Biomarkers Predict Progression of Acute Kidney Injury after Cardiac Surgery

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ABSTRACT

Being able to predict whether AKI will progress could improve monitoring and care, guide patient counseling, and assist with enrollment into trials of AKI treatment. Using samples from the Translational Research Investigating Biomarker Endpoints in AKI study (TRIBE-AKI), we evaluated whether kidney injury biomarkers measured at the time of first clinical diagnosis of early AKI after cardiac surgery can forecast AKI severity. Biomarkers included urinary IL-18, urinary albumin to creatinine ratio (ACR), and urinary and plasma neutrophil gelatinase-associated lipocalin (NGAL); each measurement was on the day of AKI diagnosis in 380 patients who developed at least AKI Network (AKIN) stage 1 AKI. The primary end point (progression of AKI defined by worsening AKIN stage) occurred in 45 (11.8%) patients. Using multivariable logistic regression, we determined the risk of AKI progression. After adjustment for clinical predictors, compared with biomarker values in the lowest two quintiles, the highest quintiles of three biomarkers remained associated with AKI progression: IL-18 (odds ratio=3.0, 95% confidence interval=1.3–7.3), ACR (odds ratio=3.4, 95% confidence interval=1.3–9.1), and plasma NGAL (odds ratio=7.7, 95% confidence interval=2.6–22.5). Each biomarker improved risk classification compared with the clinical model alone, with plasma NGAL performing the best (category-free net reclassification improvement of 0.69, $P<0.0001$). In conclusion, biomarkers measured on the day of AKI diagnosis improve risk stratification and identify patients at higher risk for progression of AKI and worse patient outcomes.

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AKI is a common and serious complication of cardiac surgery that has been shown to correlate with all manner of adverse patient outcomes.[1,2] Recently, standardized clinical definitions of AKI have been implemented through the validation of the Risk Injury Failure Loss End Stage (RIFLE) and Acute Kidney Injury Network (AKIN) criteria.[3,4] These definitions have aided in ascertainment of the true incidence of AKI after cardiac surgery and shown that AKI, regardless of severity or stage, is associated with longer length of intensive care unit (ICU) and hospital stay, increased cost of hospitalization, and increased patient mortality.[5,6]

In recent years, several studies have shown that novel biomarkers can detect acute tubular injury earlier than serum creatinine in the setting of AKI.[7–11] Despite attempts to select patients at high risk for severe AKI, the majority of patients in these prospective studies do not develop clinical AKI. Additionally, most of those patients who develop AKI experience a milder form of AKI (*e.g.*, AKIN stage 1 or RIFLE R) with transient bumps in serum creatinine and do not

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progress to more severe AKI stages (AKIN stage 2 or 3 or RIFLE F) or require acute dialysis.

Although several risk stratification scoring systems have been constructed to predict who will develop AKI before surgery, few tools exist for assessing the risk for progression of AKI when early AKI is evident in the postoperative setting. Diagnostic mainstays such as the fractional excretion of sodium and urea have been repeatedly shown to be suboptimal in a variety of clinical settings, including cardiac surgery.[10,12–15] Similarly, in the specific setting of cardiac surgery, the use of urinalysis with microscopy has not been found to be effective.[16] Currently, there are no clinical tools available at the time of early clinical AKI diagnosis (*e.g.*, ≥50% or ≥0.3 mg/dl increase in serum creatinine) to predict the patients who will progress to more severe AKI after cardiac surgery.

Assessment of biomarkers of structural renal tubular injury (*e.g.*, neutrophil gelatinase-associated lipocalin [NGAL] and IL-18) at the time of creatinine-based diagnosis of AKI may offer prognostic information that may improve monitoring and care, guide patient counseling, and assist in the identification of patients for trials of AKI treatment. We have recently shown that urine IL-18 and plasma NGAL peak within 6 hours of adult cardiac surgery and forecast the future development of AKI in the early postoperative period.[17] Additionally, at this early time point, these biomarkers were associated with longer length of in-hospital and ICU stay and higher risk of dialysis or death. The current study serves to expand on these data by investigating the role of biomarkers at the time of creatinine-based AKI diagnosis.

We used blood and urine from the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE AKI) study, a large prospective, multicenter international cohort of adult patients undergoing cardiac surgery, to evaluate the prognostic utility of traditional and novel plasma and urinary biomarkers measured on the morning that creatinine-based AKI was detected. The four biomarkers that we examined were urinary IL-18, urinary albumin to creatinine ratio (ACR), and plasma and urine NGAL.

