Risk prediction for severe acute kidney injury by integration of urine output, glomerular filtration, and urinary cell cycle arrest biomarkers

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Acute kidney injury (AKI) is common in critically-ill patients admitted to the intensive care unit (ICU), and is associated with high morbidity and mortality in the critically ill population.[1-3] Its pathogenesis is still poorly understood, with limited ability to predict its occurrence.[4,5] Failure to improve outcomes of patients with AKI may be linked to delays in its detection.

AKI is defined and staged by international guidelines using two variables: serum creatinine (sCr) and urine output (UO).[6] While UO is easily measured every hour, sCr is often measured every 24 hours. Such infrequent measurement of sCr may contribute to diagnostic delays. It is plausible that small acute increases in sCr over short periods (eg, 6 h), together with UO assessment, may help predict subsequent AKI. As creatinine levels can now be measured with high frequency using low cost high validity point-of-care technology, this approach seems feasible. However, additional predictive information may also be obtained from novel biomarkers.[7]

Cell cycle arrest biomarkers (CCABs), such as tissue inhibitor of metalloproteinase type 2 (TIMP-2) and insulin-like growth factor binding protein type 7 (IGFBP-7), are secreted by tubular cells and could reflect major cell stress at the earliest stages of AKI.[8] Recently, CCAB urinary levels have been shown to accurately predict severe AKI in a broad spectrum of critical presentations, including sepsis, cardiac surgery and post-cardiac arrest patients.[9-12]

Thus, acute changes in UO and sCr and the assessment of tubular CCABs may help improve early detection of AKI. In particular, their combination may prove particularly informative and help design interventions aimed at AKI prevention.[13] Accordingly, we conducted a prospective observational study of 6-hourly UO, 6-hourly sCr changes, and urinary concentrations of CCABs in a general ICU population deemed at risk of AKI, aiming to evaluate their predictive performance for severe AKI at 12 hours after enrolment.

ABSTRACT

Background: Frequent assessment of urine output (UO), serum creatinine (sCr) and urinary cell cycle arrest biomarkers (CCAB) may improve acute kidney injury (AKI) prediction.

Objective: To study the performance of UO, short term sCr changes and urinary CCAB to predict severe AKI.

Methods: We measured 6 hours of UO, 6-hourly sCr changes, and urinary CCABs in all critically ill patients with cardiovascular or respiratory failure or early signs of renal stress between February and October 2018. We studied the association of such measurements, and their combination, with the development of AKI Stage 2 or 3 of the Kidney Disease: Improving Global Outcomes (KDIGO) definition at 12 hours. We evaluated predictive performance with logistic regression, area under the receiver operating characteristic (AUROC) curve, and net reclassification indices. We computed an optimal cut-off value for each biomarker.

Results: We assessed 622 patients and, as per the exclusion criteria, we enrolled 105 critically ill patients. After 12 hours of enrolment, AKI occurred in 32 patients (30%). UO, sCr change over 6 hours and CCABs were significantly associated with severe AKI at 12 hours, with all variables achieving an AUROC > 0.7 after adjustment. Combination of any of the two or three variables achieved an AUROC > 0.7 for subsequent severe AKI at 12 hours. The optimal predictive high specificity cut-off values were ≤ 0.4 mL/kg/h for UO, variation of +15 μmol/L over 6 hours in sCr, and ≥ 1.5 $(ng/mL)^2/1000$ for CCABs.

Conclusion: In this prospective study, an integrative approach using UO, short term sCr change and/or urinary CCABs showed a satisfactory performance for the prediction of severe AKI development at 12 hours.

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Methods

This prospective, observational, investigator-initiated study was approved by the Austin Health Human Research Ethics Committee (Melbourne, VIC, Australia; approval No. LNR/18/Austin/151 and LNRSSA/18/Austin/315), who waived the need for informed consent due to study design.

Study cohort

We prospectively assessed for eligibility all adult critically ill patients (aged ≥ 18 years) admitted to the Austin Hospital department of intensive care, within 48 hours of their admission, and presenting one of the following inclusion criteria in the preceding 6 hours:

- cardiovascular Sequential Organ Failure Assessment (SOFA) score of 1 or over;
- respiratory SOFA score of 2 or over;
- increase in sCr greater than 8 μmol/L between two creatinine measurements performed during the 6-hour period; or
- UO below 0.5 mL/kg/h over 4 hours.[14]

In eligible patients, time of inclusion (H_0) corresponded to the time at which a 10 mL urine sample was collected from the indwelling vesical catheter by research staff for CCAB measurement.

