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TIMP2•IGFBP7 Biomarker Panel Accurately Predicts Acute Kidney Injury In High Risk Surgical Patients

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Abstract

Background: Acute kidney injury (AKI) is an important complication in surgical patients. Existing biomarkers and clinical prediction models underestimate risk for developing AKI. We recently reported data from two trials of 728 and 408 critically ill adult patients in whom urinary TIMP2•IGFBP7 (NephroCheck, Astute Medical) was used to identify patients at risk of developing AKI. Here we report a pre-planned analysis of surgical patients from both trials to assess whether urinary tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) accurately identifies surgical patients at risk of developing Acute Kidney Injury (AKI).

Study Design: We enrolled adult surgical patients at risk for AKI who were admitted one of 39 intensive care units across Europe and North America. The primary endpoint was moderate-severe AKI (equivalent to KDIGO stage 2-3) within 12 hours of enrollment. Biomarker performance was assessed using the area under the receiver operating characteristic curve (AUC), integrated discrimination improvement and category-free net reclassification improvement.

Results: 375 patients were included in the final analysis of whom 35 (9%) developed moderate-severe AKI within 12 hours. The AUC for [TIMP-2]•[IGFBP7] alone was 0.84 (95% CI 0.76-0.90, $p<0.0001$). Biomarker performance was robust in sensitivity analysis across pre-defined subgroups (urgency and type of surgery).

Conclusions: For postoperative surgical ICU patients, a single urinary TIMP2•IGFBP7 test accurately identified patients at risk for developing AKI within the ensuing 12 hours and its inclusion in clinical risk prediction models significantly enhances their performance.

Level of Evidence: Level 1, prognostic

Key words: Acute kidney injury, surgical, tissue inhibitor of metalloproteinase-2, critical

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INTRODUCTION

Acute kidney injury (AKI) is a significant public health problem,[1,2] and is common in surgical patients of all types.[3,4] AKI occurring after surgery is associated with short-term morbidity, an increased risk of de novo or progressive chronic kidney disease (CKD), adverse cardiovascular events and it also decreases hospital survival rates and increases hospital costs.[5-10] Surgical inpatients, particularly those with co-morbid disease and those undergoing complex procedures are at increased risk for developing AKI.[5,7,11] Patients who develop AKI in the postoperative period are at high risk for fluid overload, infection (surgical site, pulmonary and urinary tract), and other complications that may directly affect outcome after surgery.[3,4,12] In comparison to other risk factors for AKI (e.g. sepsis and nephrotoxic antibiotics), complex surgical procedures expose patients to a combination of pathophysiological processes including inflammation, oxidative stress, iron and heme proteins, tissue injury, blood product transfusion, and hemodynamic instability.[13-18]

Although recent international Kidney Disease Improving Global Outcomes (KDIGO) guidelines have further advanced consensus regarding uniform diagnostic criteria and preventive strategies for AKI[19] the inability to identify AKI early at a potentially reversible stage is still the rate-limiting step.[1,20] Traditional biomarkers and physiologic indicators such as serum creatinine and urine output are understood to be late and nonspecific markers of renal dysfunction—yet in the absence of better alternatives, they are in widespread clinical use.[21] Even with the new KDIGO guidelines, the impact of AKI on surgical outcomes is still not fully recognized as demonstrated by a recent study showing the underestimation of risk associated with AKI in postoperative patients.[3]

Surgical patients represent an unique population for early AKI risk stratification because of the presence of readily modifiable risk factors (e.g. fluid type and amount, blood product administration, hemodynamic management). However, the etiological differences in surgical AKI patients when compared with their non-surgical peers means it is important to demonstrate that novel biomarkers of AKI risk perform in a useful and robust fashion in patients whose main risk for AKI is their surgical procedure and its attendant management. Accordingly, we conducted a *preplanned* analysis of surgical patients using data from two multicenter clinical studies that successfully identified ICU patients at risk for developing AKI within the ensuing 12 hours of biomarker assessment.[22,23] Our goal for this new analysis was to test the hypothesis that the TIMP2•IGFBP7 test would correctly identify a broad range of critically ill surgical patients at high risk for developing AKI.

