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Urinary Tissue Inhibitor of Metalloproteinase-2 and Insulin-Like Growth Factor-Binding Protein 7 for Risk Stratification of Acute Kidney Injury in Patients With Sepsis

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Dr. Shi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Design and conduct of the study: Dr. Kellum and Dr. Chawla. Data collection and interpretation: all authors reviewed the data and participated in discussions related to interpretation. Preparation, review or approval of the article: Dr. Honore, Dr. Nguyen, Dr. Shi, and Dr. Kellum wrote the article. All authors reviewed and edited the article and have seen and approved the final draft.

DOI: 10.1097/CCM.0000000000001827

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccmjournal).

Supported by Astute Medical.

Clinical Trials Registration: ClinicalTrials.gov # NCT01209169 and #NCT01573962.

Dr. Honore had also received research grants from Baxter, AM Pharma, Bellco, and Pfizer. Dr. Nguyen's institution received funding from Astute Medical. Dr. Gong is supported by grants from the National Institutes of Health. Dr. Gong's institution received funding from Astute. Dr. Chawla is employed by La Jolla Pharmaceutical Company and has received consulting fees from Astute Medical, Alere Medical, AM Pharma, and Baxter Medical, and has licensed unrelated technologies through George Washington University to Astute Medical. Dr. Bagshaw is supported by a Canada Research Chair in Critical Care Nephrology. Dr. Bagshaw has received consulting fees from Baxter Healthcare, Masimo. Dr. Artigas reports consultation fees from Baxter, Rubio and Grifols. Dr. Artigas received funding from Rubio Co and Grifols (consultancy and lectures) and disclosed other support (Ferrer farma). His institution received funding from Hill-Rom research grant. Dr. Shi received consulting fees from Astute Medical and has family disclosures (her husband also received consulting fees from Astute Medical). Dr. Joannes-Boyau received consulting fees from Gambro Baxter, B. Braun, and Merck Sharp & Dohme. His institution received funding from Astute. Dr. Kellum has received consulting fees from Astute Medical, Alere, Aethlon, AM Pharma, Cytosorbents, Bard, Fresenius, Baxter, Abbott Diagnostics, and Spectral Diagnostics. Dr. Kellum has also received research grants from Astute Medical, Alere, Cytosorbents, Bard, Baxter, and Spectral Diagnostics and has licensed unrelated technologies through the University of Pittsburgh to Astute Medical, and Cytosorbents; received funding from Astute Medical, Alere, Aethlon; other: Received consulting fees AM Pharma, Cytosorbents, Bard, Fresenius, Baxter, Abbott Diagnostics, and Spectral Diagnostics. He disclosed other support (Licensed unrelated technologies through University of Pittsburgh to Astute Medical and Cytosorbents). His institution received funding from Astute Medical, Alere, and Cytosorbents. Dr. Vincent disclosed that he does not have any potential conflicts of interest.

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Objectives: To examine the performance of the urinary biomarker panel tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 in patients with sepsis at ICU

admission. To investigate the effect of nonrenal organ dysfunction on tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 in this population.

Method: In this ancillary analysis, we included patients with sepsis who were enrolled in either of two trials including 39 ICUs across Europe and North America. The primary endpoint was moderate-severe acute kidney injury (equivalent to Kidney Disease Improving Global Outcome stage 2–3) within 12 hours of enrollment. We assessed biomarker performance by calculating the area under the receiver operating characteristic curve, sensitivity, specificity, and negative and positive predictive values at three cutoffs: 0.3, 1.0, and 2.0 $(ng/mL)^2/1{,}000$. We also calculated nonrenal Sequential Organ Failure Assessment scores for each patient on enrollment and compared tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 results in patients with and without acute kidney injury and across nonrenal Sequential Organ Failure Assessment scores. Finally, we constructed a clinical model for acute kidney injury in this population and compared the performance of the model with and without tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7.

Results: We included 232 patients in the analysis and 40 (17%) developed acute kidney injury. We observed significantly higher urine tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 in patients with acute kidney injury than without acute kidney injury in both patients with low and high nonrenal Sequential Organ Failure Assessment scores ($p < 0.001$). The area under the receiver operating characteristic curve (95% CI) of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 was 0.84 (0.73–0.92) and 0.85 (0.76–0.94), in low and high nonrenal Sequential Organ Failure Assessment score subgroups. Performance of the tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 test was not modified by nonrenal Sequential Organ Failure Assessment ($p = 0.70$). In multivariate analysis, the addition of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 significantly improved the performance of a clinical model for predicting acute kidney injury ($p = 0.015$).

Conclusion: Urinary tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 accurately predicts acute kidney injury in septic patients with or without other organ failures. (*Crit Care Med* ; 44:1851–1860)

Key Words: acute kidney injury; insulin-like growth factor-binding protein 7; organ dysfunction; risk prediction; sepsis; tissue inhibitor of metalloproteinase-2

Acute kidney injury (AKI) is one of the most common complications of sepsis and is associated with an increased ICU and hospital mortality (1–7). The benefit of preventive and therapeutic measures for AKI has been difficult to confirm because treatments are often initiated when renal injury is already established (8, 9). Perhaps as a result, the prognosis for sepsis patients with AKI remains poor.

