Research Article

Serial Urinary Tissue Inhibitor of Metalloproteinase-2 and Insulin-Like Growth Factor-Binding Protein 7 and the Prognosis for Acute Kidney Injury over the Course of Critical Illness

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Keywords

Acute kidney injury · Critical illness · Biomarker · Nephrotoxicity · Exposures · Cell-cycle arrest markers

Abstract

Introduction: Over the course of critical illness, there is a risk of acute kidney injury (AKI), and when it occurs, it is associated with increased length of stay, morbidity, and mortality. The urinary cell-cycle arrest markers tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP7) have been utilized to predict the risk of AKI over the next 12 h from the time of sampling. The aim of this analysis was to evaluate the utility of [TIMP-2] × [IGFBP7] measured serially to anticipate the occurrence of AKI over the first 7 days of critical illness. **Methods:** This analysis is from a prospective, blinded, observational, international study of patients admitted to intensive care units. We designed the analysis to emu-

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late a clinician-driven serial testing strategy. Urine samples collected every 12 h up to 3 days from 530 patients were considered for analysis. We evaluated [TIMP-2] × [IGFBP7] results for the first 3 measurements (baseline, 12 and 24 h) and continued to evaluate additional results if any of the first 3 were positive >0.3 (ng/mL)²/1,000. Patients were stratified by number of [TIMP-2] × [IGFBP7] results >0.3 (ng/mL)²/1,000 and number of results >2.0 (ng/mL)²/1,000. The primary endpoint was AKI stage 2–3 defined by the Kidney Disease: Improving Global Outcomes (KDIGO) criteria. **Results:** The median (interquartile range) age was 64 (53–74) years, 61% were men, and 79% were Caucasian. The median APACHE III score was 71 (51–93), and 82% required mechanical ventilation. Baseline serum creatinine was 0.8 mg/dL and 164/530 (31%) developed the primary endpoint by day 7 with a median time from baseline to stage 2/3 AKI of 26 (8–56) h. In patients with negative values for the first 3 tests (≤ 0.3 (ng/mL)²/1,000), the cumulative incidence of the primary endpoint at 7 days was 13.0%. Conversely, for those with one, two, or three strongly positive values (>2.0 (ng/mL)²/1,000), the cumulative incidence for the primary endpoint at 7 days was 57.7, 75.0, and 94.4%, respectively, $p < 0.001$ for trend. There were 3.4% with test results between 0.3 and 2.0 (ng/mL)²/1,000 at all measurements; one third of those patients developed the primary endpoint. We observed a graded increase in the primary endpoint in Kaplan-Meier plots for successively positive test results over time. **Conclusion:** Serial urinary [TIMP-2] × [IGFBP7] at baseline, 12 and 24 h, and up through 3 days are prognostic for the occurrence of stage 2/3 AKI over the course of critical illness. Three consecutive negative values (≤ 0.3 (ng/mL)²/1,000) are associated with very low (13.0%) incidence of stage 2/3 AKI over the course of 7 days. Conversely, emerging or persistent, strongly positive results [>2.0 (ng/mL)²/1,000] predict very high incidence rates (up to 94.4%) of stage 2/3 AKI. There was a low rate of test results between 0.3 and 2.0 (ng/mL)²/1,000, where the primary endpoint was observed in a third of cases.

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Introduction

Acute kidney injury (AKI) is one of the most common complications of critical illnesses occurring in 50–60% of patients admitted to the intensive care unit (ICU) and is associated with serious short- and long-term complications, including increased mortality and significant healthcare costs [1]. Daily assessment of serum creatinine (Cr), blood urea nitrogen (BUN), and hourly urine output are common ICU standards; however, while helpful in recognizing AKI once it has occurred, these methods do not allow for anticipation of AKI or changes in management before AKI is recognized. The measurement of urinary tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP7) and interpretation of risk based on the product of urinary concentrations [TIMP-2] × [IGFBP7] has advanced the field with a single measurement in the prediction of moderate to severe AKI defined as $\geq$ Stage 2 Kidney Disease Improving Global Outcomes (KDIGO) AKI over the next 12 h [14]. However, exposures to conditions which can result in AKI continue through the ICU stay [2], and thus, serial measurement of [TIMP-2] × [IGFBP7] would be a clinically intuitive complement to daily serum Cr and BUN which are commonly obtained in biochemical profiles in ICU patients [3].