The first two inclusion criteria were the same as those used in the landmark SAPPHIRE study, which validated the prognostic value of CCABs. Such criteria were used to identify cohort A.[9] The last two inclusion criteria were selected to target another group of patients at risk of AKI (cohort B) and were derived from the AKI Stage 1 definition of the Kidney Disease: Improving Global Outcome (KDIGO) guidelines, with the aim of identifying a potentially small yet significant change in renal function over a limited period, which would portend subsequent development of Stage 2 or Stage 3 AKI.[6]

We excluded patients with anuria, known Stage 2 or 3 AKI at the time of enrolment (including patients on renal replacement therapy [RRT]), Stage 4 or 5 chronic kidney disease (including renal transplant recipient or maintenance dialysis), a history of urinary tract surgery, an expected length of stay below 48 hours, or patients undergoing end-of-life care.

Short term urine output evaluation

UO was monitored in all patients, by means of an indwelling catheter. Treating staff recorded the hourly UO volume, which was then recorded over the 6 hours preceding inclusion ($UO_{H-6,H0}$). UO was weight-corrected and expressed in mL/kg/h. This assessment protocol is presented in Figure 1.

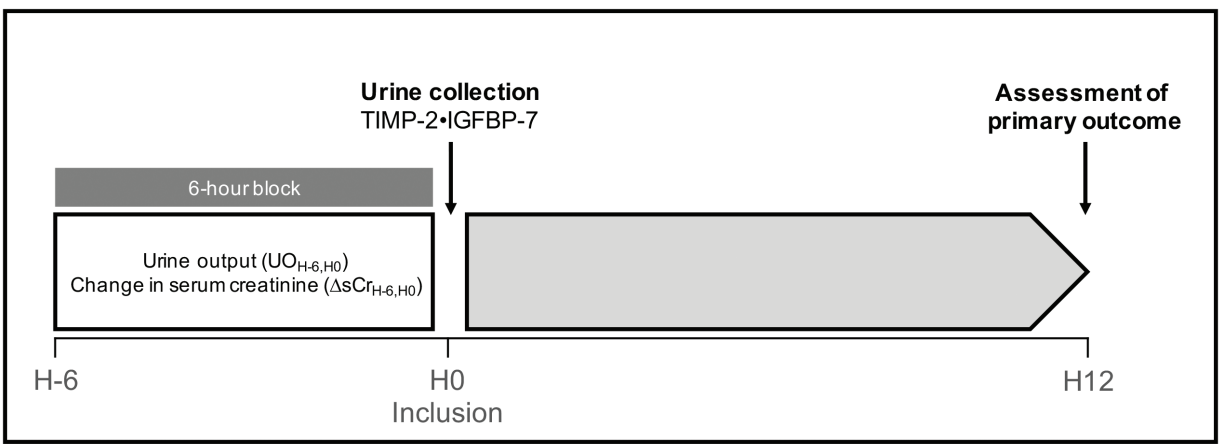
Acute biomarkers of glomerular filtration change

We recorded the acute variation of sCr values over the 6 hours preceding study inclusion ($\Delta sCr_{H-6,H0}$), corresponding to the difference between sCr at 6 hours pre-inclusion, and the sCr closest to inclusion, allowing a ± 2-hour window around each time point. The sCr levels were measured with our point-of-care blood gas analyser (Radiometer ABL 800, Radiometer Medical, Brønshøj, Denmark) (repeatability coefficient of variation [CV], 1.3%; reproducibility CV, 3.6%).[7]

Urine cell cycle arrest biomarkers

Immediately after urine collection (H_0), the 10 mL sample was centrifuged at 1500 × g for 10 minutes, following the manufacturer's recommendations. Then, 5 mL of urine

Figure 1. Study protocol



IGFBP-7: insulin-like growth factor binding protein type 7; TIMP-2: tissue-inhibitor metalloproteinase type 2.

supernatants were carefully transferred into aliquots and stored in a –20°C freezer. Urinary CCABs (TIMP-2 and IGFBP-7) were measured using this sample with an end-user-ready kit (NephroCheck, Astute Medical, San Diego, CA, USA) on the Cobas 8000 analyzer (Roche Diagnostics, Indianapolis, IN, USA). For each biomarker, the results were expressed in ng/mL of urine. A combined value, resulting from the product of the biomarkers' concentration (TIMP-2*IGFBP-7) of a given sample was automatically calculated by the assay, and expressed in $(ng/mL)^2/1000$.

