

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] levels>0.3 were associated with death or RRT only in subjects who developed AKI (compared with levels≤0.3: HR, 1.44; 95% CI, 1.00 to 2.06 for levels>0.3 to ≤2.0; $P=0.05$ and HR, 2.16; 95% CI, 1.32 to 3.53 for levels>2.0; $P=0.002$). In conclusion, [TIMP-2]·[IGFBP7] measured early in the setting of critical illness may identify patients with AKI at increased risk for mortality or receipt of RRT over the next 9 months.

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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} 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.⁸ To date, little is known about the relationship between these novel

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biomarkers and long-term outcomes,^{9,10} with only one large-scale multicenter investigation linking some biomarkers collected around the time of AKI with long-term mortality.¹

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.¹⁰ 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]

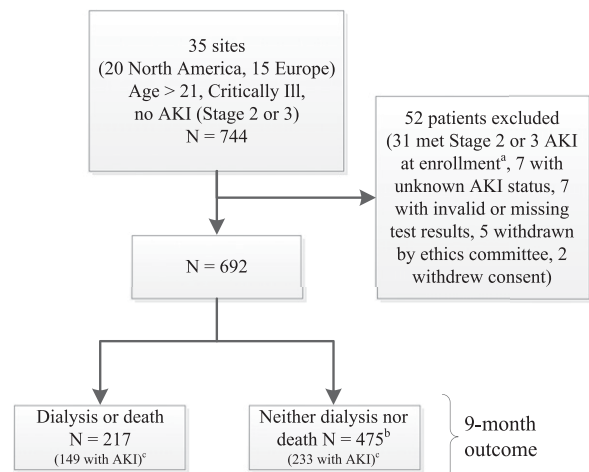


Figure 1. Flow diagram showing patient recruitment, enrollment and long-term outcomes in the Sapphire study. ^aThe Sapphire study enrolled patients not known to have moderate or severe AKI (744 patients),¹⁰ but 31 enrolled patients were found to have already met criteria for moderate or severe AKI (unbeknownst to investigators) at the time of enrollment, leaving 692 subjects from the original Sapphire study eligible for the primary analysis. ^bIn total, 140 of 475 patients in the neither death nor dialysis group did not have complete 9-month follow-up and were censored at the time of their last contact. Of these 140 subjects, 87 (62.1%) patients were known to be alive and free of dialysis at 3 months, and 115 (82.1%) patients were known to be alive and free of dialysis at 1 month. ^cKDIGO stage 1 or higher AKI within 72 hours after urine sample collection. The proportion of patients with AKI was higher ($P<0.001$) in the cohort with the death or dialysis end point (69% AKI) than in the cohort without the end point (49% AKI). Serum creatinine samples collected at the same time as urinary [TIMP-2]·[IGFBP7] samples were available for 683 patients.

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

Characteristic	Death or Dialysis	Neither Death Nor Dialysis	P Value
All patients	217	475	
Men	131 (60)	292 (61)	0.80
Age (yr)	71 (59–78)	61 (51–70)	<0.001
Body mass index (kg/m ²)	26 (23–31)	27 (24–31)	0.09
Race			0.79
Black or African American	24 (11)	61 (13)	
Other/unknown/missing	21 (10)	43 (9)	
White or Caucasian	172 (79)	371 (78)	
Chronic comorbidities			
CKD	29 (13)	31 (7)	0.005
Diabetes mellitus	67 (31)	128 (27)	0.32
Congestive heart failure	43 (20)	72 (15)	0.15
Coronary artery disease	67 (31)	136 (29)	0.59
Hypertension	134 (62)	271 (57)	0.28
Chronic obstructive pulmonary disease	56 (26)	97 (20)	0.12
Cancer	64 (29)	110 (23)	0.09
Liver disease	8 (4)	22 (5)	0.69
Reason for ICU admission			
Respiratory	126 (58)	168 (35)	<0.001
Surgery	46 (21)	192 (40)	<0.001
Cardiovascular	75 (35)	155 (33)	0.66
Sepsis	46 (21)	79 (17)	0.17
Cerebrovascular	25 (12)	41 (9)	0.26
Trauma	10 (5)	43 (9)	0.05
Burn	7 (3)	7 (1)	0.15
Other	38 (18)	82 (17)	>0.99
Enrollment serum creatinine (mg/dl)	1.0 (0.71–1.4)	0.9 (0.7–1.1)	0.001
APACHE III nonrenal	70 (50–93)	54 (40–75)	<0.001
Maximum KDIGO stage within 72 h			<0.001
No AKI	68 (31)	242 (51)	
KDIGO 1	78 (36)	160 (34)	
KDIGO 2	46 (21)	70 (15)	
KDIGO 3	25 (12)	3 (1)	
Outcomes			
ICU length of stay (d)	9 (5–18)	6 (4–12)	<0.001
Hospital length of stay (d)	15 (9–26)	13 (8–21)	0.11
In-hospital ^a RRT	40 (18)	0 (0)	<0.001
In-hospital ^a mortality	108 (50)	0 (0)	<0.001
Postdischarge RRT	2 (1)	0 (0)	0.10
Postdischarge mortality	92 (42)	0 (0)	<0.001