Conditions such as sepsis, heart failure, or shock can be associated with AKI which can progress even in an optimally managed patient. It is also recognized that during critical illness, patients can incur a large number of additional exposures with nephrotoxic potential, often in combination and succession. For example, exposure to intravascular iodinated contrast is common upon admission as well as when there is a change in clinical status due to the use of contrast-enhanced computed tomography and angiography [4–6]. Additionally,

Fig. 1. Patient flow diagram. The Sapphire study enrolled critically ill adults without known stage 2–3 AKI. For the primary analysis of serial [TIMP-2] × [IGFBP7] test results, patients missing a urine collection for [TIMP-2] × [IGFBP7] measurement at enrollment or 12 ± 6 or 24 ± 6 h following enrollment were excluded. Of the remaining 538 patients considered for analysis, 115 (21%) had [TIMP-2] × [IGFBP7] ≤ 0.3 (ng/mL)²/1,000 at all 3 time points. For the 423 (79%) patients with [TIMP-2] × [IGFBP7] > 0.3 (ng/mL)²/1,000 at one or more time points, an additional two urine collections at 12 ± 6 and 24 ± 6 h following the earliest observation of [TIMP-2] × [IGFBP7] > 0.3 (ng/mL)²/1,000 were assessed. Thus, a total of 530 patients had test results at least at the 0, 12, and 24 h time points. Patients missing either of the additional time points were excluded from the analysis of consecutive [TIMP-2] × [IGFBP7] > 0.3 (ng/mL)²/1,000.

about 20% of the drugs prescribed in the ICU have the potential for nephrotoxicity [7, 8]. A large fraction of ICU patients receive intravenous fluids both before and after AKI ensues [9]. Fluids along with inotropic agents may confound the clinical picture and make it difficult to discern risk for serious AKI based on the clinical and laboratory examination [10]. Finally, many patients receive intravenous diuretics which further impair the clinical interpretation of azotemia and urine output. Thus, there is a strong rationale for novel biomarkers to reliably detect risk for serious AKI in the ICU.

We previously reported the results of the Sapphire study which showed that initial urine [TIMP-2] × [IGFBP7] predicted AKI stage 2–3 within 12 h and before a rise in serum Cr [11]. Additionally, we demonstrated that exposure to multiple nephrotoxic insults is common during critical illness and associated with an increased risk of AKI which could be anticipated with serial [TIMP-2] × [IGFBP7] levels within a day of exposure [2]. Thus, we hypothesized that early serial [TIMP-2] × [IGFBP7] measurements would be informative in the prediction stage 2–3 AKI over the course of the first 7 days of critical illness.

Methods

Study Design

The Sapphire study methods and results have been previously disclosed in several publications [11, 12, 13]. In summary, 744 critically ill adults (21 years or older) were recruited at 35 sites in North America and Europe from September 2010 to June 2012 (Fig. 1), and based upon the availability of serial urine samples, 530 patients were included in the analysis. Patients were enrolled within 24 h of admission to the ICU, had cardiovascular or respiratory sequential organ failure assessment (SOFA) score ≥ 1 or ≥ 2 , respectively, and were expected to stay in the ICU for at least 48 h. The primary endpoint was de novo KDIGO stage 2/3 AKI according to serum Cr and urine output [14]. Serum Cr from enrollment through hospital discharge and urine output data from the day prior to enrollment through 7 days were collected to determine maximum KDIGO AKI stage. Exposures to nephrotoxic drugs (online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000502837), radiocontrast agents, shock (defined as vasopressor use), diuretics, sepsis (as noted in the medical record), and surgery were recorded each day.

Sample and Data Collection

Following informed consent, urine and blood samples were taken by standard methods at the time of study enrollment and at 12-h intervals up through 3 days. Serum and urine supernatants were frozen within approximately 1 h of collection, stored at -70°C or lower, and thawed immediately before analysis. All clinical data, including patient demographics, reason for ICU admission, past medical history, parameters for the Acute Physiology and Chronic Health Evaluation III (APACHE III) score, serum Cr, and hourly urine output, were collected by trained research coordinators and stored in a password-protected dataset residing on servers at independent sites (Medidata Solutions, New York, NY, USA). Baseline estimated glomerular filtration rate (eGFR) was calculated from baseline serum Cr using the CKD-EPI equation [15].