Biomarkers for AKI might allow for earlier initiation or more tailored application of renal protection measures and avoidance of iatrogenic harm (10, 11). Two novel urinary biomarkers, tissue inhibitor of metalloproteinases-2 (TIMP-2), and insulin-like growth factor-binding protein 7 (IGFBP7) have been validated for predicting moderate and severe AKI (classified as AKI stage 2 and 3 according to the Kidney Disease Improving Global Outcome [KDIGO] 2012 classification) (12) in critically ill patients. Both TIMP-2 and IGFBP7 are markers of cellular stress in the early phase of tubular cell injury caused by a wide variety of insults (inflammation, ischemia, oxidative stress, drugs, and toxins) (13–16). Furthermore, both molecules can initiate G_1 cell-cycle arrest that prevents cells from dividing when potentially injured (17). Importantly, both biomarkers also act as "alarm" proteins exerting paracrine effects on adjacent cells (18). The product of these two biomarkers ([TIMP-2]·[IGFBP7]) outperformed all other known biomarkers or biomarker combinations for predicting moderate-severe AKI (19), and the test has been validated using a clinical adjudication committee as a gold-standard for AKI (20). Finally, [TIMP-2]·[IGFBP7] measurement proved to be a highly sensitive predictor of AKI in cardiac surgery patients (21).

Importantly, performance of novel biomarkers for AKI can suffer in patients with sepsis presumably because many of the pathologic processes of sepsis can affect biomarkers even without injuring the kidney (22). Furthermore, because the mechanisms of organ injury in sepsis may not be specific for the kidney, nonrenal organ failures could mimic AKI (23). Thus, we sought to evaluate the performance of [TIMP-2]·[IGFBP7] in patients with sepsis, with or without nonrenal organ failures. We assessed this biomarker combination in a subset of patients with early sepsis from the Sapphire (19) and Topaz (20) prospective clinical trials using cutoff values of greater than 0.3, 1.0, and 2.0 $(ng/mL)^2/1{,}000$ (24). At the time of biomarker measurement and adjusting for common sepsis risk factors, we evaluated test performance in conjunction with bedside clinical parameters.

PATIENTS AND METHODS

Study Design

We conducted a preplanned subgroup analysis of critically ill patients enrolled in either of our two previously reported studies on the discovery/validation (Sapphire) (19) and subsequent secondary validation (Topaz) (20) of [TIMP-2]·[IGFBP7]. We defined sepsis based on international consensus criteria and the clinical diagnosis assigned by the treating physicians at enrolling sites (25). All patients were considered to be at high risk for AKI, characterized by respiratory or cardiovascular dysfunction as previously reported (**Fig. 1**) (19, 20). The design, execution, and reporting of this study meet the Strengthening the Reporting of Observational Studies in Epidemiology (26) and the Standards for Reporting of Diagnostic Accuracy criteria (27). Data were collected by the investigators and analyzed by independent statisticians not directly affiliated with