METHODS

Study Design

This was a pre-planned subgroup analysis of critically ill surgical patients enrolled in either of our two previously reported studies in which the discovery (Sapphire)[23] and subsequent validation (Topaz)[22] of TIMP2•IGFBP7 was performed. Surgical patients were further categorized as either cardiothoracic or non-cardiothoracic because of known differential risk factors, for example use of cardiopulmonary bypass.[24] All patients were deemed at high risk for AKI, characterized as those with respiratory or cardiovascular dysfunction as previously reported (Figure 1).[22,23] The design, execution and reporting of this study meet the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[25] and the Standards for Reporting of Diagnostic Accuracy (STARD)[26] criteria. Data were collected by the investigators and analyzed by external statisticians. Both study protocols were approved by the Western Institutional Review Board (Olympia, Washington, USA) and also by the institutional review board or ethics committee of each study site if required. All patients provided written informed consent. In this paper we present data from the Sapphire study, which defined AKI as KDIGO stage 2-3, and from the Topaz study, which used clinical adjudication for AKI, in order to examine the performance of the TIMP2•IGFBP7 test for risk assessment of AKI in surgical patients. Although these biomarkers have previously been shown to have excellent ability to identify risk of AKI in an unselected group of acutely ill adult ICU patients,[23] their performance in a broad range of surgical patients has not been previously described. Given the likely etiological differences in surgical and medical AKI, and the biological nature of these markers (indicators of cellular stress), this is an important issue that needs to be addressed before the test can be included in clinical decision making for these patients.

Biomarker Assays

Urine samples were analyzed for TIMP-2 and IGFBP7 using a clinical immunoassay (NephroCheck® Test and Astute140® Meter, Astute Medical Inc., San Diego, CA) by technicians who were unaware of the clinical data. 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 $(ng/ml)^2/1000$ (the units for all TIMP2•IGFBP7 values in this report). Each sample was tested once for TIMP2•IGFBP7 in the Sapphire study and three times in the Topaz study. The single value from Sapphire and the median of the three replicates in Topaz were used for analysis.

Statistical Analysis

The primary objective was to evaluate the ability of urinary TIMP2•IGFBP7 to identify critically ill surgical patients who went on to develop moderate or severe AKI in the immediate 12 hours after measurement. Ability to predict this event (i.e. development of moderate or severe AKI) was assessed using the area under the receiver operating characteristic curve (AUC-ROC) for urinary TIMP2•IGFBP7. As a secondary analysis, we used integrated discrimination improvement (IDI), category-free net reclassification improvement (cfNRI), and change in AUC-ROC to investigate the improvement in the prediction of the primary endpoint resulting from the addition of the TIMP2•IGFBP7 data to a clinical model.[27] Variables from Table 1 that were associated with the endpoint ($p<0.1$) were selected for inclusion in the clinical model, which was then analyzed using multivariate logistic regression. Logistic regression analyses used the logarithmic transformed and standardized serum creatinine and TIMP2•IGFBP7 test results from time-paired blood and urine samples, respectively, collected at the time of enrollment. All continuous variables in the clinical models were standardized by subtracting the mean and then

dividing by twice the standard deviation in order for their coefficients to be comparable with the coefficients of binary predictors,[28] which were coded as 0 or 1. Model performance was assessed with the Hosmer-Lemeshow test for the goodness-of-fit. Six subjects in the Sapphire study did not have a TIMP2•IGFBP7 test result at the time of enrollment, and therefore, test results from the second collection scheduled twelve hours after enrollment were used for these subjects.

Statistical analyses were performed using R 3.1.0.[29] For all analyses, two-sided p-values less than 0.05 and one-sided p-values less than 0.025 were considered statistically significant. Categorical variables were analyzed using Fisher's Exact test, chi-square test, or logistic regression. All biomarker performance statistics (AUC, sensitivity, specificity and relative risk) were calculated as empirical estimates, and their confidence intervals were obtained by bootstrap analysis.

RESULTS

Baseline Characteristics

In the Sapphire and Topaz trials there were 247 and 128 surgical patients, respectively and these were combined to form the analysis cohort of 375 patients (Figure 1). Baseline characteristics for all patients are provided in Table 1 and are shown separately by study in the supplemental appendix. Patients developing AKI had higher baseline creatinine and tended to have more comorbidities but were otherwise similar to patients without AKI. Patients in both trials had similar baseline characteristics with regards to both acute severity of illness (Acute Physiology and Chronic Health Evaluation (APACHE) III score without the renal component) and chronic disease burden. However, there were more diabetic patients in Sapphire (31% vs. 20%) and more emergency surgery patients (37% vs. 22%) and nephrotoxic medication use (88% vs. 74%) in Topaz. The overall rate of moderate to severe AKI within 12 hours of sampling was slightly higher in Topaz (12.5%) compared to Sapphire (7.7%), but this difference was not statistically significant. The 12-hour incidence of moderate to severe AKI was 9% (35 / 375) for the pooled surgical cohorts (Figure 1).