Methodology of premorbid renal function estimation

Premorbid serum creatinine level was assessed using all available data present in the electronic medical record, and corresponded to the nadir sCr measured between 365 days and 7 days before ICU admission, and closest to the latter. If such data were unavailable, we retrospectively estimated them by reporting the lowest value of stable sCr recorded during the index admission. Premorbid estimated glomerular filtration rate was systematically re-estimated using the Modification of Diet in Renal Disease (MDRD) formula to avoid bias related to differing estimation methods used in result reports.[15]

Acute kidney injury

For the purpose of the study, the primary outcome was severe AKI (Stage 2 or 3 of the KDIGO guidelines staging system) occurring within 12 hours of urine collection.[6] Thus, we recorded the 12-hour cumulative UO after enrolment, and used the sCr value measured closest to the 12-hour time point (using a ± 2-hour time window). Secondary outcomes were AKI Stage 2 or 3 at 24 hours and RRT during the index ICU admission. RRT was initiated by the clinician in charge and was a decision independent of the investigators.

Other patient characteristics

We recorded patient demographics; comorbidities; category and origin of ICU admission; severity of illness, as assessed by the Acute Physiology and Chronic Health Evaluation (APACHE) III and SOFA scores; characteristics of organ failure and support; and diuretic treatment before urine collection.[14,16]

Statistical analysis

We analysed the data using the R software (version 3.3.1, The R Foundation for Statistical Computing, Vienna, Austria), with the packages *survival*, *pROC*, *OptimalCutpoints* and *nricens*.[17-19] A *P* value below 0.05 was considered statistically significant. Continuous variables were expressed as median with interquartile range (IQR), unless specified otherwise, and categorical variables as count with percentage; comparisons between groups were done using the Wilcoxon–Mann–Whitney test and the Fisher exact test respectively.

We assessed the association of UO, sCr and CCABs with the primary and secondary outcomes using generalised linear models. Adjustment of the AKI risk was performed using the following predefined variables: age, premorbid sCr levels, and APACHE III score. Using these adjustments, we completed the analysis by calculating the net reclassification index, using a 10% risk increase in the primary outcome, and the same variables cited above as the reference risk model.[20]

To assess predictive performance, we computed the area under the receiver operating characteristic (AUROC) curve for the detection of the primary outcome. AUROC quality was defined as follows: excellent (0.9–1.0), very good (0.8 to < 0.9), good (0.7 to < 0.8), fair (0.6 to < 0.7), poor (0.5 to < 0.6), and invaluable (< 0.5).[21] Optimal cut-offs were computed by selecting the point of the ROC curve at which specificity was above 0.85. Furthermore, we combined the studied biomarkers' performance by building multivariate models to assess their association with the primary outcome, respecting the rule of thumb of one included variable for every five to ten events.[22] The best model was selected using backward stepwise selection, on the basis of both Akaike and Bayesian information criteria.

