and sCysC to a clinical model further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$). Moreover, the addition of these two to the clinical model significantly improved risk

reclassification of AKI beyond that of the clinical model alone or with single biomarker ($P < 0.05$), as measured by NRI and IDI.

Conclusions

In critically ill individuals, serum NT-proBNP, sCysC, and clinical risk factors combination improve the discriminative power for diagnosing AKI.

Keywords

Acute kidney injury, N-terminal pro-B-type natriuretic peptide, Serum cystatin C, Intensive care unit

Strengths and limitations of this study

This study is the first to evaluate the performance of serum NT-proBNP combined with sCysC in detecting AKI.

This prospective, observational study has a long duration and an adequate sample size.

We evaluated the combined efficacy of serum NT-proBNP and sCysC in the diagnosis of AKI by calculating area under the curve and category-free net reclassification index and incremental discrimination improvement.

The limitation of this study is that dynamic assessment of NT-proBNP and sCysC changes was not available as only measured their levels at admission.

Introduction

Acute kidney injury (AKI) is a predominant clinical syndrome affecting more than 50% of patients who underwent treatment at intensive care unit (ICU)[1-3]. Increased morbidity, mortality, hospitalization length, and cost are extremely related to AKI[4-6], so early recognition of AKI is critical to guiding management. Urine production and serum creatinine were employed as diagnostic criteria for AKI in accordance with the recommendations of Kidney Disease Improving Global Outcomes (KDIGO) guidelines[7]. Changes in sCr or urine production cannot recognize early renal tubular injury prior to a reduction in glomerular filtration rate [8, 9]. Therefore, early and reliable AKI biomarkers are necessary to promote timely intervention and minimize complications. However, it is impossible for an individual biomarker to adequately evaluate the risk of AKI as a complex multifactorial syndrome [3, 10]. Combining diverse biomarkers in a clinical model evaluation could enhance early detection of AKI in critically ill patients[5, 11, 12].

A hemodynamic marker stress, N-terminal pro-B-type natriuretic peptide (NT-proBNP), has recently received attention as a potential predictor of AKI in a wide diversity of clinical settings[13-17]. High serum NT-proBNP level reflects hemodynamic instability, myocardial wall stress, myocardial ischemia, volume overload, sympathetic nervous system and renin-angiotensin-aldosterone system activation, all of which may contribute to AKI incidence[18-20]. However, there are finite data on clinical use of NT-proBNP for detecting AKI among critically ill patients.

Serum Cystatin C (sCysC), a 122-amino acid low-molecular-weight protein (13 kDa),

is a marker of glomerular filtration[9]. CysC has a half-life of about one-third that of sCr, causing CysC to reach homeostasis three times faster. These properties promote sCysC as an alternative marker of renal function[8]. To date, sCysC has been displayed to be conductive for the early identification of all-cause AKI[21].

Although serum NT-proBNP and sCysC are mainly used for the prognosis assessment of heart disease and the prediction of acute cardiac events with AKI [22-26], the diagnostic accuracy of their combination for AKI in ICU remains unknown. We performed a prospective study in the present research to evaluate the performance of serum NT-proBNP and sCysC at ICU admission, both independently and in combination, for AKI determination among critically ill adults.

Methods

Study design and participants

At Guangdong Provincial People's Hospital in China, a tertiary care hospital, a prospectively recruited observational research was performed in a mixed medical-surgical ICU. From December 2016 to December 2017, we consecutively enrolled patients aged 18 and up in a row. Pregnancy, renal replacement therapy (RRT) prior to ICU admittance, nephrectomy, end-stage renal disease (ESRD), renal transplant, rejection of consent, or missing admission information were all exclusion criteria. The study protocol strengthened the reporting requirements of observational studies in epidemiology[27] and standards for the reporting of diagnostic accuracy[28]. This study protocol was authorized by ethics committee of Guangdong Provincial People's Hospital. Additionally, all procedures were carried out consistent with applicable regulations and guidelines. All patients provided written informed consent.

Data collection

Clinical baseline data were collected prospectively. Within 1 h following ICU admission, blood samples were collected contemporaneously to determine sCr, serum NT-proBNP, and sCysC. Within 24 h after collection, all samples were analyzed in the Guangdong Provincial People's Hospital central laboratory utilizing standard protocol. When a patient is admitted to the ICU, sCr was measured and subsequently done at least once a day until discharge as part of routine clinical care. Throughout admission to ICU, urine production was also been recorded. We evaluated the following clinical variables: age, gender, body mass index (BMI), preexisting clinical conditions, sepsis,

admission type, baseline sCr, baseline estimated glomerular filtration rate (eGFR), Acute Physiology and Chronic Health Evaluation (APACHE) II score, ICU mortality, in-hospital mortality, duration of ICU admittance, length of hospitalization, ICU costs, and total costs. The outcome was the incidence of AKI following ICU enrollment within 1 week. To calculate eGFR, we employed the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [29].

Definitions

The KDIGO classification criteria were utilized to define AKI: as a rise in sCr by ≥ 0.3 mg/dL (26.5μmol/L) within 48 h or a rise in sCr to ≥ 1.5 times the baseline within 1 week, or urine output < 0.5 mL/kg/h for 6 h after ICU admission[7].

Relying on following principles, ranked in descending order of preference, a baseline sCr was affirmed[30]: (1) prior to ICU admittance, the most recent pre-ICU value between 30 and 365 days; (2) a stable pre-ICU value > 365 days prior to ICU admittance for patients < 40 years of age (stable definition is being within 15% of the lowest ICU measurement); (3) pre-ICU value > 365 days prior to ICU admittance and lower than the initial sCr at ICU admission; (4) a pre-ICU value (within 3 and 39 days prior to ICU admittance) lower than or equal to the initial sCr upon ICU admittance not obviously during AKI; (5) the least sCr value obtained at initial ICU admittance, the most recent ICU value, or the lowest value achieved to a 365-day follow up.

Biomarker measurement

The levels of sCysC and sCr were quantified through the UniCel DxC 800 Synchron system usage in compliance with the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). For sCysC, the intra- and inter-assay variation coefficients were 10% and 5%, respectively. Levels of serum NT-proBNP were quantified through an electrochemiluminescence immunoassay employing a Cobas® e602 system usage (Roche Diagnostics, Germany). For NT-proBNP, the uppermost limit of normal for those who seem to be healthy (95th percentage) has been 125 pg/mL. The coefficient of interassay variation for NT-proBNP was < 5%. Each patient's clinical features were blinded to the personnel measuring the biomarkers.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Statistical analysis

For statistical analyses, we utilized SPSS version 21.0 (SPSS, Chicago, IL, USA), MedCalc version 18.2.1 (MedCalc Software, Ostend, Belgium) and R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). The mean ± standard deviation or median (25th to 75th percentage, interquartile range) had been utilized to express continuous variables. Numbers (percent) were utilized to represent categorical variables. For nonnormally distributed continuous variables, the Wilcoxon rank-sum test was employed for intergroup difference measurement, and for categorical variables, the chi-square or Fisher's exact test was deployed.

NT-proBNP concentrations were extremely skewed and therefore were log10

transformed before inclusion in the models. Areas under the receiver operating characteristic curves (AUC-ROCs) were computed. The method exploited by DeLong et al. was used to compare AUC-ROCs between groups[31]. The biomarkers' sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios of the biomarkers were calculated. Youden's index for AKI detection identified the optimal cutoff values for individual biomarkers and their combination[32].

Logistic analysis was utilized to compute the odds ratios (ORs) and 95% confidence intervals for each factor: to identify the independent risk factors of AKI, with a forward stepwise method, in which the clinical variables with $P < 0.10$ in univariate analysis were incorporated into the multivariate logistic model. We categorized NT-proBNP and sCysC levels according to their cutoff values and then performed logistic regression on the created variables. We conducted multiple logistic regression analyses to calculate the adjusted ORs of AKI, which was based on the clinical risk factors for AKI.

The performance of AKI detection after adding NT-proBNP and sCysC, or any of them, into the clinical model as categorical variables was assessed by AUC-ROC, category-free net reclassification improvement (NRI) index and integrated discrimination improvement (IDI) index, as described previously[33, 34]. To better quantify how accurately the reference and reclassification model would perform with independent data, we adopted a 10-fold cross-validation[35]. All the tests were 2-tailed, and $P < 0.05$ was regarded statistically significant.

Results

Clinical data and outcomes

A total of 150 (10.9 %) of the 1372 adult patients enrolled in the study were excluded (Figure 1). Therefore, 1222 patients were enrolled and AKI occurred in 256 patients (20.9%). Patient baseline variables and outcomes are exhibited in Table 1. In comparison to patients without AKI, patients with AKI were elderly and were observed more frequently in those with comorbidities, including chronic kidney disease (CKD), hypertension, coronary artery disease (CAD), diabetes mellitus (DM), heart failure (HF), cerebrovascular disease and chronic obstructive pulmonary disease (COPD). Increased sCr, serum NT-proBNP and sCysC levels at admission, as well as increased APACHE II scores, were more prevalent in AKI patients. The baseline sCr and eGFR did not show significant differences between the two groups.