Biomarker Performance

Urinary TIMP2•IGFBP7 performed well in detecting those subjects who developed moderate to severe AKI within 12 hours of sample collection (Figures 2-3). As reported previously for heterogeneous (medical and surgical) cohorts of critically ill patients,[22,30] when the *pre-specified* cutoff TIMP2•IGFBP7 value of 0.3 was used, the sensitivity of the test was 89% (95% CI, 77-97) with an accompanying specificity of 49% (95% CI, 43-54), a positive likelihood ratio of 1.72 (95% CI, 1.44-2.01) and a negative likelihood ratio of 0.24 (95% CI, 0.06-0.49) (see supplementary appendix for additional details). Patients with urinary

TIMP2•IGFBP7 test values greater than 0.3 had more than six times the risk for AKI compared to those with a test value at or below the 0.3 cutoff. Put another way, when the TIMP2•IGFBP7 result is less than or equal to 0.3, only 1 in 42 surgical patients (2.4% absolute risk) will develop moderate or severe AKI within the next 12 hours. At the *pre-specified* test cutoff of 2.0 (again as was the case in heterogeneous cohorts of critically ill patients),[22,30] the test specificity improves to a very robust 94% with a positive likelihood ratio of 6.8 (95% CI, 3.5-12.7) and a substantial increase in risk (full operating characteristics at various cut points are shown in the supplementary appendix).

The strong performance of this urinary TIMP2•IGFBP7 test was observed in the surgical cohorts of each individual study as well as when the patients from both cohorts are combined (Figures 2-3). This is demonstrated by the AUC (95% CI) for the Sapphire (0.80 (95% CI, 0.69-0.92), p<0.0001) and Topaz (0.88 (95% CI 0.81-0.96), p<0.0001) studies and for when cohorts are combined (0.84 (95% CI, 0.76-0.90), p<0.0001). In comparison, neither urine output nor serum creatinine predicted development of AKI during the same time period as well. Of note, the cohorts were not heterogeneous as evidenced by the heterogeneity tests for the AUCs between Sapphire and Topaz: Higgin's I^2 = 29%; Cochran's Q statistic for measuring heterogeneity is not significant (p = 0.23).

As shown in Figure 2, test characteristics vary somewhat, but remain robust, across different subgroups (cardiac/non-cardiac and emergent/elective). A multivariate clinical model was created using step-wise selection from all the variables in Table 1 that were associated (p<0.1) with the primary endpoint. This model was created with and without the inclusion of the biomarker test results. With the addition of the biomarker, the model's ability to predict

moderate-severe AKI increased, as shown by an increase in the AUC-ROC from 0.77 to 0.88 (p=0.01) (Figure 3).

To provide further assessment of the ability of the biomarker to enhance clinical risk prediction, we performed integrated discrimination improvement (IDI) and category-free (continuous) Net Reclassification Improvement (cfNRI) analyses as described in Table 2. The addition of the biomarker to the clinical model resulted in a significant increase in overall ability to predict AKI; IDI = 0.15 (0.09-0.21), p<0.001, and cfNRI = 0.95 (0.59-1.30), p<0.001 (Table 2) (see also eFigure 1 in the supplementary appendix).

DISCUSSION

To our knowledge, this is the first report using the cell-cycle arrest biomarkers TIMP-2 and IGFBP7 to predict the development of moderate to severe AKI in a large cohort of heterogeneous, critically ill, postoperative surgical patients. In our study, we used a single measurement of urinary TIMP2•IGFBP7 soon after ICU admission and found an AUC-ROC of 0.84 for development of moderate-severe AKI within 12 hours. Using a *pre-specified* cutoff value of 0.3 we found sensitivity and specificity of 89% (77%-97%) and 49% (43%-54%) respectively; using a *pre-specified* cutoff value of 2.0 we found sensitivity and specificity of 40% (23%-57%) and 94% (92%-96%). We have demonstrated reassuringly robust performance of a new biomarker test in the prediction of an important clinical outcome, and further shown that it is able to significantly enhance clinical risk prediction techniques alone. Importantly, we have also shown that the results of this test remain robust in cardiac and non-cardiac surgery, and in elective as well as emergent surgery.

Recently Meersch and colleagues examined the sensitivity and specificity of TIMP2•IGFBP7 for AKI (stage 1 or greater) in a group of patients undergoing cardiac surgery.[31] These investigators found a sensitivity of 0.92 and specificity of 0.81 for a cutoff value of 0.5 (AUC-ROC of 0.84) using the highest urinary TIMP2•IGFBP7 concentration achieved in the first 24 hours following surgery (composite time point). However, here we report the performance of the test in surgical patients when it is used as a single measure at or near the time of ICU admission to predict development of moderate-severe AKI in the next 12-24 hours. This may provide context to clinicians who wish to use the test to help in deciding the immediate care plan for patients in their surgical ICU practice.