RESULTS

Among 1219 adults who underwent cardiac surgery, 426 (34.9%) patients developed AKI after cardiac surgery[4]; 380 of 426 (89.2%) had biomarkers measured from the blood and urine collected on the first day of AKI diagnosis. Among the patients with AKI, 45 patients (11.8% of AKI cases) progressed to a higher severity AKI during their postoperative hospital stay; 15 (33.3%) of these 45 progressors received acute dialysis. Three hundred twenty-nine patients (86.5% of those patients with AKI) persisted in stage 1 AKI, and six patients (1.6%) were diagnosed and persisted in stage 2 AKI; 23 individuals (6.1%) progressed from stage 1 to stage 2, 17 individuals (4.5%) progressed from stage 1 to stage 3, and 5 individuals (1.3%) progressed from stage 2 to stage 3. Figure 1 displays the postoperative days when AKI was first diagnosed for both progressors and nonprogressors; 66% of patients developed AKI within the first 2 postoperative days, and 93.4% of patients developed AKI by day 3.

Table 1 describes the pre- and intraoperative clinical characteristics of patients with AKI that progressed compared with those patients with AKI that did not progress. AKI progressors had more nonelective surgeries, higher use of intraoperative, intra-aortic balloon pumps, and significantly longer cardiopulmonary bypass and aortic cross-clamp times. There was no statistical difference in Thakar or Mehta preoperative AKI risk assessment scores between those patients with and without AKI progression.[18,19]

Table 2 describes the postoperative time of creatinine-based AKI and outcomes of patients in which AKI progressed compared with nonprogressors. There was no statistically significant difference in the proportion of those patients with decreased urine output on the first day of AKI. Diuretic use, on the day before or day of AKI, did not differ between progressors and nonprogressors, despite progressors having a higher percent change in serum creatinine from baseline at the time of clinical diagnosis (Table 2). Clinical outcomes were much worse for AKI progressors; they had longer ICU and hospital stays, and they had higher inpatient mortality (Table 2).

Traditional Biomarkers of Renal Function Patterns and Performance

Mean serum creatinine (mg/dl [SD]) at the time of creatinine-based AKI diagnosis trended to be higher in those patients

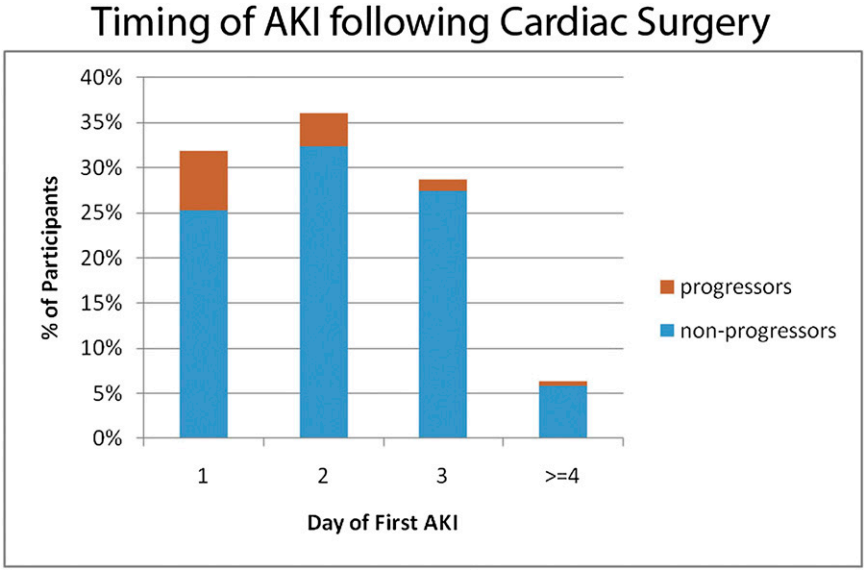

Figure 1. Timing of AKI after cardiac surgery. The graph displays when those patients with and without AKI progression were first diagnosed with AKI after cardiac surgery; 66% of patients developed AKI within the first 2 postoperative days.