Indeed, AKI patients had a higher risk of adverse outcomes, a higher percentage of ICU and in-hospital mortality, higher expenses, a longer hospitalization duration, and a longer stay in ICU ($P < 0.001$) compared to those without AKI.

Detective abilities of the two biomarkers for AKI

To demonstrate the ability of these biomarkers for AKI detection, we used AUC-ROCs to calculate the two biomarkers, respectively, and in combination. AUC-ROCs for NT-proBNP and sCysC were computed for AKI detection (0.821 and 0.766, respectively). For AKI detection, NT-proBNP had a sensitivity of 78% and a specificity of 75%, while sCysC had high specificity but limited sensitivity. The cutoff values for NT-proBNP and sCysC were 204 pg/mL and 1.02 mg/L, respectively, yielding good sensitivity and specificity. We included NT-proBNP and sCysC in a multivariate logistic regression model to derived their combined AUC for comparison with the single biomarker's. The AUC-ROCs for AKI presented a better

performance by NT-proBNP and sCysC (0.832) than any individual biomarker ($P < 0.05$, Table 2 and Figure 2).

Multivariate logistic regression analyses of the two biomarkers for AKI detection

We stratified patients based on the two cutoff values of serum NT-proBNP and sCysC levels into two categories, respectively. Compared to those with serum NT-proBNP < 204 pg/mL, patients with serum NT-proBNP ≥ 204 pg/mL on admission exhibited a higher incidence of AKI (7.2% versus 45.1%, $P < 0.001$). AKI was found to have higher incidence (46.5%) in patients with sCysC ≥ 1.02 mg/L compared to those with sCysC < 1.02 mg/L (10.2%) ($P < 0.001$). Following clinical variables adjustment (including CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores, Table 3), a level of serum NT-proBNP ≥ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with NT-proBNP level below the cutoff value ($P < 0.001$). Similarly, patients with sCysC ≥ 1.02 mg/L were linked to 2.6-fold greater odds for AKI compared to those with sCysC < 1.02 mg/L ($P < 0.001$) (Table 4).

Discrimination and reclassification of the combination of biomarkers and clinical models for AKI

Considering the effect of adding NT-proBNP and sCysC, or any of them, to a clinical model as categorical variables for AKI detection, logistic regression analysis was employed. On ICU admittance for AKI diagnosis, potential available variables including age, sepsis, admission type, sCr, and APACHE II scores as well as comorbidities, including hypertension, DM, CKD, cerebrovascular disease, COPD, CAD, and HF were considered. The clinical model for detecting AKI involved CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores (Table 3). The cross-validated baseline performance characterized by accuracy and

Kappa for the clinical model was 0.844 and 0.448.

To evaluate the enhancement of discriminative capacity, a panel of NT-proBNP and sCysC was introduced to the above-mentioned model. As shown in Table 5, compared with the clinical model, the addition of NT-proBNP to the clinical model had a higher AUC-ROC ($P < 0.05$), and no statistically significant variation existed when sCyC was added to the clinical model. However, the risk reclassification was markedly improved through the addition of NT-proBNP or sCyC to the clinical model, as measured by category-free NRI and IDI ($P < 0.05$). Adding NT-proBNP and sCysC to a clinical model for AKI detection further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$) (Figure 3). The cross-validated baseline performance characterized by accuracy and Kappa for the clinical model enriched with NTproBNP and Cystatin C was 0.848 and 0.475. Moreover, this panel addition to the clinical model significantly enhanced the risk reclassification of AKI beyond that of the clinical model with or without any individual biomarkers ($P < 0.05$), with maximum NRI (0.531) and IDI (0.038).

Table 1 Clinical data and outcomes

Characteristics	Total (n=1222)	AKI (n=256)	No AKI (n=966)	*P* value
Age, years	57 (45-67)	63.5 (52-72)	55 (44-65)	<0.001
Male, n (%)	666 (54.5)	151 (59.0)	515 (53.3)	0.105
BMI, kg/m^2	22 (19.6-24.7)	22.3 (19.9-25.0)	21.9 (19.6-24.6)	0.395
Preexisting clinical conditions				
Hypertension, n (%)	252 (20.6)	81 (31.6)	171 (17.7)	<0.001
Diabetes mellitus, n (%)	121 (9.9)	52 (20.3)	69 (7.1)	<0.001
Chronic kidney disease, n (%)	26 (2.1)	21 (8.2)	5 (0.5)	<0.001
Cerebrovascular disease, n (%)	127 (10.4)	50 (19.5)	77 (8.0)	<0.001
Chronic obstructive pulmonary disease, n (%)	35 (2.9)	18 (7.0)	17 (1.8)	<0.001
Coronary artery disease, n (%)	53 (4.3)	24 (9.4)	29 (3.0)	<0.001
Heart failure, n (%)	31 (2.5)	22 (8.6)	9 (0.9)	<0.001
Cancer, n (%)	227 (18.6)	50 (19.5)	177 (18.3)	0.659
Sepsis, n (%)	98 (8.0)	70 (27.3)	28 (2.9)	<0.001
Admission type, n (%)				<0.001
Elective surgical, n (%)	863 (70.6)	84 (32.8)	779 (80.6)	
Emergency surgical, n (%)	83 (6.8)	33 (12.9)	50 (5.2)	
Medical, n (%)	276 (22.6)	139 (54.3)	137 (14.2)	
Baseline serum creatinine, mg/dL	0.78 (0.64-0.96)	0.76 (0.58-1.09)	0.79 (0.65-0.94)	0.949
Baseline eGFR, mL/min/1.73 m^2	119.4 (97.6-145.4)	122.9 (83.3-158.7)	118.7 (100.4-142.9)	0.950
Parameters at ICU admission				
sCr, mg/dl	0.87 (0.72-1.07)	0.97 (0.72-1.55)	0.85 (0.72-1.02)	<0.001
NT-proBNP, pg/mL	97.1 (29.4-464.4)	871.0 (225.8-2919.3)	66.2 (23.7-206.2)	<0.001
sCysC, mg/L	0.78 (0.61-1.06)	1.15 (0.83-1.85)	0.75 (0.58-0.94)	<0.001
APACHE Ⅱ score	7 (4-10)	11 (8-16)	6 (4-9)	<0.001
UP, ml/kg/h	1.67 (1.20-2.26)	1.37 (0.89-2.20)	1.73 (1.26-2.27)	<0.001
Outcomes				
ICU mortality, n (%)	40 (3.3)	33 (12.9)	7 (0.7)	<0.001
In-hospital mortality, n (%)	53 (4.3)	40 (15.6)	13 (1.3)	<0.001
ICU stay, days	2 (2-4)	6 (3-13.3)	2 (2-3)	<0.001
Hospital stay, days	16 (12-23)	22 (13-33.3)	15 (11-21)	<0.001
ICU costs, CNY	39461.7 (28966.2-59437.2)	66140.1 (36526.0-131843.8)	37667.5 (27881.5-48238.4)	<0.001
Total costs, CNY	58295.3 (44201.6-98602.8)	128945.6 (71506.8-199685.4)	52773.8 (40856.2-78718.9)	<0.001

Abbreviations: AKI, acute kidney injury; BMI, body mass index; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; sCr, serum creatinine, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score; UP, Urine production first 24 h after admission; CNY, Chinese yuan.

Continuous variables are expressed as mean ± standard deviation or median (25th to 75th percentile, interquartile range). Categorical variables are expressed as a n (%).

Table 2 Detective characteristics of the two biomarkers for AKI

Logistic regression models	AUC-ROC[a] (95%CI)	Cutoff [b]	Sensitivity	Specificity	(+) LR	(-) LR	PPV	NPV
sCysC	0.766 (0.741-0.789)	1.02 mg/L	0.66	0.80	3.28	0.43	0.47	0.90
NT-proBNP	0.821 (0.799-0.842) [c]	204.00 pg/mL	0.78	0.75	3.12	0.29	0.45	0.93
NT-proBNP+ sCysC	0.832 (0.809-0.852) [d]	0.15[e]	0.84	0.69	2.69	0.23	0.42	0.94

Abbreviations: AKI, acute kidney injury; AUC-ROC, area under the receiver operating characteristic curve; CI, confidence interval; (+) LR, Positive likelihood ratio; (-) LR, negative likelihood ratio; PPV, Positive predictive value; NPV, Negative predictive value; sCysC, Serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

[a] Values are presented as AUC-ROC (95% CI)

[b] Ideal cutoff value according to Youden's index

[c] $P < 0.05$ vs. sCysC (P=0.0011)

[d] $P < 0.05$ vs. NT-proBNP (P=0.0145), sCysC(P<0.0001)

[e] Cutoff points of the biomarker panels were the predicted probabilities generated from the multiple logistic regression model

Table 3 Logistic analyses of clinical risk factors for AKI detection

Variables	Univariate analysis		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.029 (1.019-1.038)	<0.001		
Hypertension	2.152 (1.577-2.937)	<0.001		
Diabetes mellitus	3.314 (2.242-4.898)	<0.001		
Chronic kidney disease	17.175 (6.410-46.023)	<0.001	4.182 (1.250-13.998)	0.020
Cerebrovascular disease	2.802 (1.903-4.126)	<0.001		
Chronic obstructive pulmonary disease	4.222 (2.143-8.316)	<0.001		
Coronary artery disease	3.342 (1.910-5.849)	<0.001		
Heart failure	9.997 (4.544-21.997)	<0.001	3.487 (1.365-8.911)	0.009
Sepsis	12.608 (7.914-20.084)	<0.001	5.033 (2.914-8.692)	<0.001
Admission type		<0.001		<0.001
Elective surgical	1.0 (Referent)		1.0 (Referent)	
Emergency surgical	6.121 (3.735-10.030)	<0.001	3.493 (2.023-6.032)	<0.001
Medical	9.409 (6.791-13.037)	<0.001	3.237 (2.157-4.858)	<0.001
sCr	3.135 (2.385-4.120)	<0.001	1.538 (1.149-2.058)	0.004
APACHE Ⅱ score	1.215 (1.182-1.249)	<0.001	1.101 (1.066-1.137)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval; sCr, serum creatinine; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score.

