Combination of Biomarker with Clinical Risk Factors for Prediction of Severe Acute Kidney Injury in Critically ill Patients

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Abstract

**Background:** Acute kidney injury (AKI) occurs commonly in the intensive care unit (ICU). Insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase-2 (TIMP-2), known as [TIMP-2] x [IGFBP7] (NephroCheck), have been identified as novel biomarkers for the prediction of AKI risk. However, disease biomarkers require appropriate clinical context to be used effectively. We conducted a cohort study to find risk factors and assess the performance of the combination of NephroCheck with risk factors to provide feasible information for AKI prediction.

**Methods:** All patients who admitted to the ICU (June 2016 to July 2017) participated in the study. The primary outcome was severe AKI within the first 7 days of the ICU. The predictors were separated into 3 categories: chronic risk factors, acute risk factors and biochemical indicators.

**Results:** The study included 577 patients. 96 patients developed to severe AKI (16.6%) within 7 days. In addition to NephroCheck (+) (OR=2.139, 95% CI (1.260-3.630), P =0.005), age >65 years (OR=1.961, 95% CI (1.153-3.336), P=0.013), CKD (OR=2.573, 95% CI (1.319-5.018), P=0.006) and PCT (+) (OR=3.223, 95% CI (1.643-6.321), P=0.001) were also independent predictors of severe AKI within 7 days. Compared to NephroCheck (+) only (AUC=0.66, 95% CI:0.60-0.72), the combination of NephroCheck (+) and risk factors (age>65years, CKD and PCT positive) (AUC=0.75, 95% CI:0.70-0.81) led to a significant increase in the area under ROC curve for severe AKI prediction within 7 days.

**Conclusions:** Although NephroCheck is an effective screening tool for recognizing patients at high risk, we found that combination with biomarker and risk factors (age>65years, CKD, procalcitonin positive) for risk assessment of AKI has the greatest significance for patients with uncertain disease trajectories.

**Background**

Acute kidney injury (AKI) is a common clinical condition occurring in intensive care unit (ICU) patients and is confirmed as a strong independent risk factor for high mortality. 50% of ICU patients will develop AKI and more than 20% of critically ill patients will reach severe AKI, stage 2 and 3 (Kidney Disease: Improving Global Outcomes, KDIGO) [1]. However, AKI is usually unpredictable. In a large proportion of patients, the development of AKI has no obvious warnings or symptoms and remains clinically silent until a sudden drop in renal function[2].

AKI could be identified by reduced urine output (urine volume < 0.5 mL/kg/h for 6 hours) and increased serum creatinine (SCr) level (≥ 26.5 µmol/L within 48 hours), however, they have been shown to be a lagging marker[3]. Owing to the limitations of SCr and urine output, much efforts have been made to find biomarkers that can be used as "kidney troponin", which ideally predicts the severity and prognosis of AKI before markers of nephrological function change [4]. Therefore, many studies were conducted to discover and validate new AKI biomarkers. Until September 2014, following the publications of two multicenter ICU cohort studies, the combination of insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase-2 (TIMP-2), known as [TIMP-2] x [IGFBP7] (NephroCheck), has been
approved for marketing by the US Food and Drug Administration (FDA)[5–7]. This is the first biomarker for AKI risk assessment to help intensive care physicians and nephrologists make early predictions for AKI in intensive care settings and optimize the timing of resuscitation and promote supportive care for patients at risk for AKI.

Recent research has focused on the use of biomarkers for AKI to recognize high risk patients, however, most of these studies have not integrated with clinical risk factors of AKI. The random and non-directional use of any biomarker will reduce its effectiveness[5, 6, 8, 9]. Basu et al[10] have shown that combining clinical data with biomarkers can improve the accuracy of predicting risk of severe AKI in pediatric ICU patients. Disease biomarkers require appropriate clinical context to be used effectively[11]. We hypothesized that the combination of the biomarker NephroCheck with risk factors would provide feasible information for the assessment of AKI and promote early intervention to improve clinical outcomes.

