**Tissue Inhibitor Metalloproteinase-2 (TIMP-2)·IGF-Binding Protein-7 (IGFBP7) Levels Are Associated with Adverse Long-Term Outcomes in Patients with AKI**

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**ABSTRACT**

Tissue inhibitor metalloproteinase-2 (TIMP-2) and IGF-binding protein-7 (IGFBP7) have been validated for risk stratification in AKI. However, the association of urinary TIMP-2 and IGFBP7 with long-term outcomes is unknown. We evaluated the 9-month incidence of a composite end point of all-cause mortality or the need for RRT in a secondary analysis of a prospective observational international study of critically ill adults. Two predefined [TIMP-2][IGFBP7] cutoffs (0.3 for high sensitivity and 2.0 for high specificity) for the development of AKI were evaluated. Cox proportional hazards models were used to determine risk for the composite end point. Baseline [TIMP-2][IGFBP7] values were available for 692 subjects, of whom 382 (55.2%) subjects developed stage 1 AKI (defined by Kidney Disease Improving Global Outcomes guidelines) within 72 hours of enrollment and 217 (31.4%) subjects met the composite end point. Univariate analysis showed that [TIMP-2][IGFBP7].2.0 was associated with increased risk of the composite end point (hazard ratio [HR], 2.11; 95% confidence interval [95% CI], 1.37 to 3.23; \( P < 0.001 \)). In a multivariate analysis adjusted for the clinical model, [TIMP-2][IGFBP7].0.3 was associated with increased risk of the composite end point (hazard ratio [HR], 2.11; 95% confidence interval [95% CI], 1.37 to 3.23; \( P < 0.01 \)). Over the last decade, there has been intense investigation of biomarkers of kidney injury in an attempt to complement and augment the diagnostic abilities of current measures of kidney function. In hospitalized patients, AKI is associated with increased risk of both short- and long-term adverse outcomes, including death and end stage kidney disease.1–7 To date, little is known about the relationship between these novel biomarkers of kidney injury in an attempt to complement and augment the diagnostic abilities of current measures of kidney function.8


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biomarkers and long-term outcomes, with only one large-scale multicenter investigation linking some biomarkers collected around the time of AKI with long-term mortality.

We have recently published the results of a prospective, observational international investigation (the Sapphire study) of tissue inhibitor metalloprotease-2 (TIMP-2) and IGF-binding protein 7 (IGFBP7; both markers of G1 cell cycle arrest) in the setting of critical illness. Subsequently, we validated two [TIMP-2]-[IGFBP7] cutoffs for risk assessment for development of AKI. Here, we report a secondary analysis from the Sapphire study to determine the associations between [TIMP-2]-[IGFBP7] measured at intensive care unit (ICU) admission and a 9-month composite end point of all-cause mortality and/or the receipt of RRT. Documenting the relationship between markers of kidney injury and long-term outcomes is important for understanding the biology of these markers. Similarly, understanding whether the apparent hazard detected by the markers is dependent on AKI or simply related to underlying severity of illness is crucial to interpreting the specificity of the signal.

RESULTS

In total, 744 subjects were enrolled in the original Sapphire study, and for this analysis, 52 subjects were excluded as described in Figure 1. The median (interquartile range) time from ICU arrival to collection of the first samples was 16 (7–21) hours. At least 3 months of follow-up data were available for 639 (92.3%) patients, and full 9-month outcome was known for 552 (79.8% of the initial cohort) patients, with 217 (39.3%) of those with complete follow-up) patients meeting the primary end point of death or dialysis. Table 1 describes the demographic and outcomes data of those who did and did not meet the composite end point of death or receipt of RRT during the 9-month follow-up period. Compared with those who did not meet the composite end point, those who died or received RRT were older ($P<0.001$) and had higher prevalence of pre-enrollment CKD ($P=0.005$). Subjects who met the composite end point were more likely to be admitted to the ICU for respiratory reasons ($P<0.001$) and less likely to have had a surgical procedure ($P<0.001$) or recent trauma ($P=0.05$). End point-positive patients had higher enrollment serum creatinine ($P=0.001$) and severity of illness scores (Acute Physiology, Age, Chronic Health Evaluation [APACHE] and nonrenal APACHE III; $P<0.001$). AKI within 72 hours of study enrollment was more common in subjects who developed the composite end point (69% versus 49%; $P<0.001$). Additionally, those meeting the composite end point had longer length of stay in the ICU ($P<0.001$) (Table 1). Over 42% of those meeting the composite end point did so after hospital discharge (or day 30).