Table 4 Multivariate logistic regression analyses of the two biomarkers for AKI detection

Variables	AKI, %	Unadjusted OR (95% CI)	*P* value	Adjusted OR[a] (95% CI)	*P* value
NT-proBNP (pg/mL, n)					
<204, 779	7.2	1.0 (Referent)		1.0 (Referent)	
≥204, 443	45.1	10.626 (7.639-14.781)	<0.001	3.460 (2.307-5.189)	<0.001
sCysC layered by the cut-off value (mg/L, n)					
<1.02, 861	10.2	1.0 (Referent)		1.0 (Referent)	
≥1.02, 361	46.5	7.646 (5.651-10.345)	<0.001	2.649 (1.842-3.810)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, Serum cystatin C.

a Adjusted for chronic kidney disease, heart failure, sepsis, admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Table 5 Discrimination and reclassification of the combination of biomarkers and clinical model for AKI

	AUC-ROC (95%CI)	*P* value[a]	Category-Free NRI (95%CI)	*P* value[a]	IDI (95%CI)	*P* value[a]
Clinical model[b]	0.840 (0.812-0.868)					
Clinical model + sCysC	0.847 (0.819-0.874)	0.163	0.193 (0.052-0.405)	0.036	0.017 (0.009-0.026)	<0.001
Clinical model + NT-proBNP	0.855 (0.828-0.882)	0.013	0.462 (0.196-0.747)	0.001	0.028 (0.016-0.039)	<0.001
Clinical model + NT-proBNP + sCysC	0.859 (0.832-0.885)	0.006	0.531 (0.238-0.741)	<0.001	0.038 (0.025-0.051)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + sCysC	-	0.015	0.328 (0.139-0.553)	0.002	0.021 (0.010-0.031)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + NT-proBNP	-	0.223	0.167 (0.013-0.285)	0.017	0.011 (0.004-0.018)	0.003

Abbreviations: AUC-ROC, area under the receiver operating characteristic curve; NRI, net reclassification index; IDI, incremental discrimination improvement; AKI, acute kidney injury; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

a Biomarker + clinical model versus clinical model.

b The clinical model for detecting AKI is composed of chronic kidney disease, heart failure, sepsis, Admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Hosmer–Lemeshow goodness-of-fit test: for clinical model, chi-squared value=14.249(P=0.075); for clinical model + sCysC, chi-squared value=6.971 (P=0.54); for clinical model + NT-proBNP, chi-squared value=9.362 (P=0.313); or clinical model + NT-proBNP +sCysC, chi-squared value=4.245 (P=0.834).

Discussion

The study's key finding was that the combination of NT-proBNP and sCysC yields greater discriminative ability for AKI detection at ICU admission with or without a clinical model in critically ill adults. The finding indicates that assessing both serum NT-proBNP and sCysC levels on admission may assist with the early diagnosis and risk stratification of AKI in critically ill adults.

One of the most prevalent complications occurring in a variety of clinical settings is AKI, especially for critically ill patients [4, 6, 36, 37]. The development of AKI, as the same as its severity, is strongly associated with increased mortality[3, 36]. However, early identification is challenging when sCr or urine production changes are used to detect AKI[38], and precise clinical predictors are not widely known. Numerous studies have found and confirmed the accuracy and additional clinical benefits of these renal biomarkers for early AKI diagnosis, such as insulin-like growth factor-binding protein, matrix metalloproteinase-7, tissue inhibitor metalloproteinase-2, angiotensinogen, sCysC, neutrophil gelatinase-associated lipocalin and so on[5, 9, 12, 39, 40]. However, some novel biomarkers are not being used in clinical practice, due to insufficient evidence or previously unavailable commercially. Thus, the rational application of clinically available biomarkers is more practical and economical.

NT-proBNP, a widely used marker of hemodynamic stress, is a polypeptide secreted by the ventricles and its role is to facilitate natriuresis[17, 41]. In patients with elevated CVP, the raised pressure may be transmitted to the renal veins, resulting in renal congestion and reduced glomerular filtration[14, 42, 43]. It makes sense that NT-proBNP is associated with the development of AKI. The clinical application of serum NT-proBNP in cardiac disease has been

extensively confirmed and finds its predictive value for AKI development in patients with HF, coronary angiography, or percutaneous coronary intervention [19, 23, 44, 45]. Several studies have elucidated the link between NT-proBNP and AKI incidence after cardiac or noncardiac surgery[13, 46-48]. However, its utility for AKI detection has not been fully evaluated in general ICU, and it is necessary to verify the reliability and universality of NT-proBNP in heterogeneous populations. The data demonstrated herein not only maintain NT-proBNP as a risk factor for AKI but also indicate that it can enhance the risk reclassification and discrimination for AKI in ICU.

A single biomarker is insufficient to express the multiple pathophysiological mechanisms of AKI, especially for critically ill patients, as AKI is a heterogeneous syndrome[9, 10]. There is no consensus on which specific markers should be combined to detect AKI. Numerous studies have revealed that combining different biomarkers utilized to detect AKI can improve predictive abilities[5, 49, 50]. Naruse et al. showed that combining urinary liver-type fatty-acid binding protein and serum NT-proBNP can enhance early prediction of AKI in patients in medical cardiac ICUs[15]. Similar to such studies, we identified that the combination of two markers, serum NT-probNP and sCysC, improved the diagnostic performance of AKI.

CysC is a glomerular filtration biomarker that can be utilized to anticipate the development of AKI and undesirable outcomes[8, 9]. The application and performance of CysC for AKI prediction have been demonstrated in various clinical settings[5, 11, 51-53]. In individuals with ST-segment elevation myocardial infarction, a combination of B-type natriuretic peptide and CysC may contribute to risk stratification for AKI[25]. In the present cohort, sCysC had a slightly higher specificity than NT-proBNP in the detection of AKI, but its sensitivity is limited. The

ability of sCysC for detecting AKI was fine, but its AUC-ROC was not as great as NT-proBNP. Moreover, the combination of serum NT-proBNP and sCysC at ICU admission had the highest AUC-ROC. These data indicate that the simultaneous measurement of serum NT-proBNP and sCysC at ICU admission could improve the early identification of AKI.

Biomarkers for AKI, which indicates the various underlying pathophysiological mechanisms involved in AKI incidence, might be superior to individual biomarkers alone[3, 6]. It is also important that these biomarkers profit from being easily measurable, readily accessible, comparatively cheap, and with an elevated level of sensitivity and specificity. In the present study, for AKI detection NT-proBNP acted as a hemodynamic stress biomarker with high sensitivity and specificity, and sCysC had high specificity as a functional biomarker. Even after clinical risk variables adjustment, elevated NT-proBNP is an independent risk factor for AKI. The NT-proBNP addition to the clinical model significantly enhanced risk reclassification, as demonstrated by category-free NRI and IDI. Moreover, our results identified that the addition of NT-proBNP to sCysC markedly improved their detective abilities as biomarkers. Adding biomarkers to the clinical model further improved the diagnostic accuracy of AKI, as measured by AUC-ROCs. These data suggest that a single biomarker is insufficient for early diagnosis of AKI. Therefore, the method of combining different biomarkers may be of greater use.

There are some limitations for this study. First, it was a single-center study, with an unproven external validity. Second, there were only 26 patients with CKD patients and 31 HF patients enrolled, and hence we were unable to stratify our group relying on eGFR or cardiac function at baseline. Accordingly, future studies should be administrated in these subgroups. Last, we did not accomplish routine echocardiography in all patients to associate NT-proBNP levels with

ventricular dilatation or other pathways that may promote NT-proBNP release. Despite these limitations, we believe that our findings have clinical implications and should facilitate further research to confirm our results.

Conclusion

In the present cohort, simultaneous measurement of NT-proBNP and sCysC at ICU admission increases the early identification of AKI beyond that of biomarker in isolation, and that the combination of the two biomarkers and clinical risk factors improves the discriminative ability for AKI detection in critically ill adults.