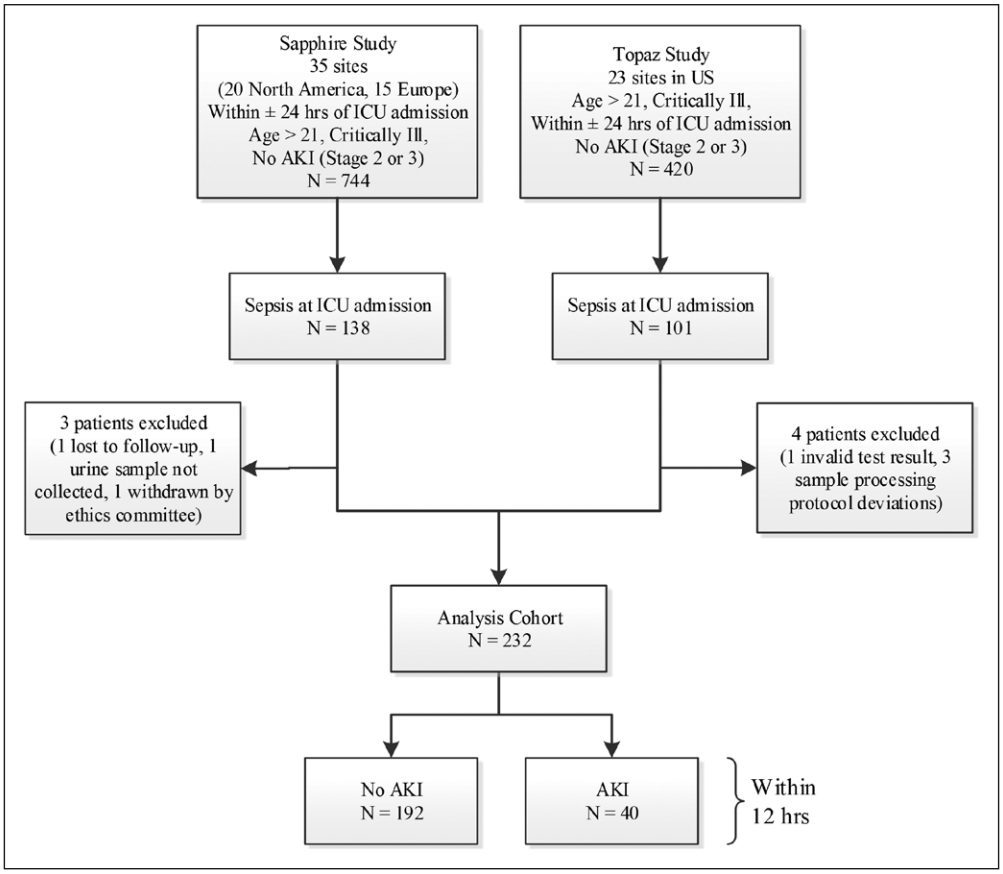

Figure 1. Study design. Acute kidney injury (AKI) defined as Kidney Disease Improving Global Outcome (KDIGO) AKI stage 2 or 3 (Sapphire) (19) and determined by clinical adjudication based on KDIGO stage 2–3 AKI (Topaz) (18).

the study. Both study protocols were approved by the Western Institutional Review Board (Olympia, Washington, DC) and also by the institutional review board or ethics committee of each study site if required. All patients (or authorized representatives) provided written informed consent. In this article, we present data from the Sapphire study, which defined AKI as KDIGO stage 2–3 (12), and from the Topaz study, which used clinical adjudication for AKI (20), in order to examine the performance of the [TIMP-2]·[IGFBP7] test for risk assessment of AKI in patients with sepsis.

Measurements

Urine and serum samples for biomarker and creatinine assessment, respectively, were obtained within 24 hours of ICU admission. TIMP-2 and IGFBP7 concentrations were measured by immunoassay with the NephroCheck Test on the Asute140 Meter (Astute Medical, San Diego, CA) by technicians blinded to clinical data. Measurements of TIMP-2 and IGFBP7 were made at Astute Medical for the Sapphire study and in triplicate at three independent laboratories (University of California at San Diego, CA; University of Louisville, KY; and ARUP Laboratories in Salt Lake City, UT) for the Topaz study. The median of the triplicate values from Topaz was used for analysis, and values were reported in units of $(ng/mL)^2/1{,}000$. Serum creatinine testing was performed as previously described (19, 20).

We assessed severity of illness and organ dysfunction/failure with the Acute Physiology and Chronic Health Evaluation (APACHE) III (28) and Sequential Organ Failure Assessment (SOFA) (29) scores. Nonrenal APACHE III and SOFA scores were calculated by subtracting the renal components from these scores.

Statistical Methods

To assess the performance of [TIMP-2]·[IGFBP7] in predicting AKI, we calculated area under the receiver operating characteristic curve (AUC), sensitivity, specificity, negative and positive predictive values (NPV and PPV), and relative risk (RR). The Delong method was used to estimate the 95% CI for AUC. Bootstrap method was used to estimate the 95% CI for sensitivity, specificity, NPV, and PPV, except for the cases where there were empty cells and the Clopper-Pearson Exact method was used instead. RR was calculated at each [TIMP-2]·[IGFBP7] stratum relative to the lowest stratum. The 95% CI for RR was calculated using bootstrap method except when there were empty cells, and an exact unconditional method was used instead (30).

To examine the effect of nonrenal organ dysfunction and AKI on the levels of [TIMP-2]·[IGFBP7], we performed a multiple linear regression analysis where the response variable was rank transformed [TIMP2]·[IGFBP7]; the explanatory variables were AKI status, subgroup status according to the median of nonrenal SOFA scores, and the interaction between them. The same analysis was also performed for each individual nonrenal SOFA component.

To assess the added value of [TIMP-2]·[IGFBP7] in predicting AKI over using clinical variables alone, we constructed two multivariable logistic regression models: one with only clinical variables as explanatory variables and the other with the addition of [TIMP-2]·[IGFBP7] as an explanatory variable besides clinical variables. A backward stepwise regression procedure with Bayesian Information Criteria was used to select which clinical variables to be included the final models. Model goodness-of-fit was assessed using the Hosmer-Lemeshow method. To quantify the added predictive ability of [TIMP-2]·[IGFBP7], we calculated integrated discrimination improvement (IDI) and category-free net reclassification improvement (cfNRI)

using R package "PredictABEL" (31). In addition, we compared the AUCs from these two models using the Delong method for paired AUCs.

A *p* value of less than 0.05 was considered to indicate statistical significance. All reported *p* values are two sided. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC) and R 3.1.0 (31).