Univariate and Multivariate Analyses Showed Strong Associations between [TIMP-2]-[IGFBP7] and the Composite End Point

The association between [TIMP-2]-[IGFBP7] and the composite end point was significant when [TIMP-2]-[IGFBP7] was analyzed as a continuous variable; each unit increase in log$_{10}$-transformed [TIMP-2]-[IGFBP7] increased risk by 49% ($P<0.001$). This association was also significant when analyzing [TIMP-2]-[IGFBP7] as a categorical variable (Table 2). Table 2 also shows univariate hazard ratios (HRs) for clinical variables shown to be significant in Table 1.

[TIMP-2]-[IGFBP7] along with all variables found to be significant in Table 1 (with the exception of outcomes) are shown in a multivariate Cox model (Table 3).

We explored the association between a history of CKD and [TIMP-2]-[IGFBP7]. In an unadjusted analysis investigating [TIMP-2]-[IGFBP7] levels as a continuous variable in those with a history of CKD ($n=60$), [TIMP-2]-[IGFBP7] levels were significantly associated with an increased risk of the 9-month composite outcome (HR, 4.24; 95% confidence interval [95% CI], 2.12 to 8.49; $P<0.001$). To a lesser degree, continuous [TIMP-2]-[IGFBP7] levels were associated with an increased risk of the end point in those without a history of CKD ($n=632$; HR, 1.4; 95% CI, 1.09 to 1.80; $P=0.008$).

Separately, we found significant interactions between AKI and two covariates: [TIMP-2]-[IGFBP7] and serum creatinine.
Table 1. Baseline patient characteristics and outcomes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Death or Dialysis</th>
<th>Neither Death Nor Dialysis</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>217</td>
<td>475</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>131 (60)</td>
<td>292 (61)</td>
<td>0.80</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>71 (59–78)</td>
<td>61 (51–70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 (23–31)</td>
<td>27 (24–31)</td>
<td>0.09</td>
</tr>
<tr>
<td>Race</td>
<td>24 (11)</td>
<td>61 (13)</td>
<td></td>
</tr>
<tr>
<td>Chronic comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD</td>
<td>29 (13)</td>
<td>31 (7)</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>67 (31)</td>
<td>128 (27)</td>
<td>0.32</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>43 (20)</td>
<td>72 (15)</td>
<td>0.15</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>67 (31)</td>
<td>136 (29)</td>
<td>0.59</td>
</tr>
<tr>
<td>Hypertension</td>
<td>134 (62)</td>
<td>271 (57)</td>
<td>0.28</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>56 (26)</td>
<td>97 (20)</td>
<td>0.12</td>
</tr>
<tr>
<td>Cancer</td>
<td>64 (29)</td>
<td>110 (23)</td>
<td>0.09</td>
</tr>
<tr>
<td>Liver disease</td>
<td>8 (4)</td>
<td>22 (5)</td>
<td>0.69</td>
</tr>
<tr>
<td>Reason for ICU admission</td>
<td>126 (58)</td>
<td>168 (35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory</td>
<td>46 (21)</td>
<td>192 (40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>75 (35)</td>
<td>155 (33)</td>
<td>0.66</td>
</tr>
<tr>
<td>Sepsis</td>
<td>46 (21)</td>
<td>79 (17)</td>
<td>0.17</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>25 (12)</td>
<td>41 (9)</td>
<td>0.26</td>
</tr>
<tr>
<td>Trauma</td>
<td>10 (5)</td>
<td>43 (9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Burn</td>
<td>7 (3)</td>
<td>7 (1)</td>
<td>0.15</td>
</tr>
<tr>
<td>Other</td>
<td>38 (18)</td>
<td>82 (17)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Enrollment serum creatinine (mg/dl)</td>
<td>1.0 (0.71–1.4)</td>
<td>0.9 (0.7–1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APACHE III nonrenal</td>
<td>50 (70–93)</td>
<td>54 (40–75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum KDIGO stage within 72 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No AKI</td>
<td>68 (31)</td>
<td>242 (61)</td>
<td></td>
</tr>
<tr>
<td>KDIGO 1</td>
<td>78 (36)</td>
<td>160 (34)</td>
<td></td>
</tr>
<tr>
<td>KDIGO 2</td>
<td>46 (21)</td>
<td>70 (15)</td>
<td></td>
</tr>
<tr>
<td>KDIGO 3</td>
<td>25 (12)</td>
<td>3 (1)</td>
<td></td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU length of stay (d)</td>
<td>9 (5–18)</td>
<td>6 (4–12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hospital length of stay (d)</td>
<td>15 (9–26)</td>
<td>13 (8–21)</td>
<td>0.11</td>
</tr>
<tr>
<td>In-hospital RRT</td>
<td>40 (18)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>108 (50)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postdischarge RRT</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>0.10</td>
</tr>
<tr>
<td>Postdischarge mortality</td>
<td>92 (42)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values shown as N (%) and median (interquartile range).