Biomarker Assays

Urinary TIMP-2 and IGFBP7 concentrations were measured by technicians who were blinded to the clinical data using a clinical immunoassay (NephroCheck® Test and Astute140® Meter; Astute Medical Inc., San Diego, CA, USA). The Astute140 Meter automatically multiplies the concentrations of the two biomarkers and divides the product by 1,000 to report a single numeric result in $(\text{ng/mL})^2/1,000$. Cr was measured in the study-specific serum samples at a central lab (LabCorp, San Diego, CA, USA) using the Jaffe method standardized to isotope dilution mass spectrometry. Urinary [TIMP-2] × [IGFBP7] results were evaluated using the validated high sensitivity and high specificity cutoffs of 0.3 (positive) and 2.0 $(\text{ng/mL})^2/1,000$ (strongly positive), respectively [13, 16]. To be included in this analysis, we required patients to have urinary [TIMP-2] × [IGFBP7] results at enrollment and 12 ± 6 and 24 ± 6 h after enrollment.

Statistical Analysis

We analyzed serial [TIMP-2] × [IGFBP7] results in 12 ± 6 h intervals starting from enrollment. The analysis proceeded in a stepwise manner based on the pattern of [TIMP-2] × [IGFBP7] values. If the first three [TIMP-2] × [IGFBP7] values for a patient were negative defined as ≤ 0.3 $(\text{ng/mL})^2/1,000$, no additional [TIMP-2] × [IGFBP7] values were analyzed for that patient. If one of the first three [TIMP-2] × [IGFBP7] values was > 0.3 $(\text{ng/mL})^2/1,000$, then additional [TIMP-2] × [IGFBP7] values were analyzed. If one of the values > 0.3 was also > 2.0 $(\text{ng/mL})^2/1,000$, two additional [TIMP-2] × [IGFBP7] values were analyzed. These patients were stratified by the number of consecutive [TIMP-2] × [IGFBP7] values > 2.0 $(\text{ng/mL})^2/1,000$. Two additional analyses were conducted in which patients were stratified by the first time at which [TIMP-2] × [IGFBP7] was either > 0.3 or > 2.0 $(\text{ng/mL})^2/1,000$. Based on the stepwise analysis method, from 3 to 7 [TIMP-2] × [IGFBP7] results could have been analyzed for each patient.

The primary endpoint was evaluated using survival analysis and displayed by Kaplan-Meier curves over 7 days. The log-rank test was used to test for differences across strata. For baseline characteristics, categorical variables were analyzed using Fisher's exact or χ^2 tests, and continuous or ordinal variables were analyzed using the Kruskal-Wallis test. Univariate statistics were reported as medians with interquartile ranges (IQR) or counts with proportions as appropriate. Two-sided p values < 0.05 and one-sided p values < 0.025 were considered statistically significant without adjustment for multiplicity. Statistical analyses were performed using R3.4.4 [17].

Table 1. Baseline patient characteristics for all patients and by [TIMP-2] × [IGFBP7] [(ng/mL)²/1,000] pattern