Table 1. Clinical characteristics in patients with and without AKI progression

	AKI Progressed		
	No (*n*=335)	Yes (*n*=45)	*P* Value
Demographics			
age, years	72.3 (9.4)	71.3 (9.9)	0.39
female	93 (28%)	13 (29%)	0.87
white race	314 (94%)	41 (91%)	0.52
patients with diabetes	144 (43%)	24 (53%)	0.19
congestive heart failure	103 (31%)	20 (44%)	0.07
ejection fraction, mean percent (SD)	49.6 (13.7)	50.5 (10.6)	0.69
preoperative creatinine, mg/dl[a]	1.14 (0.34)	1.26 (0.45)	0.12
preoperative eGFR (CKD-EPI) ml/min	64.3 (18.9)	60.7 (22.7)	0.22
<30	12 (3.6%)	4 (8.9%)	
30–60	126 (38%)	17 (38%)	
60–90	171 (51%)	19 (42%)	
>90	26 (7.8%)	5 (11%)	
preoperative medications			
β-blockers	251 (75%)	34 (77%)	0.78
ACE inhibitors/ARB	231 (69%)	30 (67%)	0.76
aspirin	245 (74%)	36 (82%)	0.24
statins	250 (75%)	28 (64%)	0.11
Preoperative biomarkers			
urine albumin to creatinine ratio (mg/g)	23.2 (10.3, 73.8)	29.6 (11.7, 69.5)	0.66
urine NGAL (ng/ml)	10.1 (5.1, 21.6)	9.8 (6.2, 17.4)	0.78
urine IL-18 (pg/ml)	14.4 (6.9, 31.4)	18.8 (9.2, 32.7)	0.66
plasma NGAL (ng/ml)	64.3 (60.0, 106.1)	67.9 (60.0, 125.1)	0.51
Operative factors			
status of the procedure			0.06
elective	246 (73%)	27 (60%)	
urgent or emergent	89 (27%)	18 (40%)	
Cardiac catheterization in the last 72 hours	31 (9.4%)	4 (8.9%)	0.91
surgery type			0.17
CABG	158 (47%)	17 (38%)	
valve	94 (28%)	11 (24%)	
CABG and valve	83 (25%)	17 (38%)	
IABP	18 (5.4%)	6 (13%)	0.051
repeat cardiac surgery	77 (24%)	8 (18%)	0.38
off CPB surgery	22 (6.6%)	4 (8.9%)	0.57
CPB time (min)[b]	120.9 (58.1)	165.2 (93.3)	<0.001
aortic cross-clamp time (min)[b]	82.4 (44.6)	112.5 (64.1)	<0.001

Mean (SD), median (interquartile range), or number (percent). AKI progression defined by worsening of AKIN stage from original diagnosis of AKI. eGFR, estimated GFR; CKD-EPI, CKD epidemiology collaboration equation; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; CABG, coronary artery bypass grafting; IABP, intra-aortic balloon pump; CPB, cardiopulmonary bypass.
[a]To convert serum creatinine values (to μmol/L), multiply by 88.4.
[b]Perfusion time is reported for the patients who had CPB and cross-clamping.

who progressed (1.9 [0.6]) compared with nonprogressors (1.6 [0.4], *P*=0.08). The highest quintile of serum creatinine had a significantly higher risk of progression in the unadjusted model; this effect was attenuated in the adjusted model (Table 3). There was no difference between the area under the curve (SE) for serum creatinine in the adjusted model and the clinical model alone (0.75 [0.04]). The clinical model consisted of variables from the Society of Thoracic Surgeons risk prediction model for AKI along with the postoperative change in serum creatinine.

There was no statistical difference in the preoperative ACR concentrations between those patients with and without progressive AKI (Table 1). Mean urinary ACR (mg/g [SD]) at the time of creatinine-based diagnosis of AKI was higher in those patients who progressed (314 [831]) compared with those patients who did not (104 [281], *P*=0.0003). There was a graded response across the quintiles that persisted after adjusting for clinical variables (Table 3). There was no difference in the percentage of progressors (15.6%, *n*=7) and nonprogressors (17.6%, *n*=59) who had preoperative ACR values above the cutoff for the fifth quintile of proteinuria (>133 mg/g) at the time of creatinine-based diagnosis of AKI (*P*=0.70).

Novel Biomarkers of Renal Injury

Median urinary IL-18 (pg/ml [interquartile range]) at the time of creatinine-based diagnosis of AKI was 40.7 (12.6–124.8) in nonprogressors and 104.3 (23.4–269.0) in progressors (*P*=0.006). There was a dose-response relationship with biomarker categories in adjusted and unadjusted analyses; those patients in the highest quintile (IL-18>185 pg/ml) had threefold odds of progression (adjusted) compared with the lowest quintiles (IL-18=0–29.6 pg/ml) (Table 3).