Abbreviations

(−) LR, negative likelihood ratios

(+) LR, positive likelihood ratios

AKI, Acute kidney injury

APACHE II score, Acute Physiology and Chronic Health Evaluation II score

AUC-ROC, area under the receiver operating characteristic curve

BMI, body mass index

CAD, coronary artery disease

CI, confidence interval

CNY, Chinese yuan

CKD, chronic kidney disease

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration

COPD, chronic obstructive pulmonary disease

DM, diabetes mellitus

eGFR, estimated glomerular filtration rate

ESRD, end-stage renal disease

HF, heart failure

ICU, intensive care unit

IDI, incremental discrimination improvement

KDIGO, Kidney Disease Improving Global Outcomes

NPV, negative predictive values

NRI net reclassification index

NT-proBNP, N-terminal pro-B-type natriuretic peptide

ORs, odds ratios

PPV, positive predictive values

RRT, renal replacement therapy

sCr, serum creatinine

sCysC, serum cystatin C

UP, Urine production first 24 h after admission

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Authors' contributions

JD, LLH, YFL and LHH equally contributed to the design of the research, analysis and interpretation of the data. CBC and JD contributed to the conception and design of the research as well as interpretation of the data, and critically revised the manuscript. JD, LLH, YFL, LHH, JX, HF and YL performed the research and collected data. All authors contributed to the acquisition and analysis of the data, drafted the manuscript, and agree to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during this study are not publicly available, owing to currently ongoing research studies, but the data are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethics committee of the Guangdong Provincial People's Hospital approved the study design, protocol, ethical issue, and data and sample collection (No. GDREC2015396H(R1)). Written informed consent was obtained from each patient or from the appropriate surrogates.

Patient consent for publication

Not required.

Consent for publication

Not applicable. No individual personal data are included in the study. All patients provided necessary consent to participate in the present study.

Competing interests

The authors declare that they have no competing interests.

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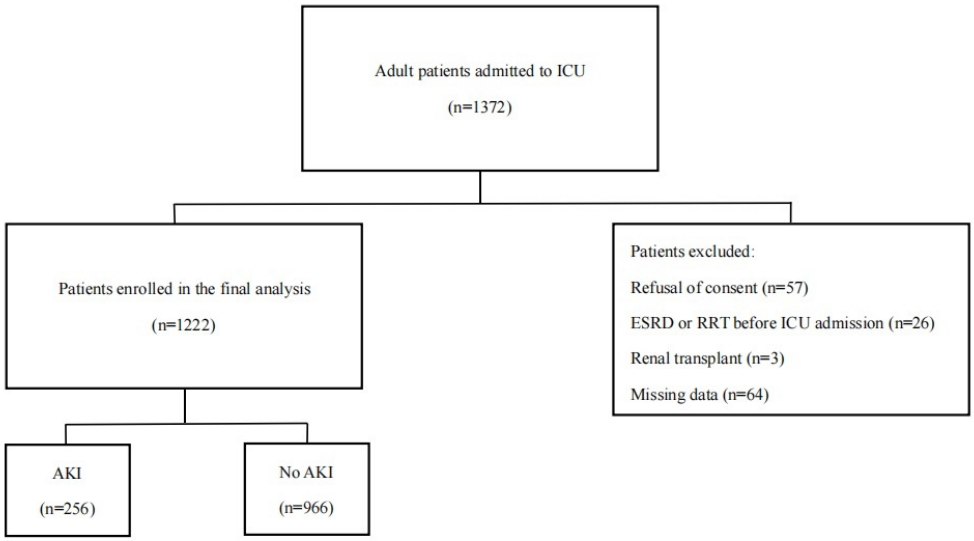
Figure legends

Figure 1. Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

Figure 2. ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

Figure 3. ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.



Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

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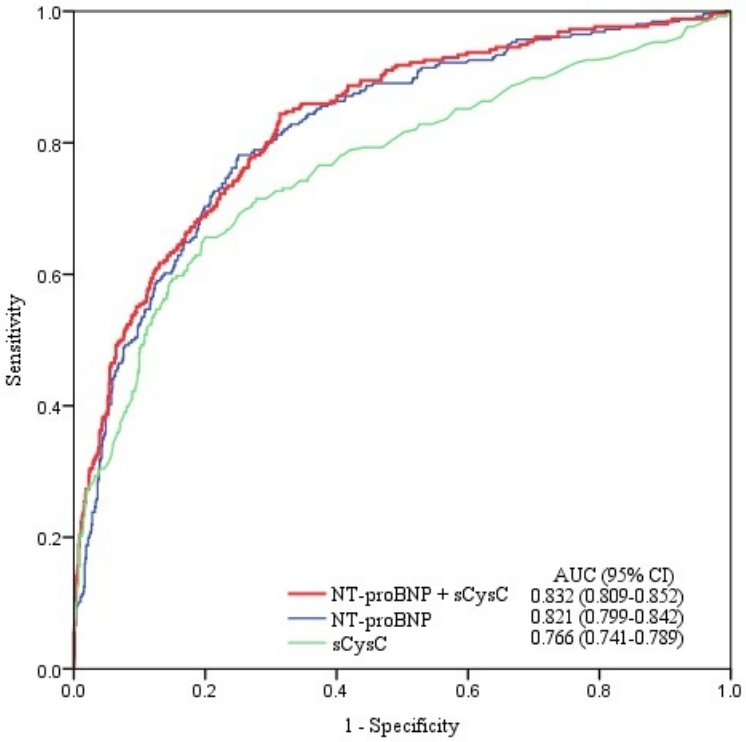

ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

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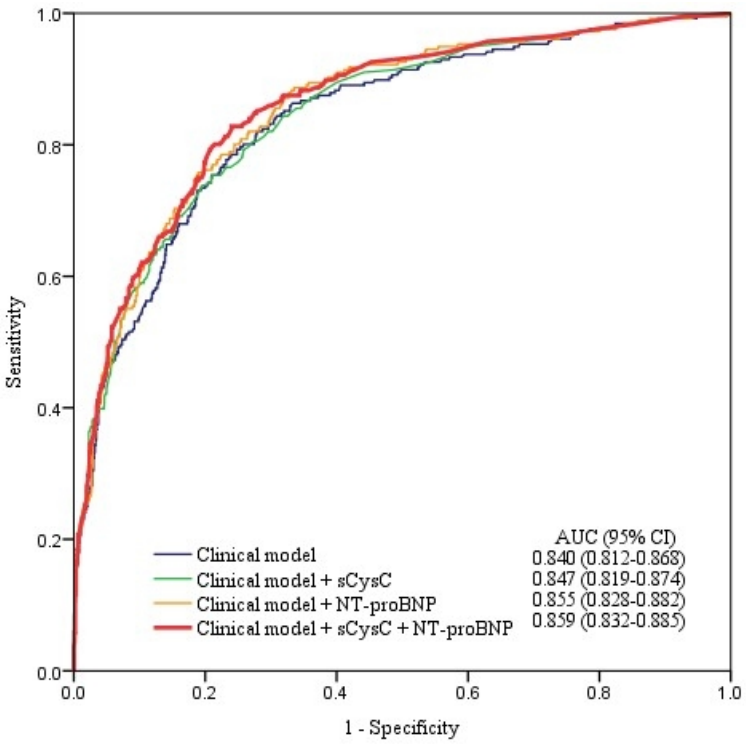

ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

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TRIPOD

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	4-5
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	7-8
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	8
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	9-10
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	9-10
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	9
	5b	Describe eligibility criteria for participants.	9
	5c	Give details of treatments received, if relevant.	NA
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	10
	6b	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	9-10
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	Explain how the study size was arrived at.	9
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	9
Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	9
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	11-12
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	12
Risk groups	11	Provide details on how risk groups were created, if done.	11-12
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	13, figure1
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	13
Model development	14a	Specify the number of participants and outcome events in each analysis.	14-15
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	14
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	13-15
	15b	Explain how to the use the prediction model.	14
Model performance	16	Report performance measures (with CIs) for the prediction model.	14-15
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	23-24
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	21-23
Implications	20	Discuss the potential clinical use of the model and implications for future research.	21-24
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	25-32
Funding	22	Give the source of funding and the role of the funders for the present study.	27

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

BMJ Open

Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults in China: A prospective, observational study

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Title

Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults in China: A prospective, observational study

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Abstract

Objective Serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cystatin C (sCysC) are available clinically and beneficial in diagnosing AKI. Our purpose is to identify the performance of their combined diagnosis for AKI in critically ill patients.

Design A prospectively-recruited, observational study was performed.

Setting Adults admitted to the intensive care unit of a tertiary hospital in China.

Participants A total of 1222 critically ill patients were enrolled in the study.

Main outcome measures To identify the performance of the combined diagnosis of serum NT-proBNP and sCysC for AKI in critically ill patients. The area under the receiver operating characteristic curve (AUC-ROC), category-free net reclassification index (NRI), and incremental discrimination improvement (IDI) were utilized for comparing the discriminative powers of a combined and single biomarker adjusted model of clinical variables enriched with NTproBNP and Cystatin C for AKI.

Results

AKI was detected in 256 out of 1222 included patients (20.9%). AUC-ROC for NT-proBNP and sCysC to detect AKI had a significantly higher accuracy than any individual biomarker ($P < 0.05$). After multivariate adjustment, a level of serum NT-proBNP $\geq$ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with those below the cutoff value. Similar results were obtained for sCysC levels ($P < 0.001$). To detect AKI, adding NT-proBNP and sCysC to a clinical model further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$). Moreover, the addition of these two to the clinical model significantly improved risk

reclassification of AKI beyond that of the clinical model alone or with single biomarker ($P < 0.05$), as measured by NRI and IDI.