*Truncated at 30 days.

As such, the adjusted HRs for [TIMP-2]-[IGFBP7] and serum creatinine were calculated separately for groups with and without AKI (Table 3).

In those subjects with AKI, [TIMP-2]-[IGFBP7] and serum creatinine remained significantly associated with long-term risk of death or RRT after adjusting for the model covariates. [TIMP-2]-[IGFBP7] values in those with AKI displayed a step-wise increase in its association with the composite end points. Compared with those with AKI and a [TIMP-2]-[IGFBP7] value <0.3, those with a [TIMP-2]-[IGFBP7] value >0.3 or <2.0 had a significantly increased risk of death or dialysis (HR, 1.44; 95% CI, 1.00 to 2.06; P=0.05), whereas [TIMP-2]-[IGFBP7] values >2.0 in those with AKI were at an even higher risk (HR, 2.16; 95% CI, 1.32 to 3.53; P=0.002).

Importantly, in those without AKI in the first 72 hours, neither [TIMP-2]-[IGFBP7] nor serum creatinine was significantly associated with increased risk of death or dialysis (Table 3).

Although our analysis was not intended to show that [TIMP-2]-[IGFBP7] should be used to predict long-term outcomes, we examined C-statistic improvement and reclassification for detection of death or dialysis events. The C statistic for the clinical model (Table 3) was not significantly improved with the addition of [TIMP-2]-[IGFBP7] (P=0.08). However, the addition of [TIMP-2]-[IGFBP7] to the clinical model led to a significant increase in risk stratification (total net reclassification index [NRI]=0.25; 95% CI, 0.08 to 0.40; P=0.001). The majority of this effect came from those with death or dialysis events (NRI_event=0.23; 95% CI, 0.09 to 0.36; P<0.001), whereas the NRI_nonevent was 0.02 (95% CI, −0.08 to 0.11; P=0.38). Similarly, the total integrated discrimination improvement (IDI) was significant at 0.02 (95% CI, 0.01 to 0.03; P<0.001). Both IDI_event and IDI_nonevent were significant, with estimates of 0.01 (95% CI, 0.00 to 0.02; P=0.02) and 0.01 (95% CI, 0.00 to 0.01; P=0.001), respectively.

Risk of Death or Dialysis Increased at Increasing Biomarker Cutoffs in Those with AKI

Kaplan–Meier curves for the composite end point are shown in Figure 2. Given the interaction between AKI and [TIMP-2]-[IGFBP7], we stratified those with AKI according to the previously established cutoffs and compared their 9-month outcomes with the outcomes of those with no AKI (regardless of [TIMP-2]-[IGFBP7] concentration). At 9 months, the risk of death or dialysis increased significantly across these strata (P<0.001), with 43% of those meeting the composite end point doing so after their index hospitalization (Figure 2). Given the subjective nature of the initiation of RRT, we performed an identical analysis after removing all subjects who received RRT during the first 30 days after study enrollment (Supplemental Figure 1). After removing those with in-hospital RRT, [TIMP-2]-[IGFBP7] >2.0 remained significantly associated with an increased risk of adverse patient outcomes (9 months; log-rank P<0.001).