	All patients (n = 530)	Three consecutive negative (≤0.3) [TIMP-2] × [IGFBP7] (n = 115)	One positive (>0.3) [TIMP-2] × [IGFBP7] (n = 128)	Two consecutive positive (>0.3) [TIMP-2] × [IGFBP7] (n = 76)	Three consecutive positive (>0.3) [TIMP-2] × [IGFBP7] (n = 211)	p value
Male	325 (61%)	65 (57%)	87 (68%)	44 (58%)	129 (61%)	0.27
Age, years	64 (53–74)	64 (52–73)	64 (53–72)	68 (56–76)	64 (54–74)	0.19
Race						0.03
White or Caucasian	419 (79%)	81 (70%)	100 (78%)	60 (79%)	178 (84%)	
Black of African-American	65 (12%)	25 (22%)	16 (13%)	7 (9%)	17 (8%)	
Other or unknown	46 (9%)	9 (8%)	12 (9%)	9 (12%)	16 (8%)	
Reason for ICU admission						
Cardiovascular	172 (32%)	40 (35%)	40 (31%)	20 (26%)	72 (34%)	0.59
Neurological	53 (10%)	15 (13%)	10 (8%)	7 (9%)	21 (10%)	0.60
Sepsis	91 (17%)	13 (11%)	19 (15%)	22 (29%)	37 (18%)	0.02
Respiratory	235 (44%)	52 (45%)	59 (46%)	39 (51%)	85 (40%)	0.37
Trauma	40 (8%)	9 (8%)	12 (9%)	2 (3%)	17 (8%)	0.32
Surgery	163 (31%)	27 (23%)	42 (33%)	21 (28%)	73 (35%)	0.18
Other	83 (16%)	15 (13%)	19 (15%)	16 (21%)	33 (16%)	0.51
Medical history						
COPD	128 (24%)	26 (23%)	44 (34%)	20 (26%)	38 (18%)	0.008
Cancer	135 (25%)	26 (23%)	34 (27%)	21 (28%)	54 (26%)	0.85
Chronic kidney disease	51 (10%)	17 (15%)	11 (9%)	7 (9%)	16 (8%)	0.22
Liver disease	26 (5%)	6 (5%)	6 (5%)	3 (4%)	11 (5%)	1.00
Heart failure	94 (18%)	26 (23%)	23 (18%)	11 (14%)	34 (16%)	0.44
Coronary artery disease	158 (30%)	33 (29%)	39 (30%)	26 (34%)	60 (28%)	0.80
Diabetes mellitus	152 (29%)	28 (24%)	44 (34%)	26 (34%)	54 (26%)	0.16
Hypertension	317 (60%)	70 (61%)	76 (59%)	47 (62%)	124 (59%)	0.96
Location prior to ICU admission						0.37
Emergency department	209 (39%)	50 (43%)	51 (40%)	29 (38%)	79 (37%)	
Operating room	125 (24%)	19 (17%)	32 (25%)	14 (18%)	60 (28%)	
Floor	114 (22%)	24 (21%)	23 (18%)	19 (25%)	48 (23%)	
Other ICU	4 (1%)	0 (0%)	2 (2%)	1 (1%)	1 (0%)	
Other hospital	69 (13%)	20 (17%)	17 (13%)	11 (14%)	21 (10%)	
Unknown	9 (2%)	2 (2%)	3 (2%)	2 (3%)	2 (1%)	
Mechanical ventilation at enrollment	433 (82%)	89 (77%)	105 (82%)	65 (86%)	174 (82%)	0.53
Baseline serum creatinine, mg/dL	0.8 (0.7–1.1)	0.9 (0.7–1.2)	0.8 (0.7–1.1)	0.8 (0.7–1.1)	0.8 (0.6–1.1)	0.27
APACHE III score	71 (51–93)	68 (50–92)	66 (51–89)	73 (53–95)	73 (52–97)	0.25
Non-renal APACHE III score	61 (43–83)	62 (42–81)	56 (43–82)	60 (44–81)	65 (44–87)	0.32

Results

Among 530 patients (Fig. 1), the median (IQR) age was 64 (53–74) years, 61% were men, and 79% were Caucasian (Table 1). The most common reasons for ICU admission were: 44% respiratory, 32% cardiovascular, and 31% surgical conditions. In terms of baseline risk for AKI, 29% had diabetes mellitus, 24% with eGFR <60 mL/min/1.73 m², and 2.6% had eGFR <30 mL/min/1.73 m², and the baseline serum Cr was 0.8 (0.7–1.1) mg/dL corresponding to a median eGFR of 87 (61–106) mL/min/1.73 m². The median APACHE III score was 71 (51–93), and 82% required mechanical ventilation. Most patients had nephrotoxic exposures while in the ICU, and the frequency and number of exposures declined on each subsequent day (online suppl. Fig. S1).

In patients where the first 3 [TIMP-2] × [IGFBP7] values were negative [≤ 0.3 (ng/mL)²/1,000] (online suppl. Table S2), the cumulative incidence of the primary endpoint at 7 days was 15/115 (13.0%) (Fig. 2a), and the occurrence of renal replacement therapy and death was 3.5 and 4.3%, respectively (online suppl. Table S3(A)). In this cohort, 29% had baseline eGFR <60 mL/min/1.73 m², and there tended to be lower rates of surgical conditions and sepsis. Among patients with at least 1 positive value and two additional samples for evaluation (online suppl. Table S2), the cumulative incidence for the primary endpoint at 7 days was 32/128 (25.0%), 28/76 (36.8%), and 89/211 (42.2%), respectively, for one, two, or three consecutive positive values (Fig. 2a), $p < 0.001$ (online suppl. Table S3(A)). Additionally, among the 96 patients with at least 1 strongly positive value [> 2.0 (ng/mL)²/1,000] and two additional samples for evaluation, the cumulative incidence for the primary endpoint at 7 days was 38/66 (57.7%), 9/12 (75.0%), and 17/18 (94.4%), respectively, for one, two, or three consecutive strongly positive values, $p < 0.001$ (Fig. 2b) (online suppl. Table S3(B)). Of note, in the highest risk group with three consecutive strongly positive results, 7/18 (38.9%) and 5/18 (27.8%) required renal replacement therapy and died, respectively (online suppl. Table S3(B)). There were 18 (3.4%) patients whose tests remained between 0.3 and 2.0 (ng/mL)²/1,000 at all measurements, and 6 (33.3%) of these patients developed the primary endpoint. We observed a graded increase in the primary endpoint in Kaplan-Meier plots for successively positive test results over time using the 0.3 and the 2.0 (ng/mL)²/1,000 cut points (Fig. 3a, b; $p < 0.001$ for both).