Methods

Study population

All patients (age ≥ 18 years old) who admitted in the ICU of San Bortolo Hospital (Vicenza, Italy) from June 2016 to July 2017 participated in the study. ESRD patients and patients diagnosed with severe AKI (stage 2 and 3) were excluded. This study was approved by the ethics committee of St. Bortolo Hospital, Vicenza, Italy (Comitato Etico provinciale a ULSS 8 Vicenza) (Exp. number: 03/17). The clinical research was conducted according to the principles expressed in the Declaration of Helsinki.

Study endpoint

The primary outcome was severe AKI (stage 2 and 3) within the first 7 days of the ICU. Secondary outcomes included continuous renal replacement therapy (CRRT) initiation, ICU mortality, and length of stay (LOS) in ICU.

Data collection

Urine samples for measuring [TIMP-2] x [IGFBP7] concentrations were obtained and analyzed immediately following enrollment. The concentration of the two proteins ([TIMP-2] and [IGFBP7]) was analyzed with the Vitros Platform (Ortho Clinical Diagnostics) using NephroCheck Kits (Astute Medical). Other data were collected from hospital records including demographics, anthropometry, admission diagnosis, comorbidities, simplified acute physiology score II (SAPS II) [12] on admission, mean arterial pressure (MAP) on admission, SCr levels on admission, lactate levels on admission and procalcitonin (PCT) levels on admission. A single SCr was recorded per day. In addition, data on CRRT, death and ICU discharge were recorded.
Definitions

Severe AKI was defined as 2.0 or more multiplied by baseline SCr according to KDIGO consensus guidelines. NephroCheck > 0.3 (ng/ml)²/1000 was considered positive and a value of ≤ 0.3 (ng/ml)²/1000 was considered negative. PCT > 0.5 µg/l was considered positive and a value of ≤ 0.5 µg/l was considered negative.

Risk factor profiling

By reviewing the literature[13–17], the predictors we identified were separated into 3 categories: chronic risk factors, acute risk factors and biochemical indicators. Chronic risk factors included advanced age (age > 65 years), obesity (BMI > 30 kg/m²), hypertension, diabetes mellitus (DM), chronic kidney disease (CKD), lung disease and cardiovascular disease (CVD). Acute risk factors were sepsis, high-risk surgery, MAP < 70 mmHg, patients requiring vasopressors and mechanical ventilation. Biochemical indicators were SCr level on admission, lactate levels on admission Nephrocheck levels on admission and procalcitonin (PCT) levels on admission.

Statistical Analysis

The percentage was calculated for category variable. Continuous variables are described as median (interquartile range). The categorical variables between the two groups were compared using Fisher’s exact test or chi-square test. The Mann-Whitney test was used for comparisons of 2 groups and the Kruskal-Wallis test was used for comparisons of 3 groups. In the pretreatment step, variables were pre-screened using univariate logistic regression analysis. Once the univariate predictor of the AKI is determined, then multivariate logistic regression is used to select the variables, which eliminated the collinearity and interaction of the selected predictors. Comparison of the areas under the receiver operating characteristic (ROC) curve was tested using the nonparametric method. P < 0.05 was considered statistically significant. Analysis was performed using SPSS Version 24.0 (IBM Corp, Somers, NY, USA).

Results

Study population

In consecutive 866 adult patients (age ≥ 18 years) who underwent screening, we excluded patients with end stage renal disease (ESRD) (n = 38), anuria (n = 53), and AKI stage 2 and 3 on admission (n = 102), incomplete data (n = 96). Therefore, the study included 577 patients. 96 patients developed to severe AKI (16.6%) within 7 days in ICU. The flowchart of this study and the number of patients is presented in Fig. 1.
Patient characteristics