AKI Biomarkers of Long-Term Outcomes
Table 2. Unadjusted HRs for the development of death or dialysis within 9 months from univariate Cox proportional hazards models

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKI</td>
<td>2.07 (1.55 to 2.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of CKD</td>
<td>1.69 (1.13 to 2.51)</td>
<td>0.01</td>
</tr>
<tr>
<td>Reason for ICU admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>2.18 (1.66 to 2.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Surgery</td>
<td>0.44 (0.31 to 0.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trauma</td>
<td>0.61 (0.32 to 1.30)</td>
<td>0.24</td>
</tr>
<tr>
<td>Serum creatinined</td>
<td>1.29 (1.05 to 1.15)</td>
<td>0.02</td>
</tr>
<tr>
<td>[TIMP-2]([IGFBP7]&gt;2.0c</td>
<td>1.22 (0.91 to 1.62)</td>
<td>0.18</td>
</tr>
<tr>
<td>[TIMP-2]([IGFBP7]&gt;2.0c</td>
<td>2.11 (1.37 to 3.23)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Overall P value for univariate [TIMP-2]([IGFBP7]) is 0.04 (likelihood ratio test). HRs are not provided for age and nonrenal APACHE III, because they did not satisfy the proportional hazards assumptions.

*KDIGO stage 1 or higher AKI within 72 hours after urine [TIMP-2]([IGFBP7]) sample collection.
*bLog2-transformed, the serum sample was collected at the same time as the urine sample for [TIMP-2]([IGFBP7]) testing.
*cRelative to [TIMP-2]([IGFBP7])≤0.3.

dSerum creatinine is not a clinically meaningful scale.

dLog2-transformed serum creatinine from a sample collected at the same time as the urine sample for [TIMP-2]([IGFBP7]) testing. We elected to use log2-transformed serum creatinine, because a 10-fold change in serum creatinine is not a clinically meaningful scale.

eRelative to [TIMP-2]([IGFBP7])≤0.3.

Table 3. Adjusted HRs for death or dialysis within 9 months from a multivariate Cox proportional hazards model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of CKD</td>
<td>1.41 (0.92 to 2.17)</td>
<td>0.11</td>
</tr>
<tr>
<td>Reason for ICU admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>1.74 (1.27 to 2.38)</td>
<td>0.001</td>
</tr>
<tr>
<td>Surgery</td>
<td>0.51 (0.35 to 0.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trauma</td>
<td>0.97 (0.50 to 1.88)</td>
<td>0.93</td>
</tr>
<tr>
<td>No AKI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinined</td>
<td>0.80 (0.56 to 1.13)</td>
<td>0.20</td>
</tr>
<tr>
<td>[TIMP-2]([IGFBP7]&gt;0.3 to ≤2.0c</td>
<td>0.71 (0.43 to 1.18)</td>
<td>0.19</td>
</tr>
<tr>
<td>[TIMP-2]([IGFBP7]&gt;2.0c</td>
<td>0.63 (0.15 to 2.64)</td>
<td>0.53</td>
</tr>
<tr>
<td>AKI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinined</td>
<td>1.40 (1.08 to 1.81)</td>
<td>0.01</td>
</tr>
<tr>
<td>[TIMP-2]([IGFBP7]&gt;0.3 to ≤2.0c</td>
<td>1.44 (1.00 to 2.06)</td>
<td>0.05</td>
</tr>
<tr>
<td>[TIMP-2]([IGFBP7]&gt;2.0c</td>
<td>2.16 (1.32 to 3.53)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

C-statistic=0.70 (0.67–0.74; P<0.001) under the null hypothesis of AUC=0.5 for the multivariate model.