Figure 3a and b demonstrates the cumulative probabilities of developing the primary endpoint according to the order in which the first positive test was observed at the 0.3 and 2.0 (ng/mL)²/1,000 cutoffs. At each successive sample, there was an increase in the probability of the primary endpoint when a positive test result was observed at both thresholds 0.3 ($p < 0.04$) and 2.0 (ng/mL)²/1,000 ($p < 0.01$). However, there was no significant difference in cumulative incidence of the primary endpoint within 7 days between patients for whom the test became positive at the first, second, or third sample. There was an approximate twofold overall absolute probability at each graduated time point for the 2.0 as compared to the 0.3 (ng/mL)²/1,000 cutoff point. Figure 4 gives the rates of stage 2/3 AKI according to the number of samples accrued with test results ≤ 0.3 (a) and > 2.0 (ng/mL)²/1,000 (b). Figure 5 displays the evolution of those patients with the first three samples in the intermediate range where the results were not strongly negative or positive. As shown, only 94/530 (17.7%) subjects were persistently in the intermediate range between 0.3 and 2.0 (ng/mL)²/1,000 for the first of 3 samples, and this progressively declined over subsequent sampling leaving 18 (3.4%) whose tests remained between 0.3 and 2.0 (ng/mL)²/1,000 at all measurements, and in those 18 patients, 6 (33.3%) ultimately developed the primary endpoint after several days in the ICU. The time of onset of stage 2/3 AKI in relationship to persistently positive > 0.3 and persistently strongly positive > 2.0 (ng/mL)²/1,000 results was mainly over the first and second samples (Fig. S2A, S2B; Table S4) with 38% of positive and 6% of strongly positive samples having the primary endpoint determined after the third sample was obtained (Fig. S3A, S3B).

Fig. 2. Cumulative incidence of stage 2–3 AKI over 1 week for patients stratified by number of consecutive [TIMP-2] × [IGFBP7] above the 0.3 (**a**) and 2.0 (ng/mL)²/1,000 (**b**) cutoffs. **b** All patients were required to have at least one [TIMP-2] × [IGFBP7] >0.3 (ng/mL)²/1,000, from which point [TIMP-2] × [IGFBP7] was assessed relative to the 2.0 cutoff. log-rank *p* < 0.001 for both cutoffs.

Fig. 3. Cumulative incidence of stage 2–3 AKI over 1 week for patients stratified by time of first observation of [TIMP-2] × [IGFBP7] above the 0.3 (**a**) and 2.0 (ng/mL)²/1,000 (**b**) cutoffs. First, second, and third samples were collected at enrollment, 12 ± 6 h after enrollment, and 24 ± 6 h after enrollment, respectively, for the 0.3 (ng/mL)²/1,000 cutoff. The first observation of [TIMP-2] × [IGFBP7] >0.3 (ng/mL)²/1,000 and samples collected 12 ± 6 and 24 ± 6 h after were evaluated for the 2.0 cutoff. $p = 0.32$ for the 0.3 (ng/mL)²/1,000 cutoff and $p = 0.57$ for the 2.0 (ng/mL)²/1,000 cutoff by the log-rank test.

Fig. 4. Incidence of stage 2–3 AKI within 1 week of enrollment by number of [TIMP-2] × [IGFBP7] values ≤ 0.3 (a) and > 2.0 (ng/mL)²/1,000 (b) among the first 7 measurements collected approximately 12 h apart. Number of values below or above the cutoff were not required to be consecutive.

Fig. 5. Evolution of patients with [TIMP-2] × [IGFBP7] values > 0.3 to ≤ 2.0 (ng/mL)²/1,000 over the first 7 consecutive [TIMP-2] × [IGFBP7] measurements collected approximately 12 h apart. Box shadings show number of patients who started within each [TIMP-2] × [IGFBP7] level (1st column) or decreased to ≤ 0.3 (ng/mL)²/1,000 (gray), remained at > 0.3 to ≤ 2.0 (ng/mL)²/1,000 (white), or increased to > 2.0 (ng/mL)²/1,000 (black). Missing [TIMP-2] × [IGFBP7] values were evenly divided between ≤ 0.3 and > 2.0 (ng/mL)²/1,000 for graphical purposes.