Results

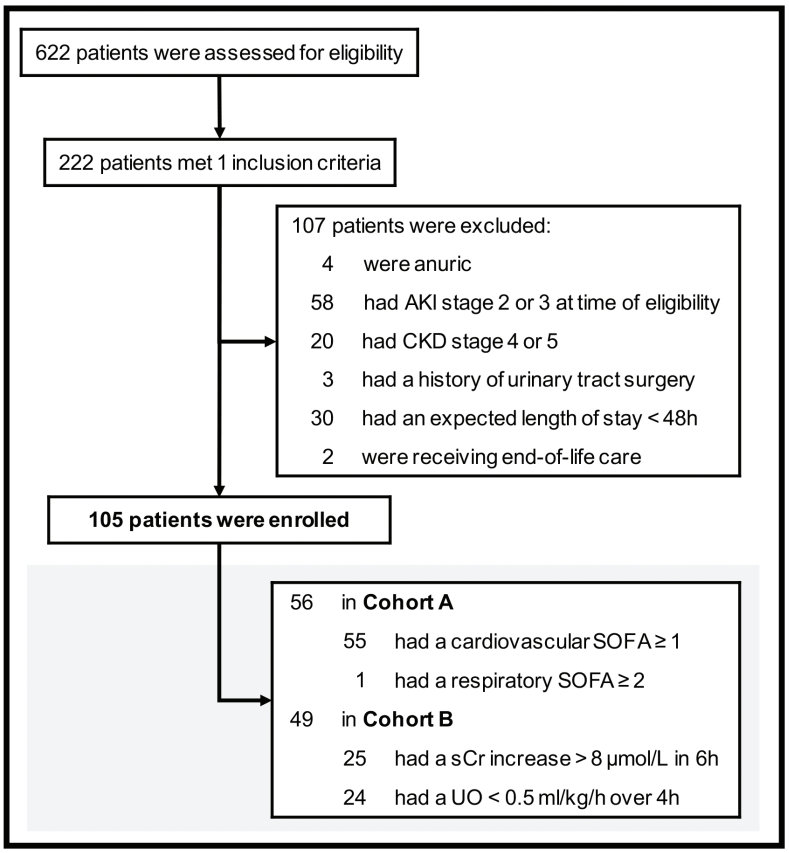
Between February 2018 and October 2018, we enrolled a convenient sample of 105 critically-ill patients (Figure 2). Enrolment occurred at a median of 14 hours (IQR, 10–17 h) into ICU admission. Patient characteristics are presented in Table 1. Patients who developed AKI had a higher weight, were more likely to be admitted after emergency surgery, had a higher baseline sCr and a higher sCr at time H_0.

The primary outcome was observed in 32 patients (30%) and was predominantly achieved by the KDIGO oliguria criterion (78%). The characteristics of patients with the primary outcome, and that of secondary outcomes are presented in the Online Appendix (supplemental table 1). The median $UO_{H-6,H0}$ was significantly lower, the median $\Delta sCr_{H-6,H0}$ was significantly higher, and the value of CCABs at enrolment was significantly greater in patients who subsequently developed Stage 2 or 3 AKI (Table 2). Diuretic use and the 6-hourly fluid balance did not differ between groups at H_0.

Association and risk prediction of biomarkers with the primary outcome

$UO_{H-6,H0}$, $\Delta sCr_{H-6,H0}$ and CCABs were significantly associated with the primary outcome in univariate analysis (Table 3). Before adjustment, the AUROC for the risk prediction of severe AKI at 12 hours was greatest for $UO_{H-6,H0}$ (Figure 3). The optimal cut-offs of the studied biomarkers are

Figure 2. Study flowchart



AKI = acute kidney injury; CKD = chronic kidney disease; sCr = serum creatinine; SOFA = Sequential Organ Failure Assessment; UO = urine output.

presented in the Online Appendix (supplemental table 2); they show that the optimal cut-off value for CCAB was 1.5 $(ng/mL)^2$/1000 or greater. After adjustment by the predefined reference model, $UO_{H-6,H0}$, $\Delta sCr_{H-6,H0}$ and CCABs remained significantly and similarly associated with the primary outcome (Table 3), with all AUROCs values being 0.70 or greater (Table 3).

Association and risk prediction of biomarkers with secondary outcomes

$\Delta sCr_{H-6,H0}$ was the only biomarker significantly associated with severe AKI risk at 24 hours and risk of RRT requirement during ICU stay (Online Appendix, supplemental table 3). It was also the only marker with an AUROC for the risk prediction of secondary outcomes (Online Appendix, supplemental figure 1).

Combination of biomarkers for acute kidney injury risk prediction

The two-variable model using $UO_{H-6,H0}$ and $\Delta sCr_{H-6,H0}$ had similar Akaike and Bayesian information criteria to the other tested models, and it was non-significantly different from the three-variable model inclusive of CCABs (Table 4). None had an AUROC of 0.80 or greater.

Effect of population selection on biomarker performance

Cohorts A and B differed in terms of vasopressor requirement, baseline lactate levels, and severity of disease at inclusion (Online Appendix, supplemental table 4). In cohort A, $UO_{H-6,H0}$ had the highest AUROC for the prediction of the primary outcome, while this was true for CCABs in cohort B (Online Appendix, supplemental table 5). The optimal threshold of CCABs in cohort A was 1.76 $(ng/mL)^2$/1000 or greater (sensitivity, 0.36 [95% CI, 0.13–0.65]; specificity, 0.86 [95% CI, 0.71–0.95]), and was 0.94 $(ng/mL)^2$/1000 or greater in cohort B (sensitivity, 0.50 [95% CI, 0.26–0.74]; specificity, 0.84 [95% CI, 0.66–0.95]).