Conclusions

In critically ill individuals, serum NT-proBNP, sCysC, and clinical risk factors combination improve the discriminative power for diagnosing AKI.

Keywords

Acute kidney injury, N-terminal pro-B-type natriuretic peptide, Serum cystatin C, Intensive care unit

Strengths and limitations of this study

This prospective observational study established a cohort with an adequate sample size.

Rather than solely focusing on the effects of one biomarker, this study also assessed the combine efficacy of serum NT-proBNP and sCysC in detection of AKI.

In this study, multiple logistic regression prediction models were established to detect AKI.

We evaluated the combined efficacy of serum NT-proBNP and sCysC in the diagnosis of AKI by calculating area under the curve and category-free net reclassification index and incremental discrimination improvement.

Dynamic assessment of NT-proBNP and sCysC changes was not available due to only measured their levels at admission.

Introduction

Acute kidney injury (AKI) is a predominant clinical syndrome affecting more than 50% of patients who underwent treatment at intensive care unit (ICU)[1-3]. Increased morbidity, mortality, hospitalization length, and cost are extremely related to AKI[4-6], so early recognition of AKI is critical to guiding management. Urine production and serum creatinine were employed as diagnostic criteria for AKI in accordance with the recommendations of Kidney Disease Improving Global Outcomes (KDIGO) guidelines[7]. Changes in sCr or urine production cannot recognize early renal tubular injury prior to a reduction in glomerular filtration rate [8, 9]. Therefore, early and reliable AKI biomarkers are necessary to promote timely intervention and minimize complications. However, it is impossible for an individual biomarker to adequately evaluate the risk of AKI as a complex multifactorial syndrome [3, 10]. Combining diverse biomarkers in a clinical model evaluation could enhance early detection of AKI in critically ill patients[5, 11, 12].

A hemodynamic marker stress, N-terminal pro-B-type natriuretic peptide (NT-proBNP), has recently received attention as a potential predictor of AKI in a wide diversity of clinical settings[13-17]. High serum NT-proBNP level reflects hemodynamic instability, myocardial wall stress, myocardial ischemia, volume overload, sympathetic nervous system and renin-angiotensin-aldosterone system activation, all of which may contribute to AKI incidence[18-20]. However, there are finite data on clinical use of NT-proBNP for detecting AKI among critically ill patients.

Serum Cystatin C (sCysC), a 122-amino acid low-molecular-weight protein (13 kDa),

is a marker of glomerular filtration[9]. CysC has a half-life of about one-third that of sCr, causing CysC to reach homeostasis three times faster. These properties promote sCysC as an alternative marker of renal function[8]. To date, sCysC has been displayed to be conductive for the early identification of all-cause AKI[21].

Although serum NT-proBNP and sCysC are mainly used for the prognosis assessment of heart disease and the prediction of acute cardiac events with AKI [22-26], the diagnostic accuracy of their combination for AKI in ICU remains unknown. We performed a prospective study in the present research to evaluate the performance of serum NT-proBNP and sCysC at ICU admission, both independently and in combination, for AKI determination among critically ill adults.

Methods

Study design and participants

At Guangdong Provincial People's Hospital in China, a tertiary care hospital, a prospectively recruited observational research was performed in a mixed medical-surgical ICU. From December 2016 to December 2017, we consecutively enrolled patients aged 18 and up in a row. Pregnancy, renal replacement therapy (RRT) prior to ICU admittance, nephrectomy, end-stage renal disease (ESRD), renal transplant, rejection of consent, or missing admission information were all exclusion criteria. The study protocol strengthened the reporting requirements of observational studies in epidemiology[27] and standards for the reporting of diagnostic accuracy[28]. This study protocol was authorized by ethics committee of Guangdong Provincial People's Hospital. Additionally, all procedures were carried out consistent with applicable regulations and guidelines. All patients provided written informed consent.

Data collection

Clinical baseline data were collected prospectively. Within 1 h following ICU admission, blood samples were collected contemporaneously to determine sCr, serum NT-proBNP, and sCysC. Within 24 h after collection, all samples were analyzed in the Guangdong Provincial People's Hospital central laboratory utilizing standard protocol. When a patient is admitted to the ICU, sCr was measured and subsequently done at least once a day until discharge as part of routine clinical care. Throughout admission to ICU, urine production was also been recorded. We evaluated the following clinical variables: age, gender, body mass index (BMI), preexisting clinical conditions, sepsis,

admission type, baseline sCr, baseline estimated glomerular filtration rate (eGFR), Acute Physiology and Chronic Health Evaluation (APACHE) II score, ICU mortality, in-hospital mortality, duration of ICU admittance, length of hospitalization, ICU costs, and total costs. The outcome was the incidence of AKI following ICU enrollment within 1 week. To calculate eGFR, we employed the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [29].

Definitions

The KDIGO classification criteria were utilized to define AKI: as a rise in sCr by ≥ 0.3 mg/dL (26.5μmol/L) within 48 h or a rise in sCr to ≥ 1.5 times the baseline within 1 week, or urine output < 0.5 mL/kg/h for 6 h after ICU admission[7].

Relying on following principles, ranked in descending order of preference, a baseline sCr was affirmed[30]: (1) prior to ICU admittance, the most recent pre-ICU value between 30 and 365 days; (2) a stable pre-ICU value > 365 days prior to ICU admittance for patients < 40 years of age (stable definition is being within 15% of the lowest ICU measurement); (3) pre-ICU value > 365 days prior to ICU admittance and lower than the initial sCr at ICU admission; (4) a pre-ICU value (within 3 and 39 days prior to ICU admittance) lower than or equal to the initial sCr upon ICU admittance not obviously during AKI; (5) the least sCr value obtained at initial ICU admittance, the most recent ICU value, or the lowest value achieved to a 365-day follow up.

Biomarker measurement

The levels of sCysC and sCr were quantified through the UniCel DxC 800 Synchron system usage in compliance with the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). For sCysC, the intra- and inter-assay variation coefficients were 10% and 5%, respectively. Levels of serum NT-proBNP were quantified through an electrochemiluminescence immunoassay employing a Cobas® e602 system usage (Roche Diagnostics, Germany). For NT-proBNP, the uppermost limit of normal for those who seem to be healthy (95th percentage) has been 125 pg/mL. The coefficient of interassay variation for NT-proBNP was < 5%. Each patient's clinical features were blinded to the personnel measuring the biomarkers.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Statistical analysis

For statistical analyses, we utilized SPSS version 21.0 (SPSS, Chicago, IL, USA), MedCalc version 18.2.1 (MedCalc Software, Ostend, Belgium) and R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). The mean ± standard deviation or median (25th to 75th percentage, interquartile range) had been utilized to express continuous variables. Numbers (percent) were utilized to represent categorical variables. For nonnormally distributed continuous variables, the Wilcoxon rank-sum test was employed for intergroup difference measurement, and for categorical variables, the chi-square or Fisher's exact test was deployed.

NT-proBNP concentrations were extremely skewed and therefore were log10

transformed before inclusion in the models. Areas under the receiver operating characteristic curves (AUC-ROCs) were computed. The method exploited by DeLong et al. was used to compare AUC-ROCs between groups[31]. The biomarkers' sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios of the biomarkers were calculated. Youden's index for AKI detection identified the optimal cutoff values for individual biomarkers and their combination[32].

Logistic analysis was utilized to compute the odds ratios (ORs) and 95% confidence intervals for each factor: to identify the independent risk factors of AKI, with a forward stepwise method, in which the clinical variables with $P < 0.10$ in univariate analysis were incorporated into the multivariate logistic model. We categorized NT-proBNP and sCysC levels according to their cutoff values and then performed logistic regression on the created variables. We conducted multiple logistic regression analyses to calculate the adjusted ORs of AKI, which was based on the clinical risk factors for AKI.

The performance of AKI detection after adding NT-proBNP and sCysC, or any of them, into the clinical model as categorical variables was assessed by AUC-ROC, category-free net reclassification improvement (NRI) index and integrated discrimination improvement (IDI) index, as described previously[33, 34]. To better quantify how accurately the reference and reclassification model would perform with independent data, we adopted a 10-fold cross-validation[35]. All the tests were 2-tailed, and $P < 0.05$ was regarded statistically significant.

Results

Clinical data and outcomes

A total of 150 (10.9 %) of the 1372 adult patients enrolled in the study were excluded (Figure 1). Therefore, 1222 patients were enrolled and AKI occurred in 256 patients (20.9%). Patient baseline variables and outcomes are exhibited in Table 1. In comparison to patients without AKI, patients with AKI were elderly and were observed more frequently in those with comorbidities, including chronic kidney disease (CKD), hypertension, coronary artery disease (CAD), diabetes mellitus (DM), heart failure (HF), cerebrovascular disease and chronic obstructive pulmonary disease (COPD). Increased sCr, serum NT-proBNP and sCysC levels at admission, as well as increased APACHE II scores, were more prevalent in AKI patients. The baseline sCr and eGFR did not show significant differences between the two groups.