*Age and APACHE III were included in the model as stratification terms to stratify the baseline hazard function and were not regression terms, because these variables violated the proportional hazards assumption; therefore, no HRs are reported for these variables.
*bCovariate-adjusted HR for each variable.
*cAKI is defined as KDIGO stage 1 or higher within 72 hours after urine [TIMP-2]([IGFBP7]) sample collection.
*dLog2-transformed serum creatinine from a sample collected at the same time as the urine sample for [TIMP-2]([IGFBP7]) testing. We elected to use log2-transformed serum creatinine, because a 10-fold change in serum creatinine is not a clinically meaningful scale.
*Relative to [TIMP-2]([IGFBP7])≤0.3.

DISCUSSION

Our results show that [TIMP-2]([IGFBP7]) tested in a large international prospective observational study and measured early in the setting of critical illness was associated with the 9-month composite outcome of all-cause mortality or receipt of RRT. This finding builds on the growing literature linking novel biomarkers of AKI with long-term outcomes subsequent to the seminal hospitalization for AKI.1,11 Additionally, we show that the link between these biomarkers and the composite end point is specific to subjects who develop AKI within 72 hours of measurement. We conclude that [TIMP-2]([IGFBP7]) is associated with adverse patient outcomes, specifically in those who develop AKI.

[TIMP-2]([IGFBP7]) has been shown to predict the impending development of severe AKI (KDIGO stage 2/3), providing an area under the curve of 0.80–0.82.9,10 Additionally, Aregger et al.12 recently showed that IGFBP7 levels predicted mortality, renal recovery, and severity/duration of AKI in a smaller cohort of critically ill adults. These biomarkers have been shown to be involved in a diverse array of biologic processes, including apoptosis/cellular senescence,13–15 inflammation,16 tubular regeneration,17 and cell cycle arrest.18–22 In the setting of cell cycle arrest, these proteins signal the activation of the p-protein cascade (p53, p21, and p27) which in turn, blocks the effect of the cyclin-dependent protein kinase complexes.18–22 The intricacies of these pathways have been elegantly elucidated in the setting of AKI/cisplatin cytotoxicity, where p21 is known to inhibit cyclin-dependent protein kinase-2 and phosphorylation of p21 alters the cellular response to this toxin.23–25 Additionally, TIMP-2 has been shown to be an important component in the pathophysiology of ischemia–reperfusion injury,26,27 playing roles in both early tubule interstitial injury26 and tubular regeneration postinjury.17 Finally, these factors play an important role in the modulation of the inflammatory response.18,19,28,29 Thus, TIMP-2 and IGFBP7 have been associated with a variety of integral cellular pathways, thus helping to explain their use in the multifactorial setting of critical illness-associated AKI (hypotension, sepsis, and nephrotoxins).

Recently, Coca et al.1 showed that several biomarkers of AKI measured in the perioperative period after adult cardiothoracic surgery correlated with long-term risk of mortality. In their follow-up of 1199 adults from the Translational Research Investigating Biomarker Endpoints in Acute Kidney Injury (TRIBE-AKI) cohort, Coca et al.1 showed that, compared with the first tertiles, the third tertiles of peak biomarker percentages of urine neutrophil gelatinase associated lipocalin, interleukin-18, kidney injury molecule-1, liver fatty acid binding protein, and urine albumin were all associated with a significantly increased risk of mortality in those subjects who developed postoperative AKI. This effect remained significant for kidney injury molecule-1 and interleukin-18 in those in the TRIBE cohort without AKI. In our study, [TIMP-2]([IGFBP7]) measured in the setting of critical illness similarly correlated with long-term outcomes; however, our subgroup analyses clearly show that the signal detected by [TIMP-2]([IGFBP7]) is highly specific to AKI (Table 3).