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

^aTruncated at 30 days.

($P<0.05$). 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 RRT (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 ($\text{NRI}_{\text{event}}=0.23$; 95% CI, 0.09 to 0.36; $P<0.001$), whereas the $\text{NRI}_{\text{nonevent}}$ 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 $\text{IDI}_{\text{event}}$ and $\text{IDI}_{\text{nonevent}}$ 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 cutoffs⁹ 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 $P<0.001$).

Table 2. Unadjusted HRs for the development of death or dialysis within 9 months from univariate Cox proportional hazards models

Variable	Unadjusted HR (95% CI)	P Value
AKI ^a	2.07 (1.55 to 2.77)	<0.001
History of CKD	1.69 (1.13 to 2.51)	0.01
Reason for ICU admission		
Respiratory	2.18 (1.66 to 2.86)	<0.001
Surgery	0.44 (0.31 to 0.61)	<0.001
Trauma	0.61 (0.32 to 1.30)	0.24
Serum creatinine ^b	1.29 (1.05 to 1.15)	0.02
[TIMP-2]·[IGFBP7]>0.3 to ≤2.0 ^c	1.22 (0.91 to 1.62)	0.18
[TIMP-2]·[IGFBP7]>2.0 ^c	2.11 (1.37 to 3.23)	<0.001

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.

^aKDIGO 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.

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

Variable ^a	Adjusted HR ^b (95% CI)	P Value
History of CKD	1.41 (0.92 to 2.17)	0.11
Reason for ICU admission		
Respiratory	1.74 (1.27 to 2.38)	0.001
Surgery	0.51 (0.35 to 0.74)	<0.001
Trauma	0.97 (0.50 to 1.88)	0.93
No AKI ^c		
Serum creatinine ^d	0.80 (0.56 to 1.13)	0.20
[TIMP-2]·[IGFBP7]>0.3 to ≤2.0 ^e	0.71 (0.43 to 1.18)	0.19
[TIMP-2]·[IGFBP7]>2.0 ^e	0.63 (0.15 to 2.64)	0.53
AKI ^c		
Serum creatinine ^d	1.40 (1.08 to 1.81)	0.01
[TIMP-2]·[IGFBP7]>0.3 to ≤2.0 ^e	1.44 (1.00 to 2.06)	0.05
[TIMP-2]·[IGFBP7]>2.0 ^e	2.16 (1.32 to 3.53)	0.002

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

^aAge 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.

^eRelative to [TIMP-2]·[IGFBP7]≤0.3.