Discussion

Main findings

In a prospective ICU cohort at risk of AKI, we assessed the performance of UO, acute sCr change, and urinary CCABs to predict severe AKI at 12 hours of enrolment. We found that, after adjustment, all three biomarkers had acceptable predictive performances for severe AKI at 12 hours. Moreover, we found that the optimal CCAB cut-off point for such prediction was 1.5 $(ng/mL)^2$/1000, and the combination of biomarkers could improve overall AKI risk prediction to a fair degree. Finally, we found that the casemix of the studied population (defined by the two cohorts) altered the predictive performance of the studied biomarkers.

Relationship with previous studies

This is the first study to assess the concept of multimodal prediction of severe AKI in ICU patients, integrating UO,

Table 1. Baseline characteristics of patients

	Whole cohort	AKI-	AKI+*	*P*
Total number of patients	105	73	32	
Demographics				
Gender, male	56 (53%)	37 (51%)	19 (59%)	0.52
Age (years), median (IQR)	64 (51–74)	64 (52–74)	64 (51–73)	0.85
Weight (kg), median (IQR)	80 (70–90)	78 (69–89)	89 (75–96)	0.01
Admission category				0.01
Elective surgery	27 (26%)	18 (25%)	9 (28%)	
Emergent surgery	31 (30%)	16 (22%)	15 (47%)	
Medical	47 (45%)	39 (53%)	8 (25%)	
Comorbidities				
Hypertension	53 (50%)	35 (48%)	18 (56%)	0.53
Diabetes	22 (21%)	14 (19%)	8 (25%)	0.60
Ischaemic heart disease	25 (24%)	20 (27%)	5 (16%)	0.22
Congestive heart failure	10 (10%)	8 (11%)	2 (6%)	0.72
COPD	16 (15%)	12 (16%)	4 (12%)	0.77
Baseline renal function				
sCr (μmol/L), median (IQR)	76 (57–89)	69 (54–86)	86 (73–100)	< 0.01
eGFR (mL/min/1.73m^2), median (IQR)	90 (64–90)	90 (74–90)	80 (56–90)	< 0.01
Severity of disease at inclusion				
APACHE III score, median (IQR)	47 (40–64)	47 (38–64)	51 (42–61)	0.39
SOFA score, median (IQR)	6 (4–8)	6 (5–8)	6 (4–9)	0.79
Sepsis	32 (30%)	25 (34%)	7 (22%)	0.25
Vasopressor support	69 (66%)	48 (66%)	21 (66%)	> 0.99
Lactate (mmol/L), median (IQR)	2.6 (1.6–4.4)	2.6 (1.6–4.2)	2.5 (1.8–4.5)	0.86
Mechanical ventilation	73 (70%)	52 (71%)	21 (66%)	0.65
sCr at H_0 (μmol/L), median (IQR)	87 (71–122)	80 (67–110)	102 (81–158)	< 0.01
Study cohorts				0.62
Cohort A	56 (52%)	42 (58%)	14 (44%)	
Cohort B	49 (48%)	31 (42%)	18 (56%)	

AKI = acute kidney injury; APACHE = Acute Physiology and Chronic Health Evaluation; COPD = chronic obstructive pulmonary disease; eGFR = estimated glomerular filtration rate; sCr = serum creatinine; SOFA = Sequential Organ Failure Assessment. * AKI+ refers to the presence of the primary outcome (severe AKI at 12 h of enrolment).

sCr changes, and CCABs. Although the conjoint use of multiple markers may theoretically improve AKI predictive ability, models have been frequently overly complex and ill-adapted to the clinical setting.[23,24]

First, episodes of oliguria defined over 6 hours (and their repetition) have been previously identified as having a higher sensitivity for subsequent AKI and mortality.[25-27] Here, a urine output of 0.4 mL/kg/h was associated with a high specificity for the prediction of severe AKI at 12 hours. However, this in not in agreement with the post hoc findings of a large observational study.[28] We hypothesise that these different findings are a consequence of casemix and AKI phenotype choice (creatinine-based *v* mainly UO-based AKI). For example, in our cohort, severe AKI was more frequently diagnosed based on the UO criterion. Therefore, it would come as no surprise that pre-enrolment oliguria would be strongly associated with a primary outcome also defined by oliguria. This may also explain why patients with a higher weight were more likely to have AKI, given that the probability of meeting the oliguria criteria for