Importantly, in this analysis, we examined the relationship between [TIMP-2]([IGFBP7]) and long-term outcomes in the presence or absence of any AKI (Kidney Disease Improving Global Outcomes [KDIGO] stage 1 or greater) within 72 hours.
In the setting of even mild AKI (KDIGO stage 1; over 34% of our total cohort), elevated \([\text{TIMP-2}]z[\text{IGFBP7}]\) is a signal for poor prognosis. It also suggests that not all cases of mild AKI are benign and that these markers seem to be detecting a biologically important signal, even when it only manifests acutely as mild functional change. Our analyses also show that, in the absence of any functional changes in glomerular filtration measured by a change in either serum creatinine or urine output, \([\text{TIMP-2}]z[\text{IGFBP7}]\) is not associated with adverse outcomes, thus supporting the specificity of \([\text{TIMP-2}]z[\text{IGFBP7}]\) for AKI.

In addition to being specific to the setting of critical illness-associated AKI, we show that \([\text{TIMP-2}]z[\text{IGFBP7}]\) remains significantly associated with the composite end point of death or dialysis. In our multivariate Cox proportional model, which was adjusted for serum creatinine, \([\text{TIMP-2}]z[\text{IGFBP7}]\) concentrations in those who developed KDIGO AKI were associated with a 44% ([\text{TIMP-2}]z[\text{IGFBP7}]=0.3–2.0) or 116% ([\text{TIMP-2}]z[\text{IGFBP7}]>2.0) increased risk of death or dialysis, respectively, in the 9-month follow-up (Table 3). Importantly, neither serum creatinine nor \([\text{TIMP-2}]z[\text{IGFBP7}]\) was associated with adverse outcomes in those without AKI; thus, the \([\text{TIMP-2}]z[\text{IGFBP7}]\) improvement in clinical risk assessment was specific to AKI. This improvement was further evidenced by NRI_{event} (0.23; 95% CI, 0.09 to 0.36; \(P<0.001\)) and IDI_{event} (0.01; 95% CI, 0.00 to 0.02; \(P=0.02\)), both of which remained significant after adjusting for the clinical model that contained serum creatinine.

Increasingly, researchers are attempting to enroll subjects into AKI therapeutic trials; however, currently, we lack the tools to determine which patients will develop AKI and progress to severe outcomes, such as death or need for RRT. Our results indicate that patients with AKI and \([\text{TIMP-2}]z[\text{IGFBP7}]\>2.0\) are approximately two times as likely to die or receive dialysis than patients with AKI and \([\text{TIMP-2}]z[\text{IGFBP7}]\leq0.3\). These results may be useful in designing therapeutic trials for AKI by targeting patients most likely to benefit.

Our study has a number of strengths. Biomarkers assays were all performed as part of a large international multicenter observational investigation. Investigators were blinded with respect to the biomarker results and remained blinded throughout the follow-up period. We used standardized internationally accepted definitions of AKI (KDIGO) and definitive, patient-centered clinical end points for our analysis. Limitations include follow-up at 9 months. However, we have 9-month data on 80% of the cohort and 3-month data on 92% of the cohort, and all available clinical data on the basis of each subject’s last validated/known outcome were included in the survival model. Additionally, we do not know whether patients had subsequent episodes of AKI, and we did not measure serum creatinine during follow-up visits; as such, we are unable to determine changes in eGFR over time or the development of CKD. Nevertheless, we found strong interaction between change in renal function (criteria for AKI) and biomarker expression. Although this relationship requires additional exploration, it suggests that the association between the biomarkers accounting for serum creatinine.

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**Figure 2.** Kaplan–Meier curves for death or dialysis within 9 months after study enrollment. Cumulative death or dialysis within 9 months (within 30 days is shown in inset) for patients without AKI (no AKI; solid line, n=310) and patients with AKI (KDIGO stage 1 or higher AKI within 72 hours after urine \([\text{TIMP-2}]z[\text{IGFBP7}]\) sample collection) with urinary \([\text{TIMP-2}]z[\text{IGFBP7}]\) ranges of ≤0.3 (long dash line), >0.3 to ≤2.0 (short dash line), and >2.0 (short-long dash; n=178, n=160, and n=44 patients, respectively). For both 9 months and 30 days, log-rank \(P<0.001\). The non-AKI cohort is not categorized by urinary \([\text{TIMP-2}]z[\text{IGFBP7}]\), because urinary \([\text{TIMP-2}]z[\text{IGFBP7}]\) was not significant in Cox analysis of this cohort.
of acute kidney distress and long-term adverse outcomes is
caused by important underlying renal biology.