The performance of AKI biomarkers can sometimes be diminished in the presence of chronic disease states and also in circumstances where there is activation of the inflammatory cascade.[32-39] However, TIMP2•IGFBP7 appears to retain its performance in this surgical cohort and also specifically in the cardiac surgery subgroup (Figure 2). Current mechanistic thinking in AKI is moving towards the concept of AKI as a secondary injury occurring as danger and pathogen associated molecular pattern (DAMPs, PAMPs) molecules are delivered to the renal tubule via both glomerular filtration and the blood stream.[37] These molecules are detected by pattern recognition receptors on the tubular cell surface where they signal a number of cell responses including alterations in cell-cycle progression. When prolonged, cell-cycle arrest may lead to senescence and/or apoptosis. However, cell-cycle arrest is, itself, a protective mechanism that prevents cells from dividing when they may be injured. The fact that these biomarkers perform so well in surgical patients, and that they are seemingly robust to type and urgency of surgery, suggests that this mechanism of AKI is determined much more by the host response to stress rather than by the type of operation itself.

Both TIMP2 and IGFBP7 have been implicated in the G_1 cell-cycle arrest phase noted to occur during the very early stages of cellular stress.[38-41] Specifically, it has been shown that renal tubular cells go through this G_1 cell cycle arrest phase following stress due to a variety of insults.[42] In surgical patients, including those undergoing a period of cardiopulmonary bypass, it is reassuring that these markers perform well given the involvement of the innate immune system as a fundamental part of the host response to environmental (surgical) stress.[43,44] It is reasonable therefore to suggest that biomarkers whose physiological origin is in the pathways modulating this response might prove useful as predictors of outcome in this patient population. TIMP2 and IGFBP7 are both implicated in cell-cycle arrest and signal transduction during innate immune

activation, and as such it is not surprising that they apparently perform better than previously described biomarkers in surgical patients.

In addition to TIMP-2 and IGFBP7, a host of other AKI biomarkers have been previously investigated within the context of surgery.[45-50] The majority of these studies have been conducted in post-cardiac surgery patients,[45,48] the most comprehensive being the NIH sponsored TRIBE AKI trial wherein multiple AKI biomarkers were tested in over 1,200 post-cardiac surgery patients.[47] These studies have demonstrated that plasma NGAL, urinary NGAL, and urinary IL-18 all performed modestly well in the prediction of AKI, with AUC-ROC values between 0.67-0.74. When the biomarkers were added to a clinical model its performance improved to 0.73-0.76. These results are consistent with other cohorts of cardiac and non-cardiac surgery patients.

Our study has several limitations. First, our limited sample size only permits exploratory analysis within different surgical sub-groups, such as those undergoing cardiac or non-cardiac surgery. Second, although consistent with other reports, our overall event rate does not permit large multivariate analyses that may uncover the contribution of other important risk factors such as blood transfusion, fluid administration, or different hemodynamic management strategies. These questions are the focus of our ongoing studies. In spite of these limitations, we believe our data provide busy clinicians caring for surgical patients with reassuring evidence that the TIMP2•IGFBP7 test is able to improve their clinical decision making in the context of AKI risk assessment and stratification. In the future it will be important to demonstrate which clinical interventions and therapeutic maneuvers are associated with good (and bad) outcomes in patients stratified by biomarker test results. If confirmed in prospective trials, these candidate interventions may then be considered therapeutic goals in patients classified as high risk.

CONCLUSIONS

We have previously shown that the TIMP2•IGFBP7 test accurately identifies a cohort of critically ill adult patients who are at increased risk for developing AKI within the subsequent 12-24 hours. Here we provide evidence that this finding is just as accurate, in fact more so, in surgical patients. This is important both because of unique exposures and the presence of several potentially modifiable risk factors in this patient population. Future interventional trials focused on AKI prevention using urinary TIMP2•IGFBP7 as an early marker of renal cellular stress are warranted.

Conflict of Interest: KK, AA-K, AB, ML, JS, and MW report no conflicts of interest. LC has consulting agreements with Abbott Medical, Affymax Medical, Alere Medical, AM Pharma, Astute Medical, Covidien Medical, Gambro Medical, Nxstage, Sanofi, Bonner Kiernana Law Offices. LC has applied for research support from Eli Lilly and owns stock in MAKO Corporation for an orthopedic surgical robot. KG has received research grants from Spectral Diagnostics. AS has received fees for expert testimony from Abbott Laboratories and is a Medical Advisory Board member for FAST diagnostics for Optical GFR measurement and a Scientific Advisory Board member for NxStage Medical for CRRT in the ICU. JK has received consulting fees from Astute Medical, Alere, Opsona, Aetholon, AM Pharma, Cytosorbents, VenBio, Gambro, Baxter, Abbott, Roche, Spectral Diagnostics, Sangart and Siemens. JK has also received research grants from Astute Medical, Alere, Cytosorbents, Gambro, Baxter, Kaneka, Grifols, CR Bard and Spectral Diagnostics, and has license unrelated technologies through the University of Pittsburgh to Astute Medical, Cytosorbents and Spectral Diagnostics.