RESULTS

Baseline and Clinical Characteristics

We included the 232 sepsis patients in the analysis, including 40 (17%) who developed AKI within 12 hours of testing. Baseline characteristics of all patients stratified by AKI status are shown in **Table 1**. Almost half of the patients were

TABLE 1. Baseline Characteristics of Patients Grouped Acute Kidney Injury Versus No Acute Kidney Injury

Variables	AKI	No-AKI	*p*
All patients	40	192	
Male	17 (43%)	102 (53%)	0.23
Age (yr)[a]	64 (16)	62 (17)	0.46
Body mass index (kg/m^2)[b]	31 (26–39)	27 (24–32)	0.008
Race, *n* (%)			0.49
Black	5 (13)	17 (9)	
Caucasian	29 (73)	154 (80)	
Other/unknown	6 (15)	21 (11)	
Medical history, *n* (%)			
Chronic kidney disease	4 (10)	11 (6)	0.30
Diabetes mellitus	13 (33)	52 (27)	0.56
Congestive heart failure	11 (28)	25 (13)	0.03
Coronary artery disease	8 (20)	42 (22)	0.99
Hypertension	24 (60)	111 (58)	0.86
Chronic obstructive pulmonary disease	5 (13)	32 (17)	0.64
Cancer	16 (40)	61 (32)	0.36
Liver disease	6 (15)	8 (4)	0.02
Acute exposures and susceptibilities, *n* (%)			
Emergency surgery	1 (3)	22 (11)	0.14
Radiocontrast agents	13 (33)	67 (35)	0.86
Nephrotoxic drugs	39 (98)	171 (89)	0.14
Hematocrit < 30%	17 (43)	109 (57)	0.12
Nonrenal Acute Physiology and Chronic Health Evaluation III	73 (56–97)	61 (47–81)	0.008
Nonrenal Sequential Organ Failure Assessment[c]	9 (7–11)	7 (5–9)	0.02
Admitted to ICU			0.55
Emergency department	21 (53)	86 (45)	
Ward	7 (18)	47 (24)	
Operating room	2 (5)	23 (12)	
Other hospital	9 (23)	31 (16)	
Other ICU	0 (0)	2 (1)	
Unknown	1 (3)	3 (2)	

(*Continued*)

TABLE 1. (*Continued*). Baseline Characteristics of Patients Grouped Acute Kidney Injury Versus No Acute Kidney Injury

Variables	AKI	No-AKI	*p*
Baseline variables			
Body temperature (°C)[b]	36.3 (35.6–38.5)	36.6 (36.0–38.5)	0.21
Heart rate (beats/min)[b]	119 (100–137)	116 (103–130)	0.96
Respiration rate (breaths/min)[b]	30 (25–36)	29 (15–36)	0.63
Mean arterial pressure (mmHg)[b]	56.0 (49.2–62.7)	61.0 (54.0–68.0)	0.007
WBC count (109/L)[b]	15.2 (8.6–24.1)	13.8 (7.7–19.9)	0.39
24-hr fluid input (mL)[b]	4245 (3124–7097)	4280 (2616–5757)	0.35
24-hr fluid output (mL)[b]	975 (413–2251)	2195 (1500–3030)	< 0.001
% Fluid overload (1st 24 hr)[b,d]	3.8 (1.3–7.4)	1.9 (0.2–5.1)	0.009
Mechanical ventilation	29 (73%)	121 (63%)	0.28
Vasopressor use	34 (85%)	128 (67%)	0.02
Blood transfusion	16 (40%)	58 (30%)	0.26
Primary source of infection, *n* (%)			0.92
Abdomen	6 (15)	22 (11)	
Lung	14 (35)	72 (38)	
Skin or soft tissue	4 (10)	19 (10)	
Urinary tract	6 (15)	37 (19)	
Other/unspecified	10 (25)	42 (22)	
Time from ICU admission to biomarker sample collection (hr)[b]	12 (7–18)	15 (8–21)	0.15
Enrollment serum creatinine (mg/dL)[b,e]	1.4 (1.0–1.9)	0.9 (0.7–1.3)	< 0.001
Tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 [(ng/mL)^2/1,000]	2.1 (1.1–4.3)	0.4 (0.2–1.0)	< 0.001

[a]Average (SD).
[b]Median (interquartile range).
[c]Sequential Organ Failure Assessment score on the day of enrollment.
[d][Fluid input – output (L)/body weight (kg)] × 100 on the day of enrollment.
[e]Hospital value taken closest to the time of enrollment.

referred from the emergency department with relatively short delay between hospital and ICU admission (median [intraquartile range] time from hospital to ICU admission was 2 hr [0–8 hr]). Exposure to radiocontrast agents and nephrotoxic drugs and the presence of risk factors such as diabetes mellitus, coronary artery disease, and hypertension were similar in patients who did or did not develop AKI. However, history of congestive heart failure and liver disease were more common in those patients developing AKI. Patients with AKI also had lower mean arterial pressure and were more often treated with vasopressors. Baseline serum creatinine and severity of illness as judged by baseline nonrenal APACHE III and SOFA scores were also greater for patients developing AKI (Table 1).