Because nephrotoxic insults could have influenced test results and outcomes once hospitalized, we analyzed the data according to exposure to potentially injurious factors. We identified the proportion of patients who were exposed to one or more potentially nephrotoxic insults on each day from enrollment through 7 days after accrual, including nephrotoxic drugs (online suppl. Table S1), radiocontrast agents, shock (defined as the need for vasopressors), diuretics, sepsis, and surgery [2]. Over 90% of patients received at least one exposure on the day of enrollment with a mean of 3.5 exposures (online suppl. Fig. S1). Patients continued to receive exposures throughout the week, although the proportion and mean decreased steadily to 60% of patients receiving at least one exposure and a mean of 1.4 exposures on the 7th day after enrollment.

Discussion

We have demonstrated that serial measurement and interpretation of urine [TIMP-2] × [IGFBP7] in critically ill patients every 12 h for at least 3 samples provided an approach to predict the progressive risk of stage 2/3 AKI up to 7 days in the ICU. Additionally, there appeared to be value in the utilization of both the 0.3 and 2.0 (ng/mL)²/1,000 cut points with persistently negative and emerging or persistently positive/strongly positive results anticipating very low or progressively higher rates of the primary endpoint, respectively. Very few patients (3.4%) remained in an intermediate range between 0.3 and 2.0 (ng/mL)²/1,000 in all the samples assayed, and in this small group the test results provided less diagnostic certainty.

We believe our results extend the observation of others which has largely relied upon baseline risk assessment with clinical factors and novel biomarkers including [TIMP-2] × [IGFBP7], neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule-1, interleukin-18, and L-type fatty acid-binding protein [3]. Recently Tecson et al. [18] demonstrated that the daily measurement of NGAL, either in blood or urine, predicted stage 2/3 AKI on a rolling basis from day to day over the course of an ICU stay. Serial perioperative [TIMP-2] × [IGFBP7] results in patients undergoing cardiac surgery were recently reported by Cummings et al. [19] who demonstrated a bimodal elevation in [TIMP-2] × [IGFBP7] in those patients who developed stage 2–3 AKI, with the first peak occurring intraoperatively and the second peak postoperatively. The authors postulated the first peak is caused by kidney exposures/stress occurring during surgery and the second peak by additional exposures/stress occurring after surgery during postoperative care. Concordant with our results, the predictive ability of [TIMP-2] × [IGFBP7] was highest when measurements at both times were utilized, as would be expected for two independent episodes of stress caused by sequential kidney exposures.

Our results are the first report of sequential measurements of [TIMP-2] × [IGFBP7] in a large multicenter international cohort of heterogeneous critically ill patients. Because critically ill patients often have ongoing and/or sequential new kidney exposures that can cause AKI, biochemical profiles including serum Cr and BUN are commonly measured daily in the ICU. We approached the blinded results of our serial [TIMP-2] × [IGFBP7] values as if a clinician would be inquiring on a patient at risk for AKI over time. Our results show that sequential measurement of [TIMP-2] × [IGFBP7] can complement the information given by serum Cr in the anticipation of subsequent stage 2/3 AKI. We recognize that while urine output is part of the KDIGO definition of AKI, the trend in early removal of urinary catheters aiming to reduce rates of catheter associated infections will limit the ability to monitor and detect a reduction in urine output as an indicator of AKI in the future [20]. As a result, we can expect to have an increasing reliance on the clinical laboratory for the prediction, detection, and management of AKI. Our data suggest that predictive information gained on successive samples is somewhat cumulative for those who have not developed AKI quickly, and thus suggest the value for surveillance when the patient remains critically ill and vulnerable to AKI.