DISCUSSION

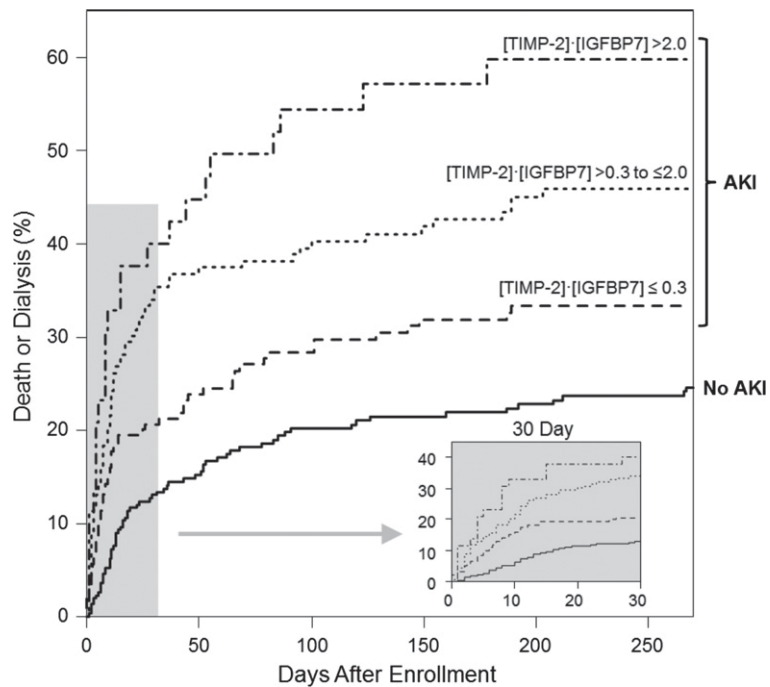
Our results show that [TIMP-2]·[IGFBP7] tested in a large international prospective observational study and measured early in the setting of critical illness was associated with the 9-month composite outcome of all-cause mortality or receipt of RRT. This finding builds on the growing literature linking

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 with 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.



	0	50	100	150	200	250	300
	Number At Risk						
No AKI	310	223	207	177	173	165	164
AKI							
≤0.3	178	118	102	95	92	92	92
>0.3 to ≤2.0	160	91	83	74	69	69	69
>2.0	44	23	20	17	16	16	16

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 [TIMP-2]·[IGFBP7] sample collection) with urinary [TIMP-2]·[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 [TIMP-2]·[IGFBP7], because urinary [TIMP-2]·[IGFBP7] was not significant in Cox analysis of this cohort.

In the setting of even mild AKI (KDIGO stage 1; over 34% of our total cohort), elevated [TIMP-2]·[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, [TIMP-2]·[IGFBP7] is not associated with adverse outcomes, thus supporting the specificity of [TIMP-2]·[IGFBP7] for AKI.

In addition to being specific to the setting of critical illness-associated AKI, we show that [TIMP-2]·[IGFBP7] remains significantly associated with the composite end point after

accounting for serum creatinine. In our multivariate Cox proportional model, which was adjusted for serum creatinine, [TIMP-2]·[IGFBP7] concentrations in those who developed KDIGO AKI were associated with a 44% ([TIMP-2]·[IGFBP7]=0.3–2.0) or 116% ([TIMP-2]·[IGFBP7]>2.0) increased risk of death or dialysis, respectively, in the 9-month follow-up (Table 3). Importantly, neither serum creatinine nor [TIMP-2]·[IGFBP7] was associated with adverse outcomes in those without AKI; thus, the [TIMP-2]·[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 trials³⁰; 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 [TIMP-2]·[IGFBP7]>2.0 are approximately two times as likely to die or receive dialysis than patients with AKI and [TIMP-2]·[IGFBP7]≤0.3. 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)³¹ and definitive, patient-centered clinical end points for our analysis. Limitations include <100% 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

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 prediction 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] concentrations were measured at the time of study enrollment. The reporting of this study follows guidelines set out in the Strengthening of 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 attempts, 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 measured 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 \log_2 -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 interaction term between \log_2 -transformed serum creatinine and AKI. Because age and nonrenal APACHE III score violated the proportional 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 improvement 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.