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Author Contributions

K.J.G., A.D.S., L.S.C., A.B., A.A-K., K.K., M.L., and J.A.K. conducted the literature search. J.A.K., K.J.G., A.B., A.D.S., L.S.C., A.A-K., and K.K. designed this study. K.J.G., A.D.S., L.S.C., A.B., A.A-K., K.K., M.L., and J.A.K. collected data on behalf of the Sapphire and Topaz investigators. J.S. and M.G.W. performed data analysis. J.A.K., K.J.G., A.D.S., L.S.C., A.B.,

A.A-K., K.K., M.L., J.S., and M.G.W. contributed to data interpretation. K.J.G., J.A.K., A.D.S.,

L.S.C., A.B., A.A-K., K.K., M.L.,J.S., and M.G.W. wrote and critically revised the manuscript.

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Figure Legends

Figure 1. Study design and patients.

Study design and number of patients in cohorts. AKI was defined as KDIGO AKI stage 2 or 3 for Sapphire and was determined by clinical adjudication for Topaz (based on KDIGO stage 2-3 AKI).

Figure 2. TIMP2•IGFBP7 biomarker performance in surgical subsets.

TIMP2•IGFBP7 box plots and area under the ROC curve (AUC-ROC). (A) TIMP2•IGFBP7 levels by surgical subgroup and AKI status within 12 hours, and (B) AUC by surgical subgroup. Two mutually exclusive subgroup pairs are shown: Non-Cardiothoracic vs Cardiothoracic and Elective vs Emergent. Boxes and whiskers show interquartile ranges and total observed ranges, censored at 1.5 times the interquartile ranges. Patients with AKI had significantly higher levels of TIMP2•IGFBP7 than patients without AKI for all surgical patients and within each subgroup shown (Wilcoxon rank sum test adjusted $P < 0.001$ in all cases). All AUC values were approximately 0.8 or greater and significantly greater than 0.5 ($p<0.001$).

Figure 3. Receiver operating characteristic curve for clinical model alone and with TIMP2•IGFBP7 and odds ratios for model variable.

ROC curves and odds ratios from a multivariate clinical model alone and the model with TIMP2•IGFBP7. Step-wise model selection was used to derive the clinical model starting from all variables from Table 1 with $p<0.1$ for the endpoint. All patients with a TIMP2•IGFBP7 value and data for all clinical variables were included (N = 353). The area under the ROC curve (AUC)

increases (one-sided p=0.008) from 0.77 (0.69-0.86) to 0.88 (0.83-0.94) when TIMP2•IGFBP7 is added to the model. *Log10 transform of APACHE III and TIMP2•IGFBP7 were used in the models. †Log2 transform of serum creatinine was used because log10 is not a clinically relevant scale for serum creatinine. Blood for serum creatinine testing was collected at the time of urine collection for TIMP2•IGFBP7 testing. §History of cirrhosis or hepatic failure. The inverse of body mass index was used in the model. All continuous variables were standardized by subtracting the mean and then dividing by two standard deviations.

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Table 1. Baseline patient characteristics shown by primary endpoint.

	Mod-Sev AKI	No AKI	P-value
All patients	35	340	
Male	23 (66%)	219 (64%)	>0.99
Age (years)*	67 (13)	64 (14)	0.27
Body Mass Index (kg/m²)†	30 (25-36)	27 (24-31)	0.02
Race			0.11
Black	1 (3%)	47 (14%)	
White	33 (94%)	273 (80%)	
Other/Unknown	1 (3%)	20 (6%)	
Medical History			
Chronic Kidney Disease	3 (9%)	37 (11%)	>0.99
Diabetes Mellitus	13 (37%)	88 (26%)	0.16
Congestive Heart Failure	7 (20%)	54 (16%)	0.48
Coronary Artery Disease	12 (34%)	133 (39%)	0.72
Hypertension	22 (63%)	224 (66%)	0.71
Chronic Obstructive Pulmonary Disease	8 (23%)	68 (20%)	0.66
Cancer	7 (20%)	96 (28%)	0.43
Liver Disease	7 (20%)	11 (3%)	<0.001
Acute Exposures and Susceptibilities			
Sepsis	4 (11%)	40 (12%)	>0.999
Radiocontrast Agents	5 (14%)	109 (32%)	0.03
Nephrotoxic Drugs	32 (91%)	263 (77%)	0.05
Hematocrit < 30%	18 (51%)	201 (59%)	0.47
Non-Renal Apache III†	70 (58-99)	55 (41-75)	0.001
Surgical Subgroups			
Cardiothoracic Surgery	14 (40%)	146 (43%)	0.86
Emergent Surgery	10 (29%)	91 (27%)	0.84
Enrollment Serum Creatinine (mg/dL)†,‡	1.3 (0.9-1.7)	0.9 (0.7-1.2)	<0.001

*Average (standard deviation)

†Median (interquartile range)

‡Hospital value taken closest to the time of enrollment.