Table 2. Studied biomarker values at time of inclusion (H_0)

	Whole cohort	AKI–	AKI+*	*P*
Total number of patients	105	73	32	
$UO_{H-6,H0}$ (mL/kg/h), median (IQR)	0.7 (0.4–1)	0.8 (0.5–1.1)	0.4 (0.3–0.7)	< 0.01
$\Delta sCR_{H-6,H0}$ (µmol/L),† median (IQR)	5 (-2–13)	3 (-2–9)	10 (3–23)	0.01
TIMP-2*IGFBP-7 (ng/mL)²/1000	0.5 (0.2–1.3)	0.4 (0.1–0.9)	0.9 (0.3–2)	< 0.01

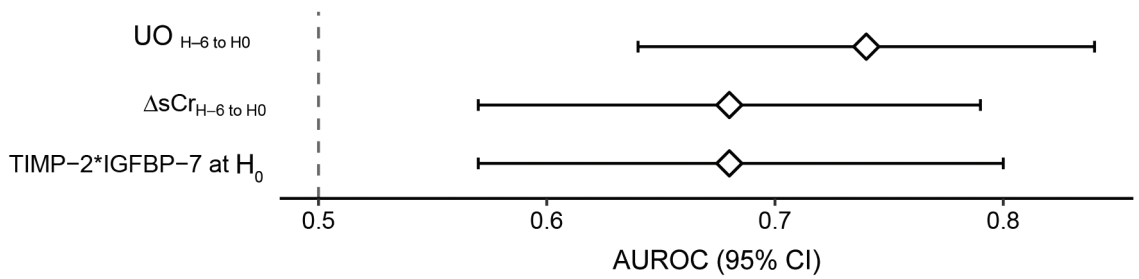
AKI = acute kidney injury; $\Delta sCr_{H-6,H0}$ = change in serum creatinine over 6 hours; IGFBP-7 = insulin-like growth factor binding protein type 7; IQR = interquartile range, OR = odds ratio; TIMP-2 = tissue inhibitor of metalloproteinase type 2; UO = urine output. * AKI+ refers to the presence of the primary outcome (severe AKI at 12 h of enrolment). † The median time between two serum creatinine measurements used to calculate ΔsCr over the 6-hour period preceding inclusion was 5.7 hours (IQR, 5.1–6.6 h). The use of Henle's loop diuretics was non-significantly different between AKI– and AKI+ patients (12 [16%] and 1 [3%] respectively; $P = 0.10$). Likewise, the cumulative fluid balance averaged over the 6 hours preceding inclusion was similar between the two groups (median, –88 mL [IQR, –407 to 258] and +87 mL [IQR, –241 to 307] respectively; $P = 0.37$).

AKI would be increased by greater weight. Nonetheless, intensive monitoring of UO improved AKI detection and was associated with better survival in a large retrospective cohort, supporting the use of this simple biomarker.[29]

Second, the use of point-of-care measurements of serum creatinine allows short term assessments of small change in glomerular filtration.[30-32] In the present study, we showed that the detection of severe AKI may be accelerated using frequent sCr measurements, which, together with other observations, challenges the once-a-day sCr measurement typical of current practice.[33] Similar to what we observed with UO, it would be expected that small change in sCr would be associated with sCr-defined AKI. Fluid balance in critically ill patients may have markedly affected sCr concentrations, and may have led to the underestimation of $\Delta sCr_{H-6,H0}$ and AKI incidence.[33,34] However, the median fluid balance varied little between two sCr measurements.

In our study, the 95% confidence intervals for the performance of urinary CCABs overlapped those presented by the SAPPHIRE investigators,[9] and this performance was also comparable to that seen in a general ICU population.[35] The predictive performance of CCABs, however, was improved when selecting patients who had signs of renal stress (cohort B). This is in line with the findings of Joannidis and colleagues[36] in a post hoc analysis of the SAPPHIRE data, which showed an improvement in risk stratification performance of urinary CCABs when combined with AKI Stage 1 indicators. We also identified an optimal CCAB cut-off value of 1.5 (ng/mL)²/1000 to have a high specificity for the primary outcome. The computed high specificity cut-off varied depending on the studied population, with a higher cut-off observed in the cohort that had the same clinical characteristics than the SAPPHIRE cohort. Furthermore, others have observed different thresholds than those initially reported; future studies should aim at better defining those thresholds and their relation to the myriad of renal-related outcomes.[37,38] Methodological and clinical differences in the characteristics of enrolled patients may also explain diverging findings