Our results also raise an important question as to whether the dynamic response of the markers to new exposures could also guide ongoing management. The results of the PrevAKI randomized trial have demonstrated that [TIMP-2] × [IGFBP7] can be used to trigger a set of care processes based on KDIGO guidelines including the use of hemodynamic monitoring and inotropic therapy, resulting in reduced rates of subsequent AKI after cardiac surgery [21]. We believe our results extend the inferences from PrevAKI and suggest continued daily surveillance for the risk of AKI has clinical value, in all three relevant [TIMP-2] × [IGFBP7] test result scenarios: persistently negative, negative transforming to positive, and persistently positive/strongly positive. In the future, interventions aimed to attenuate the

progression of AKI may benefit from the improved forecasting that can be achieved by serial measurements in the ICU. For example, patients with persistently negative [TIMP-2] × [IGFBP7] results in study appeared to be at very low risk of stage 2/3 AKI even in the presence of nephrotoxic insults. These patients would be unlikely to benefit from interventions, whereas patients with emerging or persistently positive/strongly positive [TIMP-2] × [IGFBP7] would be prime candidates for strategies to reduce the incidence, severity, duration, or complications associated with AKI. Additionally, those patients who yield three consecutive strongly positive results $>2.0 \text{ (ng/mL)}^2/1,000$, given the very high rates of stage 2/3 AKI and need for renal replacement therapy, could be considered for early nephrology consultation and planning for ultrafiltration or dialysis. Both the patient and family members could be made aware of imminent renal failure by the treating physicians. For the patients who remain between 0.3 and $2.0 \text{ (ng/mL)}^2/1,000$ (3.4%) at all measurements, it appears that [TIMP-2] × [IGFBP7] would offer less diagnostic certainty, and these intermediate range results simply call for continued vigilance and reliance on clinical judgement. We recognize that while this group was small in our study, in clinical practice it may be considerably larger due to heterogeneity in clinical presentation, duration of acute illness, and sampling frequency.

Our study has all the limitations of retrospective analyses of completed clinical trials. Because of the small sample sizes in groups, we had limited ability to find differences defined by evolving [TIMP-2] × [IGFBP7] results. Additionally, we did not account for the use of diuretics and intravenous fluids which could have influenced the serum Cr and urine output data and the determination of KDIGO stage 2/3 AKI [22]. Furthermore, we evaluated selected nephrotoxic exposures and acknowledge that a proportion of patients may have been exposed to many other nephrotoxins. We did not assess the degree of baseline proteinuria in patients and recognize that urine albumin concentrations >125 and $>3,000 \text{ mg/dL}$, respectively, can interfere with and invalidate the test results, respectively [23]. Additionally, urinary bilirubin at concentrations $>7.2 \text{ mg/dL}$ and methylene blue at concentrations $>0.49 \text{ mg/L}$ can influence the [TIMP-2] × [IGFBP7] assay and were not measured; however, we anticipate these cases were rare if they occurred at all [23].

In conclusion, serial urinary [TIMP-2] × [IGFBP7] at baseline, 12, 24 h, and up through 3 days are prognostic for the occurrence of stage 2/3 AKI over the course of critical illness. Persistently negative values [$\leq 0.3 \text{ (ng/mL)}^2/1,000$] are associated with a relatively low (13.0%) incidence of stage 2/3 AKI over 7 days. Conversely, emerging or persistently, strongly positive results ($>2.0 \text{ (ng/mL)}^2/1,000$) predict very high incidence rates (up to 94.4%). Few (3.4%) patients had tests remaining between 0.3 and $2.0 \text{ (ng/mL)}^2/1,000$ at all measurements. These results support the use of serial urinary [TIMP-2] × [IGFBP7] monitoring for the risk prediction of stage 2/3 AKI in critically ill patients.

Statement of Ethics

This study was approved by the Western Institutional Review Board (Olympia, WA, USA) and individual investigational review boards/research ethics committees of each participating institution. All subjects or their legally authorized representatives provided written informed consent prior to enrollment.

Disclosure Statement

Peter A. McCullough, MD, MPH, Lakhmir S. Chawla, MD, Jay L. Koyner, MD, Jing Shi, PhD, and John A. Kellum, MD, have been consultants to Astute Medical; J. Patrick Kampf, PhD, and Paul McPherson, PhD, are employees of Astute Medical.