Biomarker Performance

The unadjusted RR for AKI by strata of [TIMP-2]·[IGFBP7] defined by three cutoffs (0.3, 1.0, and 2.0) are shown in **Figure 2**. The absolute risk in the low stratum (≤ 0.3) was 2.7% increasing to 53.3% in the highest stratum (> 2.0) for an RR of 19.7 (95% CI, 4.3–69.4; $p < 0.001$). Patients with AKI had significantly higher levels of [TIMP-2]·[IGFBP7] than patients without AKI ($p < 0.001$). This effect of AKI on [TIMP-2]·[IGFBP7] levels was not modified by nonrenal SOFA ($p = 0.70$). In addition, nonrenal SOFA subgroup did not affect [TIMP-2]·[IGFBP7] values after adjusting for AKI status ($p = 0.29$).

The overall AUC for [TIMP-2]·[IGFBP7] for predicting AKI in this cohort was 0.84 (0.77–0.90). For patients with a nonrenal SOFA score of greater than 7, the [TIMP-2]·[IGFBP7] AUC was 0.85 (0.76–0.94) and similarly for patients with nonrenal SOFA of less than or equal to 7, the AUC was 0.84 (0.73–0.92) (**Fig. 3**). For comparison, the AUC for a serum creatinine measured at the same time as urinary [TIMP-2]·[IGFBP7] yielded AUC of 0.73 (0.59–0.85) for patients with a nonrenal SOFA score of greater than 7 and AUC of 0.77 (0.65–0.87) for

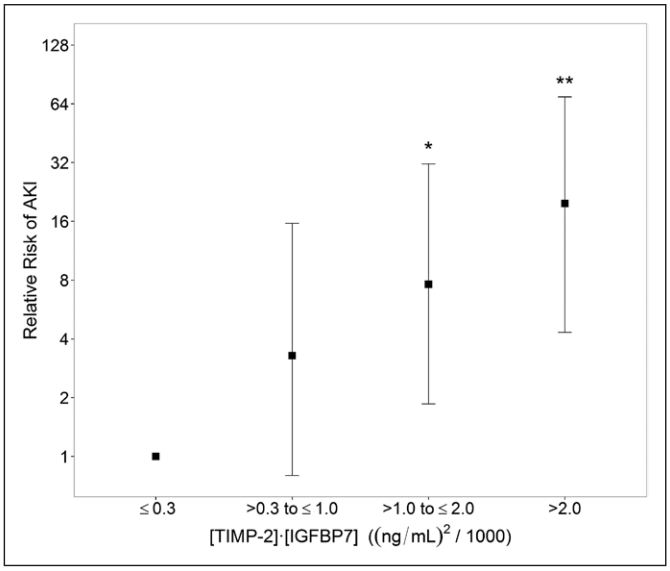

Figure 2. Unadjusted relative risk (with 95% CIs) for acute kidney injury (AKI) stratified according to tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 [TIMP-2]·[IGFBP7] concentrations in all patients. AKI risk in strata with [TIMP-2]·[IGFBP7] values between 0.3 and 1.0, 1.0 and 2.0, and greater than 2.0 relative to risk in the stratum less than or equal to 0.3. Relative risk estimates for the upper two strata are significantly greater than 1 ($^{*}p < 0.01$, $^{**}p < 0.001$). Total of 40 AKI subjects and 192 no AKI. Absolute risk in the less than or equal to 0.3 stratum was 2.7% (95% CI, 0.33–9.4%). $n = 74$, 79, 34, and 45 for strata less than 0.3, more than 0.3 to less than or equal to 1.0, more than 1.0 to less than or equal to 2.0, and more than 2.0, respectively.

patients with a nonrenal SOFA score less than or equal to 7. Similarly, estimated glomerular filtration rate yielded AUCs of 0.75 (0.61–0.87) and 0.76 (0.63–0.87).

Of note, we reported previously that for patients with sepsis in the Sapphire study (19), the individual marker performance for TIMP-2 was superior to IGFBP7, whereas the opposite was true for surgical patients. Here, in this combined cohort of sepsis patients, we again saw a better AUC for TIMP-2 0.84 (0.77–0.90) compared with that for IGFBP7 0.79 (0.72–0.86). Interestingly, these results were unchanged across low and high nonrenal SOFA subgroups.

[TIMP-2]·[IGFBP7] remained a strong predictor for AKI after adjustment for clinical variables, including severity of illness (APACHE III), nonrenal organ dysfunction (SOFA), body mass index, fluid output, and serum creatinine concentration. Addition of [TIMP-2]·[IGFBP7] to a clinical model significantly improved its predictive ability from 0.86 to 0.94 ($p = 0.015$) (**Table 2**). We also performed cfNRI and IDI. **Table S1** (Supplemental Digital Content 1, http://links.lww.com/CCM/B893) shows that [TIMP-2]·[IGFBP7] improves the overall predictive ability of the model (statistically significant AUC increase, IDI, and cfNRI). ICU and hospital outcomes stratified by biomarker results are shown in **Table S2** (Supplemental Digital Content 2, http://links.lww.com/CCM/B894).