Patient characteristics are listed in Table 1. Severe AKI group enrolled 96 (16.6%) patients and no severe AKI group enrolled 481 (83.4%) patients. The mean ages of severe AKI group and no severe AKI group were respectively 73 (58–81) and 67 (51–77) (P = 0.004). The patients of severe AKI group were older. The proportion of males in severe AKI group and no severe AKI group was respectively 66.7% and 62.6% (P = 0.448). Compared with patients of no severe AKI group, patients of severe AKI group were significantly older, had a higher body mass index and had more CKD, sepsis and hypotension (Map < 70 mmHg). ICU patients in the severe AKI group also had a higher SCr level, lactate level, NephroCheck value and PCT level on admission.
### Table 1
Baseline characteristics and outcomes of the study population by presence or absence of severe AKI within 7 days

| Variable                        | Severe AKI | No Severe AKI | P Value |
|---------------------------------|------------|---------------|---------|
| N                               | 96 (16.6)  | 481 (83.4)    |         |
| Age (years)                     | 73 (58–81) | 67 (51–77)    | 0.004   |
| Male                            | 64 (66.7)  | 301 (62.6)    | 0.448   |
| BMI (kg/m^2)                    | 26.18 (23.03–29.41) | 24.8 (22.86–27.68) | 0.037   |
| SAPSII                          | 44 (34–52) | 38 (27–50)    | 0.153   |

**Chronic risk factors**

| Variable                   | Severe AKI | No Severe AKI | P Value |
|----------------------------|------------|---------------|---------|
| Age > 65 years             | 66 (68.8)  | 256 (53.2)    | 0.005   |
| BMI > 30 kg/m^2            | 21 (21.9)  | 61 (12.7)     | 0.039   |
| Hypertension               | 48 (50)    | 212 (44.1)    | 0.49    |
| DM                         | 21 (21.9)  | 66 (13.7)     | 0.138   |
| CKD                        | 20 (20.8)  | 39 (8.1)      | < 0.001 |
| Lung diseases              | 8 (8.3)    | 43 (8.9)      | 0.848   |
| CVD                        | 10 (10.4)  | 58 (12.1)     | 0.649   |

**Acute risk factors**

| Variable                   | Severe AKI | No Severe AKI | P Value |
|----------------------------|------------|---------------|---------|
| Sepsis                     | 20 (20.8)  | 41 (8.5)      | < 0.001 |
| Surgery                    | 18 (18.8)  | 67 (13.9)     | 0.224   |
| Vasopressor                | 46 (47.9)  | 179 (37.2)    | 0.05    |
| Mechanical ventilation     | 71 (74)    | 329 (68.4)    | 0.281   |
| Map < 70 mmHg              | 40 (41.7)  | 109 (22.7)    | < 0.001 |

**Biochemical indicators**

| Variable                                                   | Severe AKI | No Severe AKI | P Value |
|------------------------------------------------------------|------------|---------------|---------|
| Serum creatinine, admission (mg/dl)                        | 1.08 (0.75–1.45) | 0.83 (0.65–1.08) | < 0.001 |
| Serum lactate, admission (mmol/l)                          | 2.1 (1.4–3.8) | 1.6 (1.2–2.6)  | 0.001   |
| NephroCheck value, admission (ng/ml)^2/1000                | 0.66 (0.23–2.49) | 0.29 (0.08–0.86) | < 0.001 |

Data are expressed as n (%) or median (interquartile range).
| Variable       | Severe AKI          | No Severe AKI        | P Value |
|---------------|---------------------|----------------------|---------|
| PCT, admission(ug/l) | 1.19(0.28–6.81)    | 0.26(0.10–1.45)      | < 0.001 |
| Nephrocheck (+)       | 69(71.9)            | 233(48.4)            | < 0.001 |
| PCT (+)               | 54(56.3)            | 115(23.9)            | < 0.001 |

**Outcomes**

|         | Severe AKI | No Severe AKI | P Value |
|---------|------------|---------------|---------|
| CRRT    | 11(11.5)   | 6(1.2)        | < 0.001 |
| Death   | 32(33.3)   | 63(13.1)      | < 0.001 |
| LOS(d)  | 5(2–8)     | 3(2–7)        | 0.034   |

Data are expressed as n (%) or median (interquartile range).