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References

- Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med*. 2015 Aug;41(8):1411–23.
- Ostermann M, McCullough PA, Forni LG, Bagshaw SM, Joannidis M, Shi J, et al.; all SAPPHERE Investigators. Kinetics of Urinary Cell Cycle Arrest Markers for Acute Kidney Injury Following Exposure to Potential Renal Insults. *Crit Care Med*. 2018 Mar;46(3):375–83.
- McCullough PA, Bouchard J, Waikar SS, Siew ED, Endre ZH, Goldstein SL, et al. Implementation of novel biomarkers in the diagnosis, prognosis, and management of acute kidney injury: executive summary from the tenth consensus conference of the Acute Dialysis Quality Initiative (ADQI). *Contrib Nephrol*. 2013;182:5–12.
- Aliaga M, Forel JM, De Bourmont S, Jung B, Thomas G, Mahul M, et al. Diagnostic yield and safety of CT scans in ICU. *Intensive Care Med*. 2015 Mar;41(3):436–43.
- Becker CR, Davidson C, Lameire N, McCullough PA, Stacul F, Tumlin J, et al.; CIN Consensus Working Panel. High-risk situations and procedures. *Am J Cardiol*. 2006 Sep;98(6 6A):37K–41K.
- McCullough PA. Contrast-Induced Nephropathy: Definitions, Epidemiology, and Implications. *Interv Cardiol Clin*. 2014 Jul;3(3):357–62.
- Mehta RL, Pascual MT, Soroko S, Savage BR, Himmelfarb J, Ikizler TA, et al.; Program to Improve Care in Acute Renal Disease. Spectrum of acute renal failure in the intensive care unit: the PICARD experience. *Kidney Int*. 2004 Oct;66(4):1613–21.
- Kane-Gill SL, Goldstein SL. Drug-induced acute kidney injury: a focus on risk assessment for prevention. *Crit Care Clin*. 2015 Oct;31(4):675–84.
- Prowle JR, Chua HR, Bagshaw SM, Bellomo R. Clinical review: volume of fluid resuscitation and the incidence of acute kidney injury - a systematic review. *Crit Care*. 2012 Aug;16(4):230.
- Townsend DR, Bagshaw SM. New insights on intravenous fluids, diuretics and acute kidney injury. *Nephron Clin Pract*. 2008;109(4):c206–16.
- Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. *Crit Care*. 2013 Feb;17(1):R25.
- Hoste EA, McCullough PA, Kashani K, Chawla LS, Joannidis M, Shaw AD, et al.; Sapphire Investigators. Derivation and validation of cutoffs for clinical use of cell cycle arrest biomarkers. *Nephrol Dial Transplant*. 2014 Nov;29(11):2054–61.
- Koyner JL, Shaw AD, Chawla LS, Hoste EA, Bihorac A, Kashani K, et al. Sapphire Investigators. Tissue Inhibitor Metalloproteinase-2 (TIMP-2)-IGF-Binding Protein-7 (IGFBP7) Levels Are Associated with Adverse Long-Term Outcomes in Patients with AKI. *J Am Soc Nephrol*. 2015 Jul;26(7):1747–54.
- Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int*. 2012;2:1–138.
- Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009 May;150(9):604–12.
- Bihorac A, Chawla LS, Shaw AD, Al-Khafaji A, Davison DL, Demuth GE, et al. Validation of cell-cycle arrest biomarkers for acute kidney injury using clinical adjudication. *Am J Respir Crit Care Med*. 2014 Apr;189(8):932–9.
- R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2018. <https://www.R-project.org/>
- Tecson KM, Erhardttsen E, Eriksen PM, Gaber AO, Germain M, Golestaneh L, et al. Optimal cut points of plasma and urine neutrophil gelatinase-associated lipocalin for the prediction of acute kidney injury among critically ill adults: retrospective determination and clinical validation of a prospective multicentre study. *BMJ Open*. 2017 Jul;7(7):e016028.
- Cummings JJ, Shaw AD, Shi J, Lopez MG, O'Neal JB, Billings FT 4th. Intraoperative prediction of cardiac surgery-associated acute kidney injury using urinary biomarkers of cell cycle arrest. *J Thorac Cardiovasc Surg*. 2019 Apr;157(4):1545–1553.e5.
- Sampathkumar P. Reducing catheter-associated urinary tract infections in the ICU. *Curr Opin Crit Care*. 2017 Oct;23(5):372–7.
- Meersch M, Schmidt C, Hoffmeier A, Van Aken H, Wempe C, Gerres J, et al. Prevention of cardiac surgery-associated AKI by implementing the KDIGO guidelines in high risk patients identified by biomarkers: the PrevAKI randomized controlled trial. *Intensive Care Med*. 2017 Nov;43(11):1551–61.
- Palazzuoli A, Ruocco G, Ronco C, McCullough PA. Loop diuretics in acute heart failure: beyond the decongestive relief for the kidney. *Crit Care*. 2015 Sep;19(1):296.
- Uettwiller-Geiger DL, Vijayendran R, Kellum JA, Fitzgerald RL. Analytical characteristics of a biomarker-based risk assessment test for acute kidney injury (AKI). *Clin Chim Acta*. 2016 Apr;455:93–8.