Figure 3. Predictive performance of studied biomarkers for severe acute kidney injury (AKI) at 12 hours of enrolment*



AUROC = area under the receiver operating characteristic; $\Delta sCr_{H-6,H0}$ = change in serum creatinine between H_{-6} and H_0; IGFBP-7 = insulin-like growth factor binding protein type 7; TIMP-2 = tissue inhibitor of metalloproteinase type 2. * The figure shows the AUROC curve, with 95% CI, of studied biomarkers for the prediction of severe AKI at 12 hours of inclusion. The dotted line represents the normal intercept, below which a biomarker is identified as being non-valuable. H_0 represents the time of inclusion, and corresponds to the time at which urine was sampled for TIMP-2 and IGFBP-7 measurements. H_{-6} corresponds to the 6 hours preceding inclusion. No significant difference between biomarkers' AUROC was observed.

Table 3. Association and adjusted predictive performance of acute kidney injury (AKI) risk biomarkers measured at inclusion, with severe AKI at 12 hours

	Unadjusted analysis		Adjusted analysis*					
Studied biomarker	OR (95% CI)	*P*	OR (95% CI)	*P*	AUROC (95% CI)	NRI+†	NRI–†	NRI
Urine output$_{H-6,H0}$ (per 1 mL/kg/h increase)	0.13 (0.03–0.42)	< 0.01	0.14 (0.03–0.47)	0.01	0.78 (0.68–0.87)‡	0.47	0.14	0.61
$\Delta sCR_{H-6,H0}$ (per 1 μmol/L increase)	1.06 (1.02–1.11)	< 0.01	1.05 (1.01–1.10)	0.03	0.72 (0.60–0.83)‡	0.10	0.08	0.18
TIMP-2*IGFBP-7 (per 1 (ng/mL)^2/1000 increase)	1.48 (1.12–2.04)	0.01	1.54 (1.11–2.20)	0.01	0.77 (0.68–0.86)‡	0.22	–0.05	0.17

AUROC = area under the receiver operating characteristic; $\Delta sCr_{H-6,H0}$ = change in serum creatinine over 6 hours; IGFBP-7 = insulin-like growth factor binding protein type 7; NRI = net reclassification index; OR = odds ratio; sCr = serum creatinine; TIMP-2 = tissue inhibitor of metalloproteinase type 2. * Adjustment variables included in the multivariate model to compute the adjusted OR, AUROC and NRIs were age, baseline creatinine, the Acute Physiology and Chronic Health Evaluation (APACHE) III score for the index intensive care unit admission, and the related biomarker. The AUROC of the reference model was 0.71 (95% CI, 0.61–0.81). † NRI+ relates to the prediction of the event (severe AKI at 12 h), and NRI– to the prediction of the non-event (absence of severe AKI at 12 h), and NRI refers to the sum of NRI+ and NRI–. ‡ No significant difference between the AUROC of the reference model compared with the AUROC of the reference model plus the studied biomarker.

Table 4. Performance of combined biomarkers for the prediction of severe acute kidney injury (AKI) at 12 hours*

Models	Observations	Events	AIC	BIC	AUROC (95% CI)	*P*†
Three-variable model						
$UO_{H-6,H0}$ + $\Delta sCr_{H-6,H0}$ + TIMP-2*IGFBP-7	91‡	26	101.5	111.5	0.77 (0.66–0.87)	–
Two-variable models						
$UO_{H-6,H0}$ + $\Delta sCr_{H-6,H0}$	91‡	26	101.0	108.6	0.76 (0.65–0.87)	0.56
$UO_{H-6,H0}$ + TIMP-2*IGFBP-7	105	32	117.6	125.6	0.74 (0.64–0.84)	0.72
$\Delta sCr_{H-6,H0}$ + TIMP-2*IGFBP-7	91‡	26	106.5	114.0	0.71 (0.60–0.83)	0.20

AIC = Akaike information criterion; AUROC = area under the receiver operating characteristic; BIC = Bayesian information criterion; $\Delta sCr_{H-6,H0}$ = change in serum creatinine over 6 hours; IGFBP-7 = insulin-like growth factor binding protein type 7; OR = odds ratio; TIMP-2 = tissue inhibitor of metalloproteinase type 2; UO = urine output. * Multicollinearity of variables was tested using the variance inflation factor and was not present in the models used. Interaction between variables was systematically tested. No such interaction existed in the presented models. † *P* value corresponding to the comparison of the AUROC to the three-variable model. ‡ A number of observations were not included in the model building process due to 14 missing values of $\Delta sCr_{H-6,H0}$.