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

DISCLOSURES

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REFERENCES

- Coca SG, Garg AX, Thiessen-Philbrook H, Koyner JL, Patel UD, Krumholz HM, Shlipak MG, Parikh CR; TRIBE-AKI Consortium: Urinary biomarkers of AKI and mortality 3 years after cardiac surgery. *J Am Soc Nephrol* 25: 1063–1071, 2014
- Coca SG, Yusuf B, Shlipak MG, Garg AX, Parikh CR: Long-term risk of mortality and other adverse outcomes after acute kidney injury: A systematic review and meta-analysis. *Am J Kidney Dis* 53: 961–973, 2009
- Bagshaw SM, Mortis G, Doig CJ, Godinez-Luna T, Fick GH, Laupland KB: One-year mortality in critically ill patients by severity of kidney dysfunction: A population-based assessment. *Am J Kidney Dis* 48: 402–409, 2006
- Chertow GM, Christiansen CL, Cleary PD, Munro C, Lazarus JM: Prognostic stratification in critically ill patients with acute renal failure requiring dialysis. *Arch Intern Med* 155: 1505–1511, 1995
- Morgera S, Kraft AK, Siebert G, Luft FC, Neumayer HH: Long-term outcomes in acute renal failure patients treated with continuous renal replacement therapies. *Am J Kidney Dis* 40: 275–279, 2002
- Hobson CE, Yavas S, Segal MS, Schold JD, Tribble CG, Layon AJ, Bihorac A: Acute kidney injury is associated with increased long-term mortality after cardiothoracic surgery. *Circulation* 119: 2444–2453, 2009
- Bihorac A, Yavas S, Subbiah S, Hobson CE, Schold JD, Gabrielli A, Layon AJ, Segal MS: Long-term risk of mortality and acute kidney injury during hospitalization after major surgery. *Ann Surg* 249: 851–858, 2009
- Koyner JL, Parikh CR: Clinical utility of biomarkers of AKI in cardiac surgery and critical illness. *Clin J Am Soc Nephrol* 8: 1034–1042, 2013
- Bihorac A, Chawla LS, Shaw AD, Al-Khafaji A, Davison DL, Demuth GE, Fitzgerald R, Gong MN, Graham DD, Gunnerson K, Heung M, Jortani S, Kleerup E, Koyner JL, Krell K, Letourneau J, Lissauer M, Miner J, Nguyen HB, Ortega LM, Self WH, Sellman R, Shi J, Straseski J, Szalados JE, Wilber ST, Walker MG, Wilson J, Wunderink R, Zimmerman J, Kellum JA: Validation of cell-cycle arrest biomarkers for acute kidney injury using clinical adjudication. *Am J Respir Crit Care Med* 189: 932–939, 2014
- Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, Bihorac A, Birkhahn R, Cely CM, Chawla LS, Davison DL, Feldkamp T, Forni LG, Gong MN, Gunnerson KJ, Haase M, Hackett J, Honore PM, Hoste EA, Joannes-Boyau O, Joannidis M, Kim P, Koyner JL, Laskowitz DT, Lissauer ME, Marx G, McCullough PA, Mullaney S, Ostermann M, Rimmelé T, Shapiro NI, Shaw AD, Shi J, Sprague AM, Vincent JL, Vinsonneau C, Wagner L, Walker MG, Wilkerson RG, Zacharowski K, Kellum JA: Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. *Crit Care* 17: R25, 2013
- Akçay AB, Özlü MF, Sen N, Cay S, Öztürk OH, Yalçın F, Bilen P, Kanat S, Karakas MF, İslayen A, Demir AD, Sogut S, Covic A, Kanbay M: Prognostic significance of neutrophil gelatinase-associated lipocalin in ST-segment elevation myocardial infarction. *J Invest Med* 60: 508–513, 2012
- Aregger F, Uehlinger DE, Witowski J, Brunisholz RA, Hunziker P, Frey FJ, Jorres A: Identification of IGFBP-7 by urinary proteomics as a novel prognostic marker in early acute kidney injury. *Kidney Int* 85: 909–919, 2014
- Severino V, Alessio N, Farina A, Sandomenico A, Cipollaro M, Peluso G, Galderisi U, Chambery A: Insulin-like growth factor binding proteins 4 and 7 released by senescent cells promote premature senescence in mesenchymal stem cells. *Cell Death Dis* 4: e911, 2013
- Benatar T, Yang W, Amemiya Y, Evdokimova V, Kahn H, Holloway C, Seth A: IGFBP7 reduces breast tumor growth by induction of senescence and apoptosis pathways. *Breast Cancer Res Treat* 133: 563–573, 2012