Finally, operating characteristics (sensitivity, specificity, PPV, and NPV) at all three cut-offs (0.3, 1.0, and 2.0) are shown in **Table 3**. Sensitivity remains near 95% and specificity near 90% for the 0.3 and 2.0 cut-offs, respectively. **Table S3** (Supplemental Digital Content 3, http://links.lww.com/CCM/B895) shows operating characteristics for [TIMP-2]·[IGFBP7] cutoffs ranging from 0.1 to 5.0 at 0.1 intervals.

DISCUSSION

For patients with sepsis, AKI is strongly associated with both short- and long-term adverse consequences (1, 2). Indeed, in a recent study of patients with septic shock, 60-day hospital mortality was 6.2% for patients without AKI, 16.8% for stage 1, and 27.7% for stage 2–3 (32). Early and adequate treatment of sepsis might prevent sepsis-induced AKI, attenuate AKI severity or might reduce the need for renal replacement therapy (RRT) (33, 34). Rapid identification of septic patients at high risk for developing AKI could substantially improve the therapeutic approach. In this context, specific and sensitive biomarkers of renal cell injury or stress could play an important role. We evaluated the performance of the novel urinary cell-cycle arrest biomarker test [TIMP-2]·[IGFBP7] in septic patients at high risk for developing AKI. We chose to assess risk of moderate to severe AKI because this severity (corresponding to KDIGO stage 2 and 3) has been shown to be associated with a significantly increased prevalence of clinically important outcomes such as receipt of RRT and in hospital death (35), as well as adverse effects on long-term survival specifically in patients with sepsis (1).

Sepsis is a challenging area for AKI biomarkers. NGAL and IL-18 have been examined as potential biomarkers of AKI, but both are strongly influenced by systemic inflammation thus degrading their specificity (36, 37). Although a small study recently reported better performance of NGAL (both plasma and urine) for AKI in patients with sepsis (AUCs, 0.83 and 0.89) (38), most studies have noted modest performance of biomarkers in this population. Recently, a sophisticated machine-learning analysis was performed using candidate biomarkers selected from extensive transcriptomic analysis to predict AKI on day 3 in pediatric patients with sepsis (37). Even after including AKI status on day 1 in the model (positive in half the cases), the AUC only reached 0.83 in the test cohort (37). These challenges are perhaps not surprising because the mechanisms of organ injury in sepsis may not be organ specific (37). However, our results indicate that the performance of [TIMP-2]·[IGFBP7] in patients with sepsis is not significantly confounded by nonrenal SOFA. Thus, clinicians can rely on these biomarkers for predicting AKI even in the presence of nonrenal organ failures. This is a notable advance in comparison with other biomarkers that are available around the world (39–42).

As alluded to in a recent study of remote ischemic preconditioning (43), biomarkers of cell-cycle arrest such as TIMP-2 and IGFBP7 may signal that the renal epithelium has been stressed and has shut down function but may still be able to recover without permanent injury to the organ. Importantly, both TIMP-2 and IGFBP7 appear to be able to signal in autocrine and paracrine fashions, thus spreading the "alarm" from the site of injury (15, 44). In terms of timing, this signal could be ideal as it may be early enough that management strategies can still alter the outcome. This is particularly important in septic AKI where delay and early timing remains a major issue (4, 32, 42).