In summary, [TIMP-2]-[IGFBP7] was strongly associated
with a composite end point of death or need for dialysis over the
ensuing 9 months. This finding further substantiates a biologic
link between these markers and clinical disease. Furthermore,
given this association, routine measurement of these biomarkers
may help to identify a population of patients in the ICU who
are at highest risk for adverse outcomes. Improving risk predic-
tion may facilitate participation in AKI trials, improve patient
care in the setting of critical illness, and guide patient decision
making and counseling with regard to treatment plans.

CONCISE METHODS

Subjects
The original Sapphire study has been described in detail elsewhere.10
Briefly, 744 critically ill subjects were enrolled at 35 sites in North
America and Europe from September of 2010 to June of 2012. Subjects
were enrolled within 24 hours of admission to the ICU, had respiratory
or cardiovascular dysfunction, were expected to be in the ICU with a
urinary catheter for at least 48 hours, and were at least 21 years of
age. For the purposes of this analysis, subjects with documented
moderate or severe AKI (KDIGO stage 2 or 3) at the time of study
enrollment were excluded (n=31). All [TIMP-2]-[IGFBP7] con-
centrations were measured at the time of study enrollment. The
reporting of this study follows guidelines set out in the Strengthening
the Reporting of Observational Studies in Epidemiology. The Sapphire
study was approved by the Western Institutional Review Board
(Olympia, WA) as well as individual investigational review boards/
ethics committees as required by each participating institution. All
subjects (or legally authorized representatives) provided written
informed consent.

Sample and Data Collection
Paired urine and blood samples were collected by standard methods at
the time of study enrollment and centrifuged. Urine supernatants and
serum were frozen within 2 hours of collection, stored at ≤ −70°C,
and thawed immediately before analysis. All clinical data, including
patient demographics, reason for ICU admission, prior health history,
serum creatinine, and hourly urine output, were collected and stored
in a password-protected dataset residing on servers at independent sites
(Medidata Solutions, New York, NY).

Clinical End Points
The primary end point of death or dialysis was determined from
hospital records or for patients discharged alive and not on dialysis,
telephone calls to the subject or family members at approximately 1, 3,
and 9 months after enrollment. If the subject was determined to
have died or been on dialysis at the time of telephone assessment, the
date of death or dialysis was recorded if known. If not known
(11 subjects), the date was approximated as the midpoint between the
last known alive/dialysis-free date and the date of assessment. Three
tries, including a written letter, were made to contact subjects or
family members at each assessment time. If subject vital status could
not be ascertained after three attempts, the subject status was classified
as no death or dialysis, and the subject was censored in the analysis after
the last date of known vital status. AKI status during the first 72 hours
after enrollment was classified using the KDIGO international
guidelines on the basis of serum creatinine and urine output.31 The
reference values for serum creatinine were obtained as previously
described.10

Laboratory Methods
As previously described, urinary TIMP-2 and IGFBP7 were analyzed
by technicians blinded to clinical data using a clinical immunoassay
(NEPHROCHECK Test and ASTUTE140 Meter; Astute Medical Inc.,
San Diego, CA) at Astute Medical Inc.9 The ASTUTE140 Meter automatically
multiplies the concentrations of the two biomarkers
together and divides this product by 1000 to report a single numerical
test result with units of (nanograms per milliliter)2/1000 (the units for
all [TIMP-2]-[IGFBP7] test values in this report). Serum creatinine for
adjustment of [TIMP-2]-[IGFBP7] in multivariate models was mea-
sured at a central laboratory (LabCorp, San Diego, CA) in serum
samples collected at the same time as the urine samples. eGFR was
calculated using the Modification of Diet in Renal Disease equation.32
All analyses were performed in singlet on the first sample collected
after enrollment.