**Severe AKI within 7 days is associated with poor outcomes in ICU patients**

Table 1 shows 11.5% of patients in severe AKI group and 1.2% of patients in no severe AKI group required CRRT (P < 0.001). Severe AKI was also associated with ICU mortality. The mortality incidence was 33.3% in severe AKI group and 13.1% in no severe AKI group respectively (P < 0.001). Severe AKI also increased LOS in ICU. LOS of severe AKI group and no severe AKI group were 5 (2–8) and 3 (2–7) respectively (P = 0.034).

**Univariate variables associated with severe AKI within 7 days**

Table 2 provides a list of significant univariate variables associated with severe AKI within 7 days. The presence of hypertension, CVD, lung disease, high-risk surgery, mechanical ventilation and SAPSII cannot predict the development of AKI in our cohort. Among the chronic risk factors, age > 65 years, BMI > 30 kg/m², DM and CKD could predict severe AKI, the relative risk was 1.934 (95% CI(1.212–3.085), P = 0.006), 1.887 (95% CI(1.085–3.282), P = 0.025), 1.748 (95% CI (1.009–3.027), P = 0.046), 2.982 (95% CI (1.651–5.388), P < 0.001). Among the acute risk factors, sepsis and MAP < 70 mmHg could predict severe AKI, with a relative risk of 2.824 (95% CI (1.570–5.081), P = 0.001) and 2.431 (95% CI (1.537–3.845), P < 0.001). Among biochemical indicators, elevated SCr level was associated with a relative risk of 1.697 of developing severe AKI (95% CI (1.263–2.28), P < 0.001). For increase in serum lactate concentration, there was a 11.5% increased relative risk of developing severe AKI (OR = 1.115, 95% CI (1.036–1.199), P = 0.003). In addition, NephroCheck (+) predicts the development of severe AKI with a relative risk of 2.72.
(95% CI (1.684–4.394), P < 0.001). PCT (+) predicts the development of severe AKI with a relative risk of 4.883 (95% CI (2.625–9.084), P < 0.001).

Table 2
Logistic regression analysis for predictor of severe AKI within 7 days

| Variable                      | Univariate     | Multivariate   |
|-------------------------------|----------------|----------------|
| **Chronic risk factors**      |                |                |
| Age > 65 years                | 1.934 (1.212–3.085) | 1.961 (1.153–3.336) |
| BMI > 30 kg/m²                | 1.887 (1.085–3.282) | NS             |
| DM                            | 1.748 (1.009–3.027) | NS             |
| CKD                           | 2.982 (1.651–5.388) | 2.573 (1.319–5.018) |
| **Acute risk factors**        |                |                |
| Sepsis                        | 2.824 (1.570–5.081) | NS             |
| MAP < 70 mmHg                 | 2.431 (1.537–3.845) | NS             |
| **Biochemical indicators**    |                |                |
| Serum creatinine, admission (mg/dL) | 1.697 (1.263–2.281) | NS             |
| Serum lactate, admission (mmol/l) | 1.115 (1.036–1.199) | NS             |
| Nephrocheck (+)               | 2.720 (1.684–4.394) | 2.139 (1.260–3.630) |
| PCT (+)                       | 4.883 (2.625–9.084) | 3.223 (1.643–6.321) |

Data are expressed as odds ratio (95% CI). NS: Nonsignificant predictors.

**Independent predictors of severe AKI within 7 days**

Multivariate logistic regression was performed with univariate variables related to severe AKI within seven days. Following the variable selection, 4 independent predictors, including age > 65 years (OR = 1.961, 95% CI (1.153–3.336), P = 0.013), CKD (OR = 2.573, 95% CI (1.319–5.018), P = 0.006), NephroCheck (+) on admission (OR = 2.139, 95% CI (1.260–3.630), P = 0.005) and PCT (+) on admission (OR = 3.223, 95% CI (1.643–6.321), P = 0.001), predicted the development of severe AKI (Table 2).