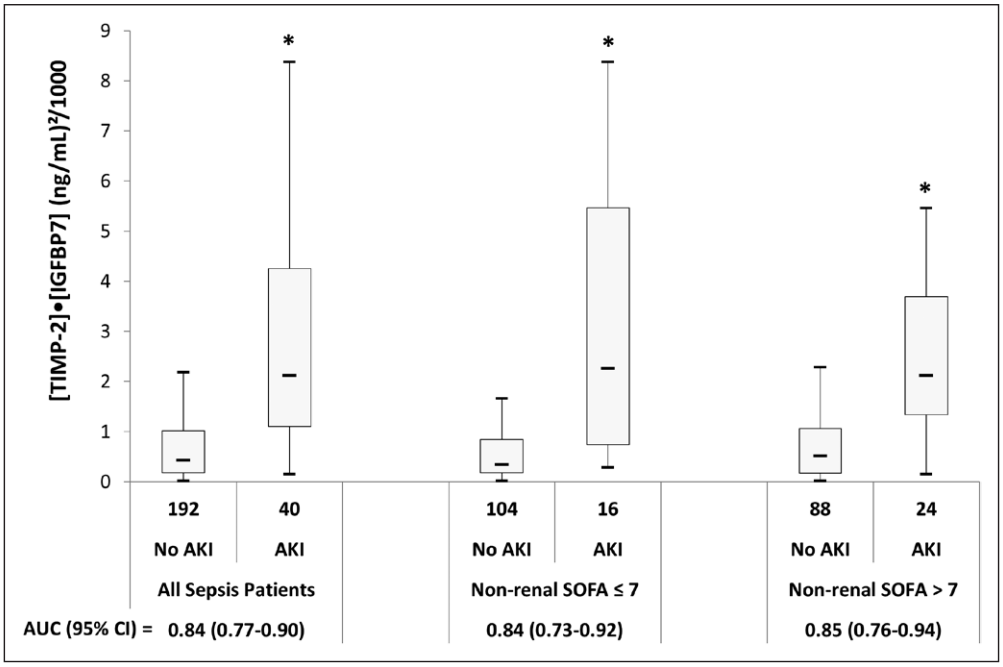

Figure 3. Tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 [TIMP-2]·[IGFBP7] and acute kidney injury (AKI) status within 12 hr for all patients and for subgroups according to nonrenal Sequential Organ Failure Assessment (SOFA) score. Boxes and whiskers show, respectively, interquartile ranges and total observed ranges, censored by 1.5 times the box range. Horizontal dashes within the boxes show the medians. SOFA scores were calculated from patient data collected on the day of enrollment. Patients with AKI had significantly higher levels of [TIMP-2]·[IGFBP7] than patients without AKI ($p < 0.001$ by Wilcoxon rank-sum test). In the linear regression model, where the response variable is rank transformed [TIMP-2]·[IGFBP7] and the explanatory variables are AKI status, SOFA subgroup status, and the interaction between them, the interaction was not statistically significant ($p = 0.70$). [TIMP-2]·[IGFBP7] was greater in patients with AKI than in those without AKI in both subgroups ($p < 0.001$), and there was no statistically significant dependence of [TIMP-2]·[IGFBP7] on SOFA subgroup regardless of the AKI status ($p = 0.29$).

Although not directly examined in these observational trials, we hypothesize that early use of biomarkers of cell-cycle arrest such as TIMP-2 and IGFBP7 could help clinicians intervene early on and thus improve outcomes for patients at risk of or with early evidence of sepsis-induced AKI (45–47). Early awareness that a patient with sepsis is about to develop a major organ failure could change a number of clinical decisions. For example, selection of antibiotics and dosing/monitoring of nephrotoxic medication (antibiotics or others) would be affected. The decision to give intravenous radiocontrast (especially intra-arterial) could be altered. Even the decision to discharge a patient or commence with a detailed diagnostic work-up would be influenced by the probability that a patient has or will have AKI (48). For example, consider a patient with sepsis admitted from the medical ward and started on broad spectrum antibiotics including empiric coverage with vancomycin. The morning after admission (9 hr later), the blood pressure and heart rate were normalized and cultures were pending. Now imagine that creatinine level of the patient was slightly elevated (1.2 mg/dL from 1.0), but urine output was adequate. If the patient had a urinary [TIMP-2]·[IGFBP7] test result more than 2.0, the risk of developing stage 2–3 AKI would be more than 50% and the patient's clinical team might well wish to stop or dose-adjust the vancomycin and perhaps keep the patient in the ICU where fluids, urine output, and hemodynamics can be monitored more carefully. Conversely, if the result were less than 0.3, then the risk would be less than 3% and thus the patient could be safely continued on current regimen.

Of course, biomarkers will not take the place of clinical judgment and the question whether they offer information in addition to clinical variables is an important one. Although clinicians are unlikely to use statistical models at the bedside, the model shown in Table 2 represents the limits of information that can be derived from clinical variables. Indeed, the model used here was developed in this dataset and is therefore likely overtrained. As such, it represents and unrealistic "benchmark" to compare the biomarker. Nevertheless, the markers show added value even in this setting. Recent work examining the role of electronic surveillance for AKI has shown that although computer programs can help identification of AKI, this alone may not improve patient outcomes. Indeed, electronic alerts may be "too late" because they are based on creatinine that is too late. Wilson et al (49) examined an electronic alert based on KDIGO stage 1 but could not demonstrate changes in physician practice or patient outcome. Physicians may already be aware of an AKI event and so do not change their management. Importantly, the [TIMP-2]·[IGFBP-7] test was developed to assess risk for stage 2–3 AKI 12 hours prior to its clinical manifestation. This has two important potential benefits over the electronic alert. First, 12 hours is a long time in the ICU and could be a decisive period to discontinue nephrotoxins, investigate the source of sepsis, and improve resuscitation. Second, as many as 70% of AKI alerts will be for patients that never progress beyond stage 1 AKI. Alert fatigue is a large factor leading to clinicians ignoring alerts. The [TIMP-2]·[IGFBP-7] test was developed for stage 2–3 AKI, and thus it does not detect stage 1 conditions that are low risk for progression. Conversely, like all diagnostics, biomarkers for AKI should not be used in patient populations for which they were not developed. Low-risk patients (e.g., stable outpatients) will exhibit far greater false-positive rates compared with critically ill patients and would not be appropriate for the [TIMP-2]·[IGFBP-7] test.