Statistical Methods
The association of [TIMP-2]-[IGFBP7] with the primary end
point of death or dialysis was investigated in several ways. Cox
proportional hazards analysis was used to determine the HR for
[TIMP-2]-[IGFBP7] in a univariate model and then, a multivariate
model adjusted for the clinical variables shown in Table 1 that have
P<0.05. These covariates included in the multivariate Cox model are
time-paired log2-transformed serum creatinine, AKI (defined as
KDIGO AKI stage 1 or higher within 72 hours of enrollment), history
of CKD, reason of ICU admission (respiratory, surgical, or trauma), an
interaction term between [TIMP-2]-[IGFBP7] and AKI, and an inter-
action term between log2-transformed serum creatinine and AKI. Be-
because age and nonrenal APACHE III score violated the propor-
tional hazards assumption (P<0.01; cox.zph function from the survival
package in R), the Cox model was stratified by them. Specifically, they were
dichotomized by their medians and combined into a new variable
with four categories, and the Cox model was stratified by this new
variable.

We also developed a clinical Cox model using the same predictors as
in the multivariate model above but leaving out [TIMP-2]-[IGFBP7].
To assess the improvement in the risk prediction performance of the
full model over the clinical model, we calculated NRI, IDI, and im-
provement in Harrell C statistic, accounting for censoring in survival
data.33,34

Statistical analyses were performed using R 3.0.0.35 Two-sided
P values were determined for the data in Table 1 and all HRs. One-
sided P values were determined for C statistic, NRI, and IDI analyses.
For all analyses, two-sided P values<0.05 were considered statistically
significant unless otherwise noted. For one-sided P values, P value=0.025 was considered statistically significant.
ACKNOWLEDGMENTS

We thank all of the patients, staff, coordinators, and investigators who were essential to the completion of this study. The study was supported by Astute Medical Inc.

Sapphire study sites were paid on the basis of patient enrollment and data entry. The study sponsor (Astute Medical Inc.) was primarily responsible for trial design, data collection, data analysis, and data interpretation with assistance from the investigators (J.L.K., A.B., and J.A.K.) and the independent biostatistician (J.S.).

A complete list of Sapphire investigators and their study/support staff is provided online at http://www.ccm.pitt.edu/sapphire-investigators.

DISCLOSURES

J.L.K., A.D.S., L.S.C., and J.A.K. have received consulting fees and research funding from Astute Medical Inc. E.A.J.H., M.H., and I.S. have received consulting fees from Astute Medical Inc. A.B. and K.K. have received research funding from Astute Medical Inc. J.A.K. also reports licensing of unrelated technologies through the University of Pittsburgh to Astute Medical Inc.

REFERENCES


AKI Biomarkers of Long-Term Outcomes

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This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2014060556/-/DCSupplemental.
Supplemental Figure 1: Kaplan-Meier curves for Death or Dialysis within the 9 months after study enrollment, excluding those who received RRT during their index hospitalization.

<table>
<thead>
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<th>Days after Enrollment</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
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<td>No AKI</td>
<td>303</td>
<td>223</td>
<td>207</td>
<td>177</td>
<td>173</td>
<td>165</td>
<td>164</td>
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<tr>
<td>AKI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.3</td>
<td>165</td>
<td>118</td>
<td>102</td>
<td>95</td>
<td>92</td>
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<tr>
<td>&gt;0.3 to ≤2.0</td>
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<td>91</td>
<td>83</td>
<td>74</td>
<td>69</td>
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<td>69</td>
</tr>
<tr>
<td>&gt;2.0</td>
<td>39</td>
<td>23</td>
<td>20</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Cumulative death or dialysis within nine months (and 30-days, inset) for non-AKI patients (No AKI, solid, N = 303) and AKI patients (KDIGO stage 1 or greater AKI within 72 hours after urine [TIMP-2][IGFBP7] sample collection) with urinary [TIMP-2][IGFBP7] ranges of ≤0.3 (long dash), >0.3 to ≤2.0 (short dash), and >2.0 (short-long dash) (N = 165, 145, and 39 patients, respectively). For both 9-months and 30-days, log-rank p < 0.0001. Patients with in-hospital RRT have been excluded from this analysis.