**NephroCheck level on admission was associated with incidence of severe AKI within 7 days and its poor outcomes**
For the 577 patients, 275 (47.7%) were NephroCheck \( \leq 0.3 \) (ng/ml)\(^2\)/1000, 220 (38.1%) were NephroCheck \( (0.3-2) \) (ng/ml)\(^2\)/1000 and 82 (14.2%) were NephroCheck \( \geq 2 \) (ng/ml)\(^2\)/1000. Severe AKI incidence within seven days, CRRT initiation and ICU mortality were highest in NephroCheck \( \geq 2 \) (ng/ml)\(^2\)/1000 group. The incidence of severe AKI within seven days increased from 9.8% in NephroCheck \( \leq 0.3 \) (ng/ml)\(^2\)/1000 patients to 19.1% in NephroCheck \( (0.3-2) \) (ng/ml)\(^2\)/1000 patients and 32.9% in NephroCheck \( \geq 2 \) (ng/ml)\(^2\)/1000 patients (compared in 3 groups, \( P < 0.001 \)). The treatment of CRRT increased from 1.1% in NephroCheck \( \leq 0.3 \) (ng/ml)\(^2\)/1000 patients to 2.7% in NephroCheck \( (0.3-2) \) (ng/ml)\(^2\)/1000 patients and 9.6% in NephroCheck \( \geq 2 \) (ng/ml)\(^2\)/1000 patients (compared in 3 groups, \( P < 0.001 \)). ICU mortality increased from 13.5% in NC (-) patients to 16.8% in NephroCheck \( (0.3-2) \) (ng/ml)\(^2\)/1000 patients and 25.6% in NephroCheck \( \geq 2 \) (ng/ml)\(^2\)/1000 patients (compared with 3 groups, \( P = 0.033 \)) (Fig. 2).

**Incorporation of risk factors augments the predictive performance of the NephroCheck**

Compared to NephroCheck (+) only (AUC = 0.66, 95% CI: 0.60–0.72), the combination of NephroCheck (+) and risk factors (age > 65 years, CKD and PCT positive) (AUC = 0.75, 95% CI: 0.70–0.81) led to a significant increase in the area under ROC curve for severe AKI prediction within 7 days (Fig. 3).

**Discussion**

AKI is a major complication of major diseases which are associated with poor outcomes, high mortality and increased resource use\(^{17–19}\). The recognition of patients at high risk for developing AKI is attracting more and more attention recently. Early identification of AKI allows for rapid therapeutic intervention to achieve significant clinical benefit. AKI animal models using ischemic, toxic, and septic models have shown that multiple therapeutic agents appear to reduce kidney injury if administered before or shortly after injury\(^{20–23}\).

Cell cycle arrest may be the first process of neurological cells activation upon stress. The proteins [TIMP-2] and [IGFBP7] associated with cell cycle arrest are promising markers for the detection of AKI. It has been verified that [TIMP-2] x [IGFBP7] are superior to other biomarkers on detecting AKI in previous cohorts study\(^{5, 6}\), which provide early warnings and allow physicians to modify risk, promote intervention and avoid further complications.

Our study demonstrated that NephroCheck is an effective screening tool for recognizing patients at high risk for AKI and indicates that early NephroCheck (+) is a good predictor of severe AKI. In this study, we found that patients with NephroCheck > 0.3 (ng/ml)\(^2\)/1000 will significantly increase predictive discrimination against subsequent severe AKI. In addition to severe AKI, we also observed that patients with NephroCheck (+) were more likely to have other poor outcomes, such as CRRT initiation and death. Furthermore, as the level of NephroCheck increases, the risk of severe AKI and its poor outcomes also
In our cohort study, we have determined predictors of severe AKI, including age > 65 years, CKD, NephroCheck (+) and PCT (+). Some risk factors identified for AKI in our study are consistent with previous literature reports: age and CKD[13, 16, 17, 26, 27]. The epidemiological association between CKD and AKI makes CKD a risk factor for AKI[13, 17]. Among the various hypotheses it is possible that patients with CKD have lost renal self-regulation and hemodynamic stability, which explains the small changes in SCr level and the predisposition to subsequent damage [11].