Indeed, AKI patients had a higher risk of adverse outcomes, a higher percentage of ICU and in-hospital mortality, higher expenses, a longer hospitalization duration, and a longer stay in ICU ($P < 0.001$) compared to those without AKI.

Detective abilities of the two biomarkers for AKI

To demonstrate the ability of these biomarkers for AKI detection, we used AUC-ROCs to calculate the two biomarkers, respectively, and in combination. AUC-ROCs for NT-proBNP and sCysC were computed for AKI detection (0.821 and 0.766, respectively). For AKI detection, NT-proBNP had a sensitivity of 78% and a specificity of 75%, while sCysC had high specificity but limited sensitivity. The cutoff values for NT-proBNP and sCysC were 204 pg/mL and 1.02 mg/L, respectively, yielding good sensitivity and specificity. We included NT-proBNP and sCysC in a multivariate logistic regression model to derived their combined AUC for comparison with the single biomarker's. The AUC-ROCs for AKI presented a better

performance by NT-proBNP and sCysC (0.832) than any individual biomarker ($P < 0.05$, Table 2 and Figure 2).

Multivariate logistic regression analyses of the two biomarkers for AKI detection

We stratified patients based on the two cutoff values of serum NT-proBNP and sCysC levels into two categories, respectively. Compared to those with serum NT-proBNP < 204 pg/mL, patients with serum NT-proBNP ≥ 204 pg/mL on admission exhibited a higher incidence of AKI (7.2% versus 45.1%, $P < 0.001$). AKI was found to have higher incidence (46.5%) in patients with sCysC ≥ 1.02 mg/L compared to those with sCysC < 1.02 mg/L (10.2%) ($P < 0.001$). Following clinical variables adjustment (including CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores, Table 3), a level of serum NT-proBNP ≥ 204 pg/mL was associated with 3.5-fold higher odds for AKI compared with NT-proBNP level below the cutoff value ($P < 0.001$). Similarly, patients with sCysC ≥ 1.02 mg/L were linked to 2.6-fold greater odds for AKI compared to those with sCysC < 1.02 mg/L ($P < 0.001$) (Table 4).

Discrimination and reclassification of the combination of biomarkers and clinical models for AKI

Considering the effect of adding NT-proBNP and sCysC, or any of them, to a clinical model as categorical variables for AKI detection, logistic regression analysis was employed. On ICU admittance for AKI diagnosis, potential available variables including age, sepsis, admission type, sCr, and APACHE II scores as well as comorbidities, including hypertension, DM, CKD, cerebrovascular disease, COPD, CAD, and HF were considered. The clinical model for detecting AKI involved CKD, HF, sepsis, admission type, sCr at admission, and APACHE II scores (Table 3). The cross-validated baseline performance characterized by accuracy and

Kappa for the clinical model was 0.844 and 0.448.

To evaluate the enhancement of discriminative capacity, a panel of NT-proBNP and sCysC was introduced to the above-mentioned model. As shown in Table 5, compared with the clinical model, the addition of NT-proBNP to the clinical model had a higher AUC-ROC ($P < 0.05$), and no statistically significant variation existed when sCyC was added to the clinical model. However, the risk reclassification was markedly improved through the addition of NT-proBNP or sCyC to the clinical model, as measured by category-free NRI and IDI ($P < 0.05$). Adding NT-proBNP and sCysC to a clinical model for AKI detection further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$) (Figure 3). The cross-validated baseline performance characterized by accuracy and Kappa for the clinical model enriched with NTproBNP and Cystatin C was 0.848 and 0.475. Moreover, this panel addition to the clinical model significantly enhanced the risk reclassification of AKI beyond that of the clinical model with or without any individual biomarkers ($P < 0.05$), with maximum NRI (0.531) and IDI (0.038).

Table 1 Clinical data and outcomes

Characteristics	Total (n=1222)	AKI (n=256)	No AKI (n=966)	*P* value
Age, years	57 (45-67)	63.5 (52-72)	55 (44-65)	<0.001
Male, n (%)	666 (54.5)	151 (59.0)	515 (53.3)	0.105
BMI, kg/m^2	22 (19.6-24.7)	22.3 (19.9-25.0)	21.9 (19.6-24.6)	0.395
Preexisting clinical conditions				
Hypertension, n (%)	252 (20.6)	81 (31.6)	171 (17.7)	<0.001
Diabetes mellitus, n (%)	121 (9.9)	52 (20.3)	69 (7.1)	<0.001
Chronic kidney disease, n (%)	26 (2.1)	21 (8.2)	5 (0.5)	<0.001
Cerebrovascular disease, n (%)	127 (10.4)	50 (19.5)	77 (8.0)	<0.001
Chronic obstructive pulmonary disease, n (%)	35 (2.9)	18 (7.0)	17 (1.8)	<0.001
Coronary artery disease, n (%)	53 (4.3)	24 (9.4)	29 (3.0)	<0.001
Heart failure, n (%)	31 (2.5)	22 (8.6)	9 (0.9)	<0.001
Cancer, n (%)	227 (18.6)	50 (19.5)	177 (18.3)	0.659
Sepsis, n (%)	98 (8.0)	70 (27.3)	28 (2.9)	<0.001
Admission type, n (%)				<0.001
Elective surgical, n (%)	863 (70.6)	84 (32.8)	779 (80.6)	
Emergency surgical, n (%)	83 (6.8)	33 (12.9)	50 (5.2)	
Medical, n (%)	276 (22.6)	139 (54.3)	137 (14.2)	
Baseline serum creatinine, mg/dL	0.78 (0.64-0.96)	0.76 (0.58-1.09)	0.79 (0.65-0.94)	0.949
Baseline eGFR, mL/min/1.73 m^2	119.4 (97.6-145.4)	122.9 (83.3-158.7)	118.7 (100.4-142.9)	0.950
Parameters at ICU admission				
sCr, mg/dl	0.87 (0.72-1.07)	0.97 (0.72-1.55)	0.85 (0.72-1.02)	<0.001
NT-proBNP, pg/mL	97.1 (29.4-464.4)	871.0 (225.8-2919.3)	66.2 (23.7-206.2)	<0.001
sCysC, mg/L	0.78 (0.61-1.06)	1.15 (0.83-1.85)	0.75 (0.58-0.94)	<0.001
APACHE Ⅱ score	7 (4-10)	11 (8-16)	6 (4-9)	<0.001
UP, ml/kg/h	1.67 (1.20-2.26)	1.37 (0.89-2.20)	1.73 (1.26-2.27)	<0.001
Outcomes				
ICU mortality, n (%)	40 (3.3)	33 (12.9)	7 (0.7)	<0.001
In-hospital mortality, n (%)	53 (4.3)	40 (15.6)	13 (1.3)	<0.001
ICU stay, days	2 (2-4)	6 (3-13.3)	2 (2-3)	<0.001
Hospital stay, days	16 (12-23)	22 (13-33.3)	15 (11-21)	<0.001
ICU costs, CNY	39461.7 (28966.2-59437.2)	66140.1 (36526.0-131843.8)	37667.5 (27881.5-48238.4)	<0.001
Total costs, CNY	58295.3 (44201.6-98602.8)	128945.6 (71506.8-199685.4)	52773.8 (40856.2-78718.9)	<0.001

Abbreviations: AKI, acute kidney injury; BMI, body mass index; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; sCr, serum creatinine, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score; UP, Urine production first 24 h after admission; CNY, Chinese yuan.

Continuous variables are expressed as mean ± standard deviation or median (25th to 75th percentile, interquartile range). Categorical variables are expressed as a n (%).

Table 2 Detective characteristics of the two biomarkers for AKI

Logistic regression models	AUC-ROC[a] (95%CI)	Cutoff [b]	Sensitivity	Specificity	(+) LR	(-) LR	PPV	NPV
sCysC	0.766 (0.741-0.789)	1.02 mg/L	0.66	0.80	3.28	0.43	0.47	0.90
NT-proBNP	0.821 (0.799-0.842) [c]	204.00 pg/mL	0.78	0.75	3.12	0.29	0.45	0.93
NT-proBNP+ sCysC	0.832 (0.809-0.852) [d]	0.15[e]	0.84	0.69	2.69	0.23	0.42	0.94

Abbreviations: AKI, acute kidney injury; AUC-ROC, area under the receiver operating characteristic curve; CI, confidence interval; (+) LR, Positive likelihood ratio; (-) LR, negative likelihood ratio; PPV, Positive predictive value; NPV, Negative predictive value; sCysC, Serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

[a] Values are presented as AUC-ROC (95% CI)

[b] Ideal cutoff value according to Youden's index

[c] $P < 0.05$ vs. sCysC (P=0.0011)

[d] $P < 0.05$ vs. NT-proBNP (P=0.0145), sCysC(P<0.0001)

[e] Cutoff points of the biomarker panels were the predicted probabilities generated from the multiple logistic regression model

Table 3 Logistic analyses of clinical risk factors for AKI detection

Variables	Univariate analysis		Multivariate model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.029 (1.019-1.038)	<0.001		
Hypertension	2.152 (1.577-2.937)	<0.001		
Diabetes mellitus	3.314 (2.242-4.898)	<0.001		
Chronic kidney disease	17.175 (6.410-46.023)	<0.001	4.182 (1.250-13.998)	0.020
Cerebrovascular disease	2.802 (1.903-4.126)	<0.001		
Chronic obstructive pulmonary disease	4.222 (2.143-8.316)	<0.001		
Coronary artery disease	3.342 (1.910-5.849)	<0.001		
Heart failure	9.997 (4.544-21.997)	<0.001	3.487 (1.365-8.911)	0.009
Sepsis	12.608 (7.914-20.084)	<0.001	5.033 (2.914-8.692)	<0.001
Admission type		<0.001		<0.001
Elective surgical	1.0 (Referent)		1.0 (Referent)	
Emergency surgical	6.121 (3.735-10.030)	<0.001	3.493 (2.023-6.032)	<0.001
Medical	9.409 (6.791-13.037)	<0.001	3.237 (2.157-4.858)	<0.001
sCr	3.135 (2.385-4.120)	<0.001	1.538 (1.149-2.058)	0.004
APACHE Ⅱ score	1.215 (1.182-1.249)	<0.001	1.101 (1.066-1.137)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval; sCr, serum creatinine; APACHE Ⅱ score, acute physiology and chronic health evaluation Ⅱ score.