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Table 2. IDI and cfNRI for addition of [TIMP-2]•[IGFBP7] to the clinical model

Statistic	Value (95% CI)	p (1-sided)
IDI	0.15 (0.09-0.21)	<0.001
IDI_event	0.14 (0.08-0.20)	<0.001
IDI_non_event	0.014 (0.004-0.025)	0.008
cfNRI	0.95 (0.59-1.30)	<0.001
cfNRI_event	0.52 (0.17-0.86)	0.003
cfNRI_non_event	0.43 (0.32-0.54)	<0.001
AUC_reference_model	0.77 (0.69-0.86)	<0.001
AUC_new_model	0.88 (0.83-0.94)	<0.001
AUC_difference	0.11 (0.03-0.19)	0.008

IDI, cfNRI, and AUC were calculated based on the reference clinical model and new risk model (addition of [TIMP-2]•[IGFBP7]) shown in Figure 3. Event = KDIGO 2 or 3 AKI within 12h or clinical adjudication for Topaz (based on KDIGO stage 2-3 AKI).

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Figure 1. Study design and patients

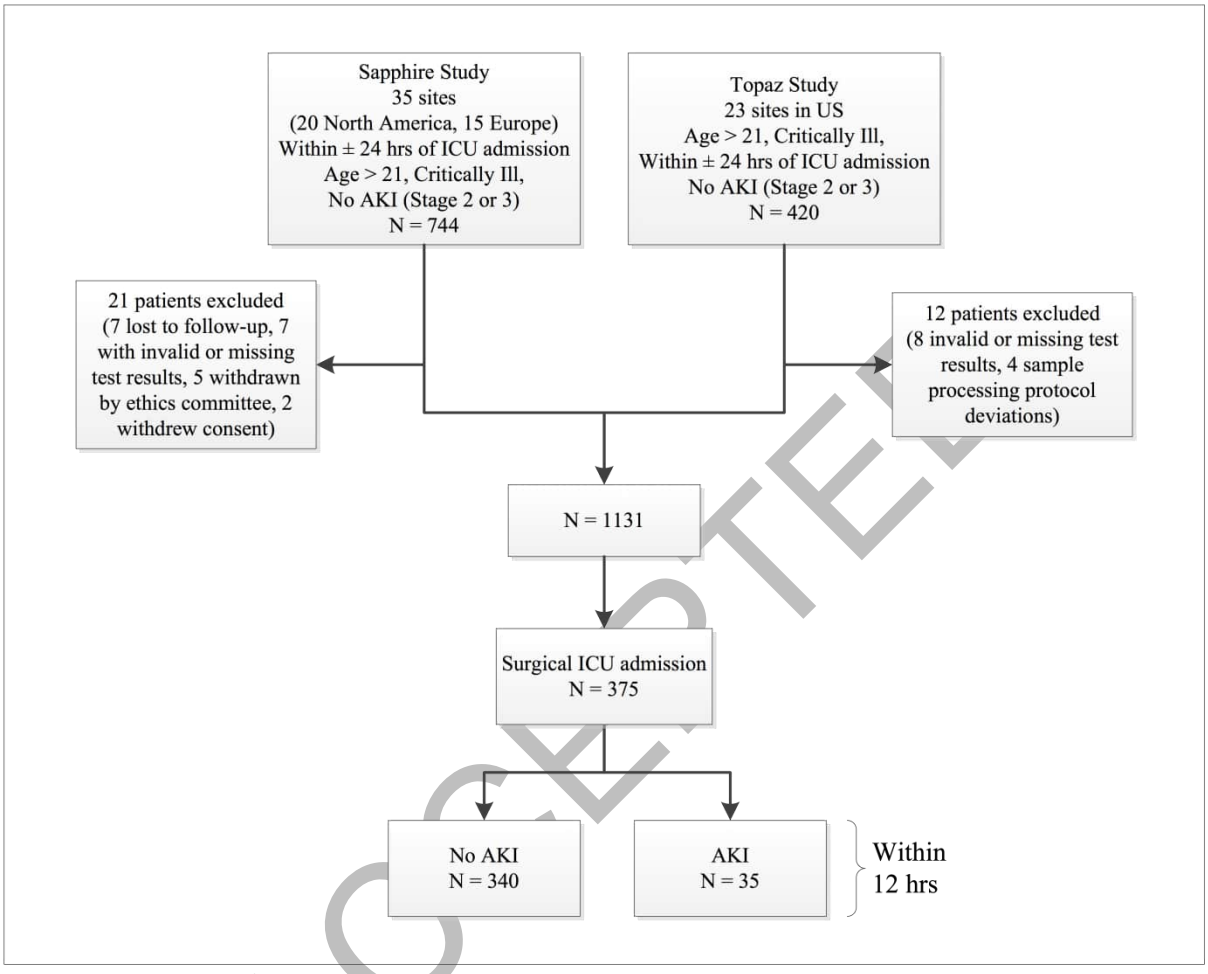

Figure 1. Study design and number of patients in cohorts. AKI was defined as KDIGO AKI stage 2 or 3 for Sapphire and was determined by clinical adjudication for Topaz (based on KDIGO stage 2-3 AKI).