Our study has important strengths. We analyzed data from two large and unrelated cohorts with two different methods of

TABLE 2. Multivariate Analysis of the Clinical Model With and Without Inclusion of Tissue Inhibitor of Metalloproteinase-2 and Insulin-Like Growth Factor-Binding Protein 7 for Predicting Acute Kidney Injury in Sepsis

	Clinical Model Alone		Clinical Model With [TIMP-2]·[IGFBP7]	
Variable	Odds Ratio (95% CI)	*p*	Odds Ratio (95% CI)	*p*
Acute Physiology and Chronic Health Evaluation III, nonrenal	1.02 (1.00–1.05)	0.04	1.03 (1.01–1.06)	0.01
Sequential Organ Failure Assessment, nonrenal	1.03 (0.84–1.27)	0.76	0.99 (0.79–1.24)	0.93
Body mass index	1.06 (1.02–1.10)	0.004	1.09 (1.03–1.14)	0.002
24-hr fluid output[a]	0.03 (0.01–0.16)	< 0.001	0.07 (0.01–0.39)	0.002
Serum creatinine[b]	1.3 (1.1–1.5)	0.002	1.3 (1.1–1.6)	0.001
[TIMP-2]·[IGFBP7][a]	…	…	7.3 (3.1–17.5)	< 0.001
Area under the receiver operating characteristic curve[c]	0.86 (0.78–0.94)	< 0.001	0.94 (0.90–0.98)	< 0.001

[TIMP-2] · [IGFBP7] = Tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7.

[a]Log10 transform.

[b]Log2 transform, serum sample collected simultaneously with urine sample for tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 ([TIMP-2]·[IGFBP7]) testing.

[c]Area under the receiver operating characteristic curve was significantly ($p = 0.015$) greater with than without adding [TIMP-2]·[IGFBP7] to the model.

TABLE 3. Operating Characteristics (95% CI) for Tissue Inhibitor of Metalloproteinase-2 and Insulin-Like Growth Factor-Binding Protein 7 Cutoffs 0.3, 1.0, and 2.0

Subgroup	Cutoff	%Subjects Above Cutoff	Sensitivity	Specificity	Negative Predictive Value	Positive Predictive Value
All	0.3	68.1 (62.5–74.1)	95 (87.5–100)	37.5 (30.2–43.8)	97.3 (93.2–100)	24.1 (21.8–26.5)
All	1.0	34.1 (28.9–39.2)	77.5 (62.6–90)	75.0 (68.2–80.7)	94.1 (90.8–97.2)	39.2 (32.1–46.6)
All	2.0	19.4 (15.1–23.7)	60 (45–75)	89.1 (84.4–92.7)	91.4 (88.5–94.4)	53.3 (41.5–65)
Nonrenal SOFA ≤ 7	0.3	60.0 (51.7–69.1)	93.7 (81.3–100)	45.2 (34.6–54.8)	97.9 (93.5–100)	20.8 (17.2–24.6)
Nonrenal SOFA ≤ 7	1.0	28.3 (20.8–36.7)	68.8 (43.8–87.5)	77.9 (69.2–86.5)	94.2 (90.1–97.8)	32.4 (22.6–44.4)
Nonrenal SOFA ≤ 7	2.0	17.5 (11.7–24.2)	62.5 (37.5–87.5)	89.4 (82.7–95.2)	93.9 (90.2–97.8)	47.6 (30.4–66.7)
Nonrenal SOFA > 7	0.3	76.8 (68.8–83.9)	95.8 (87.5–100)	28.4 (19.3–37.5)	96.2 (88.0–100)	26.7 (23.9–30)
Nonrenal SOFA > 7	1.0	40.2 (32.1–48.2)	83.3 (66.7–95.8)	71.6 (62.5–80.7)	94.0 (88.5–98.5)	44.4 (35.7–55.6)
Nonrenal SOFA > 7	2.0	21.4 (15.2–27.7)	58.3 (37.5–75)	88.6 (81.8–94.3)	88.6 (83.7–93.3)	58.3 (42.9–76)

SOFA = Sequential Organ Failure Assessment.

determining the AKI endpoint (KDIGO stage 2–3 and clinical adjudication). However, this could be viewed as limitation, and there are also other important limitations to this work. First, because the Sapphire and Topaz clinical trials were not specifically designed to examine sepsis-induced AKI, we did not collect information on the type of organism or on the timing of infection. Nonetheless, our results are consistent across both multicenter cohort studies. Second, long-term outcomes were not available or was quality of life assessed in the Topaz study. Third, and most importantly, we could not examine whether the availability of biomarker results would have changed patient management or outcomes. This question awaits further study. However, given the limited information in the literature about the performance of biomarkers in sepsis-induced AKI, despite its common occurrence, we believe that our results should inform clinical practice and future research.

CONCLUSIONS

The urinary [TIMP-2]·[IGFBP7] test provides accurate prediction of AKI in septic patients, and test performance is not affected by nonrenal organ dysfunction. As such, the test may extend the therapeutic window for renal protection and potentially enhance (future) therapeutic interventions to prevent or attenuate AKI.

ACKNOWLEDGMENTS

We acknowledge the many staff, coordinators, and investigators whose work was essential to completion of this study. A

complete list of Sapphire and Topaz investigators and support personnel is available online (http://www.ccm.pitt.edu/sapphire-investigators and http://www.ccm.pitt.edu/topaz-investigators).

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