Table 4 Multivariate logistic regression analyses of the two biomarkers for AKI detection

Variables	AKI, %	Unadjusted OR (95% CI)	*P* value	Adjusted OR[a] (95% CI)	*P* value
NT-proBNP (pg/mL, n)					
<204, 779	7.2	1.0 (Referent)		1.0 (Referent)	
≥204, 443	45.1	10.626 (7.639-14.781)	<0.001	3.460 (2.307-5.189)	<0.001
sCysC layered by the cut-off value (mg/L, n)					
<1.02, 861	10.2	1.0 (Referent)		1.0 (Referent)	
≥1.02, 361	46.5	7.646 (5.651-10.345)	<0.001	2.649 (1.842-3.810)	<0.001

Abbreviations: AKI, acute kidney injury; OR, odds ratio; CI, confidence interval, NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, Serum cystatin C.

a Adjusted for chronic kidney disease, heart failure, sepsis, admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Table 5 Discrimination and reclassification of the combination of biomarkers and clinical model for AKI

	AUC-ROC (95%CI)	*P* value[a]	Category-Free NRI (95%CI)	*P* value[a]	IDI (95%CI)	*P* value[a]
Clinical model[b]	0.840 (0.812-0.868)					
Clinical model + sCysC	0.847 (0.819-0.874)	0.163	0.193 (0.052-0.405)	0.036	0.017 (0.009-0.026)	<0.001
Clinical model + NT-proBNP	0.855 (0.828-0.882)	0.013	0.462 (0.196-0.747)	0.001	0.028 (0.016-0.039)	<0.001
Clinical model + NT-proBNP + sCysC	0.859 (0.832-0.885)	0.006	0.531 (0.238-0.741)	<0.001	0.038 (0.025-0.051)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + sCysC	-	0.015	0.328 (0.139-0.553)	0.002	0.021 (0.010-0.031)	<0.001
Clinical model + NT-proBNP + sCysC vs Clinical model + NT-proBNP	-	0.223	0.167 (0.013-0.285)	0.017	0.011 (0.004-0.018)	0.003

Abbreviations: AUC-ROC, area under the receiver operating characteristic curve; NRI, net reclassification index; IDI, incremental discrimination improvement; AKI, acute kidney injury; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

a Biomarker + clinical model versus clinical model.

b The clinical model for detecting AKI is composed of chronic kidney disease, heart failure, sepsis, Admission type, serum creatinine at admission and acute physiology and chronic health evaluation Ⅱ score.

Hosmer–Lemeshow goodness-of-fit test: for clinical model, chi-squared value=14.249(P=0.075); for clinical model + sCysC, chi-squared value=6.971 (P=0.54); for clinical model + NT-proBNP, chi-squared value=9.362 (P=0.313); or clinical model + NT-proBNP +sCysC, chi-squared value=4.245 (P=0.834).

Discussion

The study's key finding was that the combination of NT-proBNP and sCysC yields greater discriminative ability for AKI detection at ICU admission with or without a clinical model in critically ill adults. The finding indicates that assessing both serum NT-proBNP and sCysC levels on admission may assist with the early diagnosis and risk stratification of AKI in critically ill adults.

One of the most prevalent complications occurring in a variety of clinical settings is AKI, especially for critically ill patients [4, 6, 36, 37]. The development of AKI, as the same as its severity, is strongly associated with increased mortality[3, 36]. However, early identification is challenging when sCr or urine production changes are used to detect AKI[38], and precise clinical predictors are not widely known. Numerous studies have found and confirmed the accuracy and additional clinical benefits of these renal biomarkers for early AKI diagnosis, such as insulin-like growth factor-binding protein, matrix metalloproteinase-7, tissue inhibitor metalloproteinase-2, angiotensinogen, sCysC, neutrophil gelatinase-associated lipocalin and so on[5, 9, 12, 39, 40]. However, some novel biomarkers are not being used in clinical practice, due to insufficient evidence or previously unavailable commercially. Thus, the rational application of clinically available biomarkers is more practical and economical.

NT-proBNP, a widely used marker of hemodynamic stress, is a polypeptide secreted by the ventricles and its role is to facilitate natriuresis[17, 41]. In patients with elevated CVP, the raised pressure may be transmitted to the renal veins, resulting in renal congestion and reduced glomerular filtration[14, 42, 43]. It makes sense that NT-proBNP is associated with the development of AKI. The clinical application of serum NT-proBNP in cardiac disease has been

extensively confirmed and finds its predictive value for AKI development in patients with HF, coronary angiography, or percutaneous coronary intervention [19, 23, 44, 45]. Several studies have elucidated the link between NT-proBNP and AKI incidence after cardiac or noncardiac surgery[13, 46-48]. However, its utility for AKI detection has not been fully evaluated in general ICU, and it is necessary to verify the reliability and universality of NT-proBNP in heterogeneous populations. The data demonstrated herein not only maintain NT-proBNP as a risk factor for AKI but also indicate that it can enhance the risk reclassification and discrimination for AKI in ICU.

A single biomarker is insufficient to express the multiple pathophysiological mechanisms of AKI, especially for critically ill patients, as AKI is a heterogeneous syndrome[9, 10]. There is no consensus on which specific markers should be combined to detect AKI. Numerous studies have revealed that combining different biomarkers utilized to detect AKI can improve predictive abilities[5, 49, 50]. Naruse et al. showed that combining urinary liver-type fatty-acid binding protein and serum NT-proBNP can enhance early prediction of AKI in patients in medical cardiac ICUs[15]. Similar to such studies, we identified that the combination of two markers, serum NT-probNP and sCysC, improved the diagnostic performance of AKI.

CysC is a glomerular filtration biomarker that can be utilized to anticipate the development of AKI and undesirable outcomes[8, 9]. The application and performance of CysC for AKI prediction have been demonstrated in various clinical settings[5, 11, 51-53]. In individuals with ST-segment elevation myocardial infarction, a combination of B-type natriuretic peptide and CysC may contribute to risk stratification for AKI[25]. In the present cohort, sCysC had a slightly higher specificity than NT-proBNP in the detection of AKI, but its sensitivity is limited. The

ability of sCysC for detecting AKI was fine, but its AUC-ROC was not as great as NT-proBNP. Moreover, the combination of serum NT-proBNP and sCysC at ICU admission had the highest AUC-ROC. These data indicate that the simultaneous measurement of serum NT-proBNP and sCysC at ICU admission could improve the early identification of AKI.

Biomarkers for AKI, which indicates the various underlying pathophysiological mechanisms involved in AKI incidence, might be superior to individual biomarkers alone[3, 6]. It is also important that these biomarkers profit from being easily measurable, readily accessible, comparatively cheap, and with an elevated level of sensitivity and specificity. In the present study, for AKI detection NT-proBNP acted as a hemodynamic stress biomarker with high sensitivity and specificity, and sCysC had high specificity as a functional biomarker. Even after clinical risk variables adjustment, elevated NT-proBNP is an independent risk factor for AKI. The NT-proBNP addition to the clinical model significantly enhanced risk reclassification, as demonstrated by category-free NRI and IDI. Moreover, our results identified that the addition of NT-proBNP to sCysC markedly improved their detective abilities as biomarkers. Adding biomarkers to the clinical model further improved the diagnostic accuracy of AKI, as measured by AUC-ROCs. These data suggest that a single biomarker is insufficient for early diagnosis of AKI. Therefore, the method of combining different biomarkers may be of greater use.

There are some limitations for this study. First, it was a single-center study, with an unproven external validity. Second, there were only 26 patients with CKD patients and 31 HF patients enrolled, and hence we were unable to stratify our group relying on eGFR or cardiac function at baseline. Accordingly, future studies should be administrated in these subgroups. Last, we did not accomplish routine echocardiography in all patients to associate NT-proBNP levels with

ventricular dilatation or other pathways that may promote NT-proBNP release. Despite these limitations, we believe that our findings have clinical implications and should facilitate further research to confirm our results.