We also observed that PCT is a predictor of severe AKI in our cohort. PCT is closely related to the severity of systemic inflammation and bacterial infection[28]. High PCT levels have been considered a good diagnostic indicator of poor prognosis in sepsis patients[29]. Increased PCT levels in patients with pancreatitis and contrast-induced AKI are associated with the development of AKI[30, 31]. Nie et al[32] reported that PCT can be used as a predictor of AKI for infective patients.

In this study, we found that combination with biomarker NephroCheck and risk factors for risk assessment of AKI has the greatest significance for patients with uncertain disease trajectories. Future research needs to clinically or electronically identify patients at high risk of AKI development or progression to CKD or ESRD[1, 33]. We plan to develop an electronic alert system including the assessment of NephroCheck and risk factors. This risk prediction tool can automatically detect high-risk patients for AKI and help early management and individualized treatment of AKI [13, 34–38]. For example, patients identified as high-risk AKI don’t need to wait for the development of AKI, but begin to optimize volume, adjust drug dose and avoid potential nephrotoxicity based on their AKI risk profile. The aim of the tool is to reduce the severity of AKI and decrease the number of patients requiring dialysis.

Our research also has limitations. First, our cohort has a small number of severe AKI events. Second, we were unable to determine all risk variables associated with AKI. For example, insufficient blood volume, oliguria and exposure to nephrotoxic drugs. Finally, when a family member is unable to provide a medical history, it is not always possible to determine the chronic risk factors for a comatose ICU patient.
Conclusions

In this study, we have determined predictors of severe AKI, including age > 65 years, CKD, NephroCheck (+) and PCT (+). Furthermore, we found that combination with biomarker NephroCheck and risk factors (age > 65 years, CKD and PCT) for risk assessment of AKI has the greatest significance for patients with uncertain disease trajectories.

Abbreviations

AKI: Acute kidney injury
ICU: Intensive care unit
KDIGO: Kidney Disease: Improving Global Outcomes
IGFBP7: Insulin-like growth factor-binding protein 7
TIMP-2: Tissue inhibitor of metalloproteinase-2
SCr: Serum creatinine
FDA: Food and Drug Administration
CRRT: Continuous renal replacement therapy
LOS: Length of stay
SAPS II: Simplified acute physiology score II
MAP: Mean arterial pressure
PCT: Procalcitonin
DM: Diabetes mellitus
CKD: Chronic kidney disease
ESRD: End stage renal disease
CVD: Cardiovascular disease
ROC: Receiver operating characteristic

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee of St. Bortolo Hospital, Vicenza, Italy (Comitato Etico provinciale aULSS 8 Vicenza) (Exp. number: 03/17). The informed consent obtained from study participants was written.

**Consent for publication**

Not applicable.

**Availability of data and materials**

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

**Competing interests**

All the authors declared no competing interests.

**Funding**

Not applicable.

**Authors' contributions**

Lan Jia and Claudio Ronco designed the study; Anna Zamperetti, Yun Xie, Valentina Corradi, Massimo De Cal, Diego Pomarè Montin and Carlotta Caprara collected data; Lan Jia, Xiaohua Sheng and Yun Xie analyzed the data; Lan Jia and Xiaohua Sheng drafted the manuscript; Lan Jia and Shikha Chandel revised the manuscript; all authors have read and approved the manuscript.

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**Figures**
Figure 1

The flowchart of this study and the number of patients.
Figure 2

The graph shows that NephroCheck level on admission has a positive relationship with incidence of severe AKI within seven days and its poor outcomes. NC: NephroCheck. *P<0.05.
Figure 3

The ROC curves show incorporation of clinic risk factors augments the predictive performance of the NephroCheck. NC only: NephroCheck (+); NC+ risk factors: age>65years, CKD, NephroCheck (+) and PCT (+).