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Figure 2. TIMP2•IGFBP7 biomarker performance in surgical subsets.

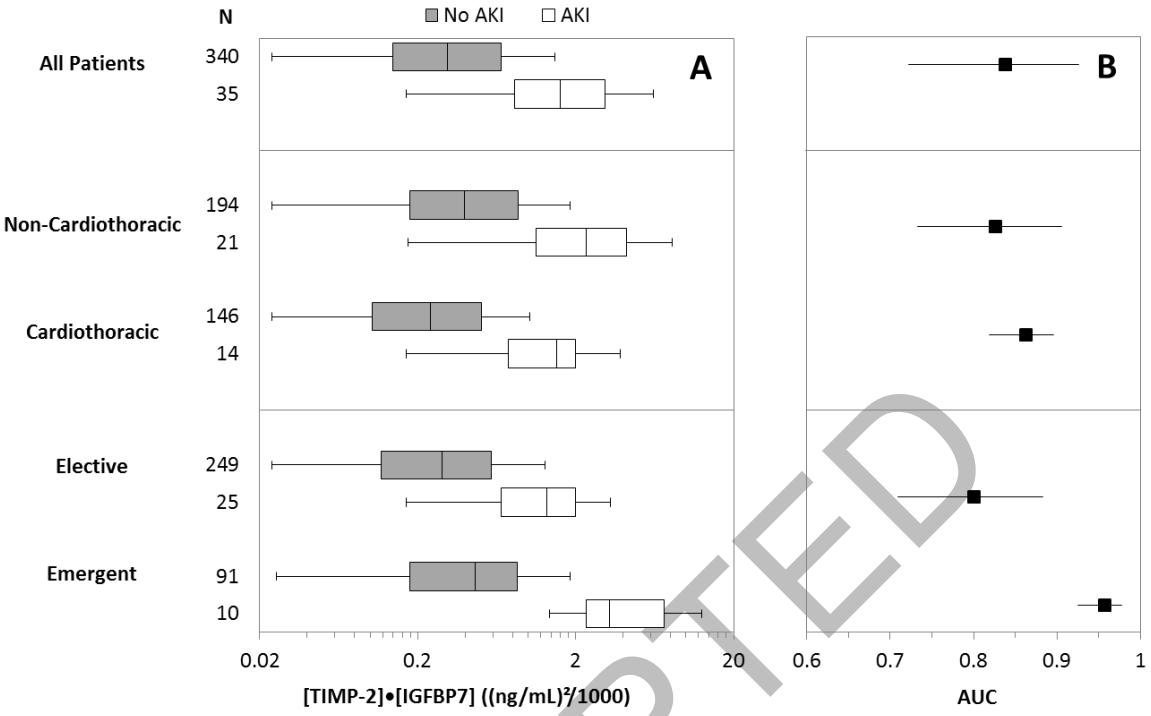

Figure 2. TIMP2•IGFBP7 box plots and area under the ROC curve (AUC-ROC). (A) TIMP2•IGFBP7 levels by surgical subgroup and AKI status within 12 hours, and (B) AUC by surgical subgroup. Two mutually exclusive subgroup pairs are shown: Non-Cardiothoracic vs Cardiothoracic and Elective vs Emergent. Boxes and whiskers show interquartile ranges and total observed ranges, censored at 1.5 times the interquartile ranges. Patients with AKI had significantly higher levels of TIMP2•IGFBP7 than patients without AKI for all surgical patients and within each subgroup shown (Wilcoxon rank sum test adjusted $P < 0.001$ in all cases). All AUC values were approximately 0.8 or greater and significantly greater than 0.5 ($p<0.001$).