Conclusion

In the present cohort, simultaneous measurement of NT-proBNP and sCysC at ICU admission increases the early identification of AKI beyond that of biomarker in isolation, and that the combination of the two biomarkers and clinical risk factors improves the discriminative ability for AKI detection in critically ill adults.

Abbreviations

(−) LR, negative likelihood ratios

(+) LR, positive likelihood ratios

AKI, Acute kidney injury

APACHE II score, Acute Physiology and Chronic Health Evaluation II score

AUC-ROC, area under the receiver operating characteristic curve

BMI, body mass index

CAD, coronary artery disease

CI, confidence interval

CNY, Chinese yuan

CKD, chronic kidney disease

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration

COPD, chronic obstructive pulmonary disease

DM, diabetes mellitus

eGFR, estimated glomerular filtration rate

ESRD, end-stage renal disease

HF, heart failure

ICU, intensive care unit

IDI, incremental discrimination improvement

KDIGO, Kidney Disease Improving Global Outcomes

NPV, negative predictive values

NRI net reclassification index

NT-proBNP, N-terminal pro-B-type natriuretic peptide

ORs, odds ratios

PPV, positive predictive values

RRT, renal replacement therapy

sCr, serum creatinine

sCysC, serum cystatin C

UP, Urine production first 24 h after admission

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Authors' contributions

JD, LLH, YFL and LHH equally contributed to the design of the research, analysis and interpretation of the data. CBC and JD contributed to the conception and design of the research as well as interpretation of the data, and critically revised the manuscript. JD, LLH, YFL, LHH, JX, HF and YL performed the research and collected data. All authors contributed to the acquisition and analysis of the data, drafted the manuscript, and agree to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during this study are not publicly available, owing to currently ongoing research studies, but the data are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethics committee of the Guangdong Provincial People's Hospital approved the study design, protocol, ethical issue, and data and sample collection (No. GDREC2015396H(R1)). Written informed consent was obtained from each patient or from the appropriate surrogates.

Patient consent for publication

Not required.

Consent for publication

Not applicable. No individual personal data are included in the study. All patients provided necessary consent to participate in the present study.

Competing interests

The authors declare that they have no competing interests.

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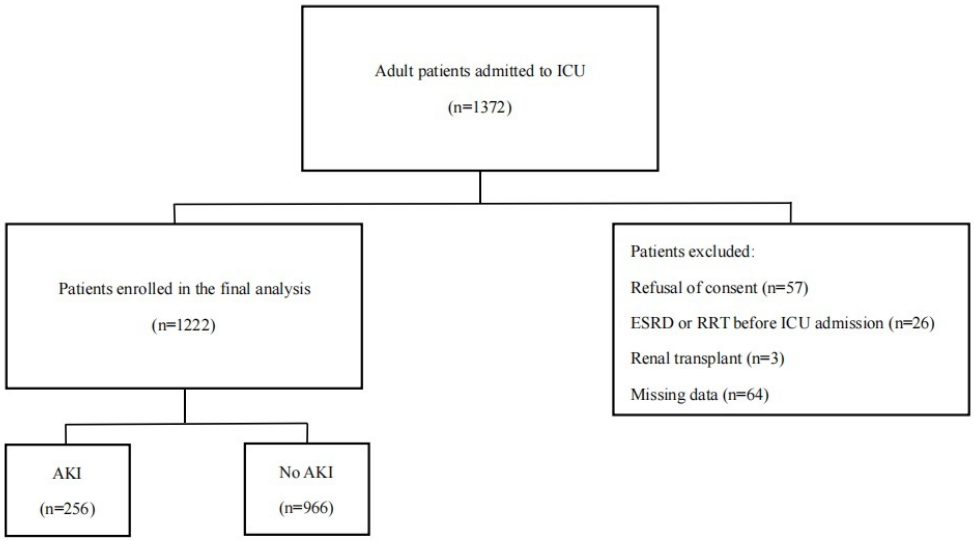
Figure legends

Figure 1. Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

Figure 2. ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

Figure 3. ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.



Flowchart for patient selection. ICU, intensive care unit; AKI, acute kidney injury; ESRD, end-stage renal disease; RRT, renal replacement therapy.

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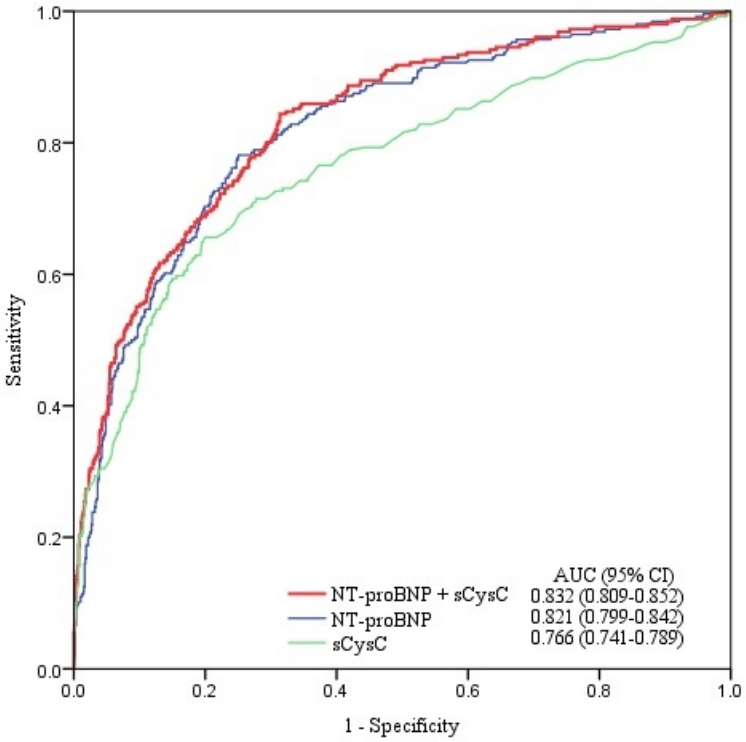

ROC analysis of the two biomarkers for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCysC, serum cystatin C.

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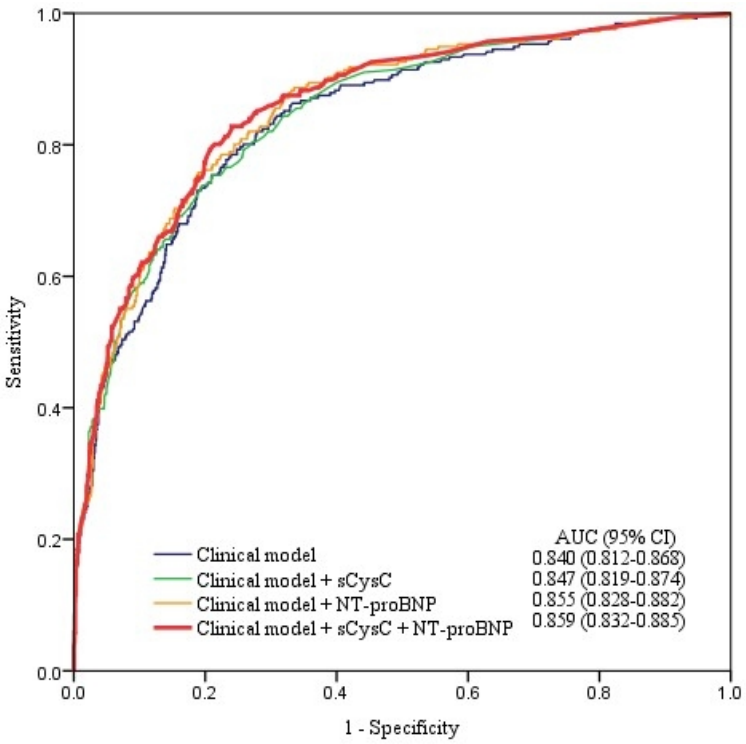

ROC analysis when two biomarkers were added to the clinical model for AKI detection. Among 1222 patients, 256 were diagnosed as AKI. ROC, receiver operating characteristic; AKI, acute kidney injury; AUC, area under the receiver operating characteristic curve; CI, confidence interval; sCysC, serum cystatin C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

165x132mm (96 x 96 DPI)

TRIPOD

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	4-5
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	7-8
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	8
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	9-10
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	9-10
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	9
	5b	Describe eligibility criteria for participants.	9
	5c	Give details of treatments received, if relevant.	NA
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	10
	6b	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	9-10
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	Explain how the study size was arrived at.	9
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	9
Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	9
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	11-12
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	12
Risk groups	11	Provide details on how risk groups were created, if done.	11-12
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	13, figure1
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	13
Model development	14a	Specify the number of participants and outcome events in each analysis.	14-15
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	14
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	13-15
	15b	Explain how to the use the prediction model.	14
Model performance	16	Report performance measures (with CIs) for the prediction model.	14-15
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	23-24
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	21-23
Implications	20	Discuss the potential clinical use of the model and implications for future research.	21-24
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	25-32
Funding	22	Give the source of funding and the role of the funders for the present study.	27

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.