compared with the SAPPHIRE study. However, the absence of details on the AKI-defining criterion (UO-only severe AKI especially), frequency of UO and creatinine assessment (hourly and 6-hourly in the present work respectively), and the patients' AKI stage at time of enrolment in the SAPPHIRE report prevent further comparison. Finally, the timing at which CCABs are measured may also have an impact on their diagnostic performance. Others have demonstrated that CCABs should be measured 24 hours after the clinical diagnosis of sepsis (compared with 12 h) to predict with high accuracy the incidence of RRT-requiring AKI.[38] Hence, it is plausible that CCABs were measured too early in the present cohort to show their optimal proficiency. Importantly, after adjustment, CCABs performed as well as sCr and UO.

Finally, we found an incidence of severe AKI within the range of that reported by previous large observational works.[1,5] This finding consolidates our results as being typically representative of the critically ill population and supports the search for novel diagnostic tools to detect severe AKI as early as possible.[13]

Implication of study findings

Our findings imply that severe AKI may be predicted using short term assessment of UO, acute sCr change, or CCAB measurements in the critically ill population. Moreover, they imply that risk prediction could be improved when combining such biomarkers, and that the prediction performance of a biomarker is inevitably affected by AKI

phenotype (sCr- or oliguria-defined), timing of measurement, and casemix. Finally, they imply that prediction based on the very variables (UO and sCr) that define the outcome represents a tautology. Given that such continuum does not apply to CCABs, their comparable predictive performance provides a degree of support for their biological plausibility.

Strengths and limitations

Our study has several strengths. The high rate of severe AKI reflects the severity of illness in our population and its exposure to AKI risk factors, as well as the adequacy of selecting the target population when testing prediction tools.[5] This is the first study to test the performance of three predictive markers: urine flow, frequent sCr and CCABs, aiming at integrating them into a multimodal assessment of AKI risk. Urinary biomarkers were measured by a laboratory investigator blinded to the primary outcome and clinical context, while all involved investigators were unaware of urinary biomarkers results at the time of AKI adjudication. Also, this was a prospective observational study, in which investigators were not involved in patient care. Furthermore, we selected the same primary outcome than the one reported by the SAPPHIRE group, allowing comparison of our results with the already available literature, especially those observed in cohort B. However, we acknowledge that secondary outcomes may have been of greater interest to clinicians, but their low incidence would have limited the assessment of the statistical association with the biomarkers of interest. Finally, we used an adequate and validated statistical methodology, such as net reclassification indices and multivariate models, to assess each biomarker's performance.

Nevertheless, we acknowledge some limitations. First, this is a single centre study, which limits the external validity of its findings. However, our ICU has all the characteristics of a tertiary teaching centre, with a recruitment of a broad spectrum of medical and postoperative patients alike. Furthermore, we observed an incidence of severe AKI similar to that reported in other studies.[5] Second, we acknowledge that no power calculation was performed before enrolment. Nonetheless, this is one of the largest studies on CCAB performance, behind those of the SAPPHIRE group. Third, this is an observational study, limiting any inference on causality. However, our findings are based on a simple rationale, directly linked to the definitions of AKI, hence strongly suggesting a physiological continuum between short term variations in sCr and/or urine flow and KDIGO-defined AKI. Fourth, our findings may have also been significantly affected by our population selection, as shown by the significant differences in performance of biomarkers in cohorts A and B. This limits the extrapolation of our results to other populations, but should also motivate cautious population selection when evaluating future biomarkers. Finally, we did not compare our results with other biomarkers such as neutrophil gelatinase-associated lipocalin or soluble urokinase-type plasminogen activator receptor.[39] However, their performance is limited, and they have not been approved by the United States Food and Drug Administration.

Conclusions

In this single centre prospective study, an integrative approach using UO, short term sCr change, and CCABs showed acceptable performances for the risk prediction of severe AKI at 12 hours. The frequent evaluation of already available markers with the addition of CCABs may help identify high risk patients in whom AKI-specific therapeutic strategies could be tested.

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Competing interests

None declared.

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