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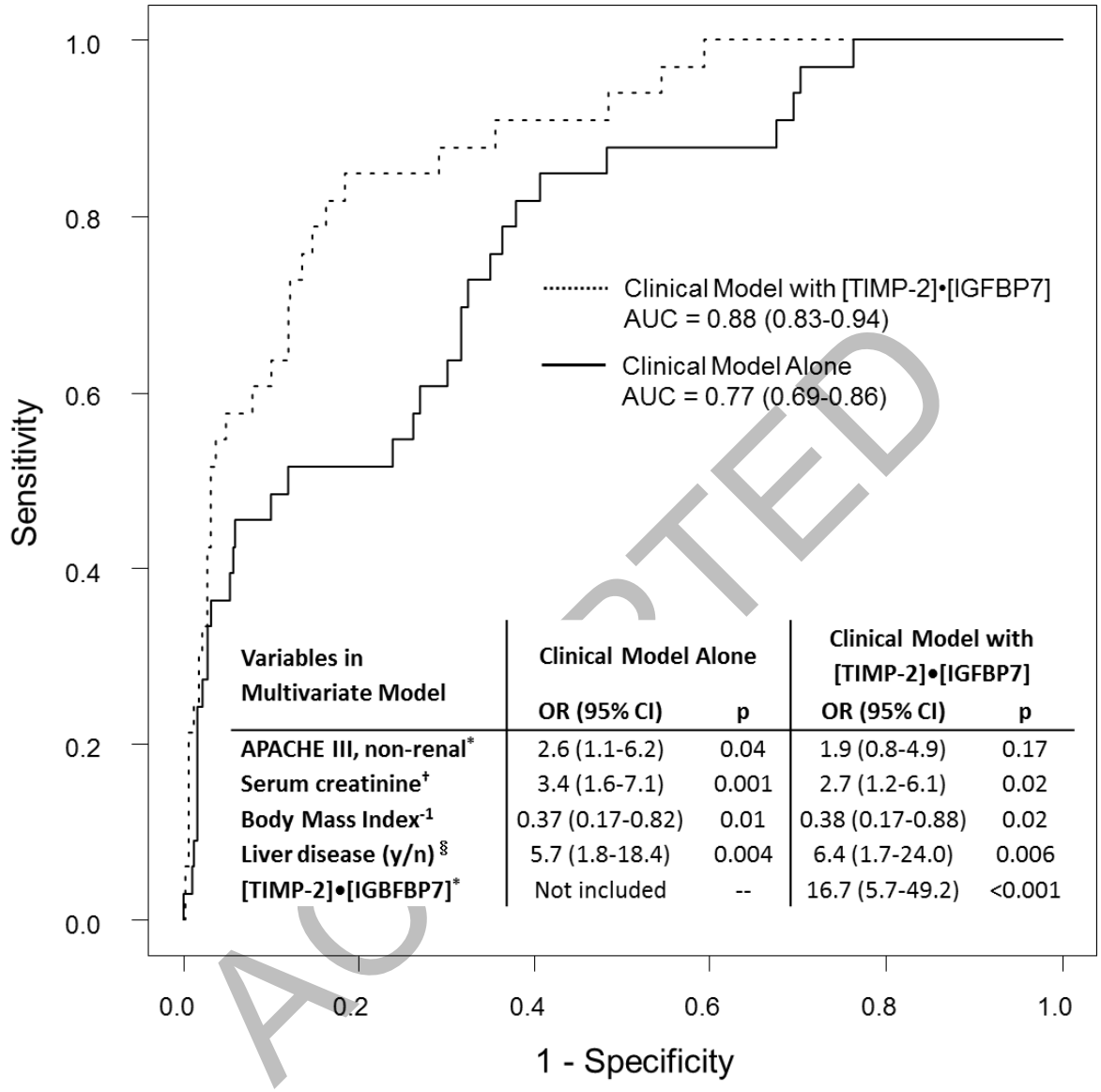
Figure 3. Receiver operating characteristic curve for clinical model alone and with TIMP2•IGFBP7 and odds ratios for model variables



Variables in Multivariate Model	Clinical Model Alone		Clinical Model with [TIMP-2]•[IGFBP7]	
	OR (95% CI)	p	OR (95% CI)	p
APACHE III, non-renal*	2.6 (1.1-6.2)	0.04	1.9 (0.8-4.9)	0.17
Serum creatinine†	3.4 (1.6-7.1)	0.001	2.7 (1.2-6.1)	0.02
Body Mass Index-1	0.37 (0.17-0.82)	0.01	0.38 (0.17-0.88)	0.02
Liver disease (y/n) §	5.7 (1.8-18.4)	0.004	6.4 (1.7-24.0)	0.006
[TIMP-2]•[IGBFBP7]*	Not included	--	16.7 (5.7-49.2)	<0.001

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