- Tomimaru Y, Eguchi H, Wada H, Kobayashi S, Marubashi S, Tanemura M, Umeshita K, Kim T, Wakasa K, Doki Y, Mori M, Nagano H: IGFBP7 downregulation is associated with tumor progression and clinical outcome in hepatocellular carcinoma. *Int J Cancer* 130: 319–327, 2012
- Musiak K, Zwolińska D: The sFas/sFasL ratio as a novel marker of inflammation in children with chronic kidney disease. *Clin Chim Acta* 414: 7–11, 2012
- Miya M, Maeshima A, Mishima K, Sakurai N, Ikeuchi H, Kuroiwa T, Hiromura K, Yokoo H, Nojima Y: Enhancement of in vitro human tubulogenesis by endothelial cell-derived factors: Implications for in vivo tubular regeneration after injury. *Am J Physiol Renal Physiol* 301: F387–F395, 2011
- Seo DW, Li H, Qu CK, Oh J, Kim YS, Diaz T, Wei B, Han JW, Stetler-Stevenson WG: Shp-1 mediates the antiproliferative activity of tissue inhibitor of metalloproteinase-2 in human microvascular endothelial cells. *J Biol Chem* 281: 3711–3721, 2006
- Seo DW, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R, Wei BY, Stetler-Stevenson WG: TIMP-2 mediated inhibition of angiogenesis: An MMP-independent mechanism. *Cell* 114: 171–180, 2003
- Stetler-Stevenson WG: Tissue inhibitors of metalloproteinases in cell signaling: Metalloproteinase-independent biological activities. *Sci Signal* 1: re6, 2008
- Wajapeyee N, Serra RW, Zhu X, Mahalingam M, Green MR: Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. *Cell* 132: 363–374, 2008
- Zuo S, Liu C, Wang J, Wang F, Xu W, Cui S, Yuan L, Chen X, Fan W, Cui M, Song G: IGFBP-rP1 induces p21 expression through a p53-independent pathway, leading to cellular senescence of MCF-7 breast cancer cells. *J Cancer Res Clin Oncol* 138: 1045–1055, 2012
- Hodeify R, Tarcsfalvi A, Megyesi J, Safirstein RL, Price PM: Cdk2-dependent phosphorylation of p21 regulates the role of Cdk2 in cisplatin cytotoxicity. *Am J Physiol Renal Physiol* 300: F1171–F1179, 2011
- Price PM, Safirstein RL, Megyesi J: The cell cycle and acute kidney injury. *Kidney Int* 76: 604–613, 2009
- Yu F, Megyesi J, Safirstein RL, Price PM: Identification of the functional domain of p21(WAF1/CIP1) that protects cells from cisplatin cytotoxicity. *Am J Physiol Renal Physiol* 289: F514–F520, 2005
- Wang Z, Famulski K, Lee J, Das SK, Wang X, Halloran P, Oudit GY, Kassiri Z: TIMP2 and TIMP3 have divergent roles in early renal tubulointerstitial injury. *Kidney Int* 85: 82–93, 2014
- Cavdar Z, Oktay G, Egrilmez MY, Genc S, Genc K, Altun Z, Islekel H, Guner G: In vitro reoxygenation following hypoxia increases MMP-2 and TIMP-2 secretion by human umbilical vein endothelial cells. *Acta Biochim Pol* 57: 69–73, 2010

28. Ruster C, Wolf G: Angiotensin II as a morphogenic cytokine stimulating renal fibrogenesis. *J Am Soc Nephrol* 22: 1189–1199, 2011
29. Ruiz-Ortega M, Lorenzo O, Suzuki Y, Ruperez M, Egido J: Proinflammatory actions of angiotensins. *Curr Opin Nephrol Hypertens* 10: 321–329, 2001
30. Faubel S, Chawla LS, Chertow GM, Goldstein SL, Jaber BL, Liu KD; Acute Kidney Injury Advisory Group of the American Society of Nephrology: Ongoing clinical trials in AKI. *Clin J Am Soc Nephrol* 7: 861–873, 2012
31. KDIGO: Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int [Suppl 2]*: 19–33, 2012
32. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. *Ann Intern Med* 130: 461–470, 1999
33. Pencina MJ, D'Agostino RB Sr., Steyerberg EW: Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 30: 11–21, 2011
34. Chambless LE, Cumiskey CP, Cui G: Several methods to assess improvement in risk prediction models: Extension to survival analysis. *Stat Med* 30: 22–38, 2011
35. R Development Core Team: *R: A Language and Environment for Statistical Computing*, Vienna, Austria, R Foundation for Statistical Computing, 2008

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