Median urinary NGAL (ng/ml [interqartile range]) at the time of creatinine-based AKI diagnosis was 28.3 (13.5–82.9) in nonprogressors and 72.1 (11.4–495.6) in progressors (*P*=0.07). The highest quintile of urinary NGAL had a significantly higher risk of progression in the unadjusted analysis; however, there was a U-shaped relationship across risk groups that was attenuated after adjusting for clinical factors (Table 3).

Plasma NGAL (ng/ml) had the strongest ability to predict the progression of AKI; the fifth quintile detected most progressors (*n*=24 of 71; 34%). The median plasma NGAL (ng/ml [interquartile range]) in nonprogressors was 176.8 (110.3–259.8) compared with 328.0 (203.6–384.1) among progressors (*P*<0.0001). Those patients in the fifth quintile (plasma NGAL [ng/ml] >322) had an 11-fold odds of progression in the unadjusted analysis that was slightly attenuated (7.7-fold odds) in an adjusted multivariate analysis.

Supplemental Table 1 provides data with respect to the absolute and relative changes in serum creatinine and novel biomarker values compared with baseline at the time of creatinine-based AKI diagnosis. There were no significant differences between the performance of the raw biomarker values and the absolute

and relative changes in biomarkers values presented in Supplemental Table 1.

Given the potential for colinearity between some of the biomarkers, the interbiomarker correlation was assessed (Supplemental Table 2). Briefly, urinary biomarkers tended to correlate better with urinary biomarkers, and serum creatinine correlated with plasma NGAL. Finally, the ability of biomarker combinations, with and without the clinical model, to forecast AKI progression was assessed; the combination of urine and plasma NGAL provided the highest area under the curve for the unadjusted and adjusted analyses (0.79 and 0.83, respectively) (Supplemental Table 3, A and B). Supplemental Table 4 provides data about the performance of biomarkers at the time of creatinine-based diagnosis of AKI for predicting the future receipt of renal replacement therapy.

Table 2. Postoperative characteristics and outcomes in patients with and without AKI progression

	AKI Progressed		
	No (*n*=335)	Yes (*n*=45)	*P* Value
Time of AKI			
serum creatinine[a]	1.6 (0.4)	1.9 (0.6)	0.08
percent change in serum creatinine day of AKI	39.5 (18.0)	54.7 (34.7)	0.01
oliguria[b] on day of AKI	13 (4%)	4 (9%)	0.25
Diuretic use			
day before the day of AKI	85 (35%)	6 (30%)	0.63
day of AKI	135 (41%)	15 (33%)	0.35
Outcomes			
repeat cardiac surgery during hospitalization	22 (6.6%)	10 (22%)	0.0018
received RRT	0 (0%)	15(33%)	<0.0001
length of ICU stay, days	3.8 (8.0)	15.7 (28.8)	<0.0001
length of hospital stay, days	9.5 (10.1)	22.9 (32.2)	<0.0001
in-hospital mortality	7 (2%)	10 (22%)	<0.0001

Mean (SD) or number (percent). AKI progression defined by worsening of AKIN stage from original diagnosis of AKI. RRT, renal replacement therapy.
[a]To convert serum creatinine values (to μmol/L), multiply by 88.4.
[b]Oliguria defined as a patient who had <500 ml in 24 hours.

Risk Classification—Category-Free Net Reclassification Improvement and the Integrated Discrimination Improvement

To evaluate the improvement of the risk prediction with the addition of biomarkers to the clinical model, we determined the category-free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) indices. All biomarkers, traditional and novel, improved risk classification over the clinical model, with plasma NGAL displaying the largest category-free NRI of

Table 3. Progression of AKI biomarker quintiles with outcome

Biomarker on AKI Day and Category	Cut Points	*N*	AKI Progression (%)	*P* for Trend	Unadjusted OR (95% CI)	Unadjusted AUC (SE)	Adjusted OR (95% CI)[a]	Adjusted AUC (SE)
Serum creatinine								
low (Q1 and Q2)	0.8–1.4	151	7	0.0007	1 (referent)	0.67 (0.04)	1 (referent)	0.75 (0.04)
medium (Q3 and Q4)	1.41–1.9	153	10		1.49 (0.67, 3.32)		1.9 (0.7, 5.18)	
high (Q5)	1.91–3.3	76	24		3.95 (1.76, 8.88)		3.55 (0.88, 14.29)	
Urine albumin to creatinine ratio								
low (Q1 and Q2)	0.00–35.0	148	5	0.0003	1 (referent)	0.67 (0.04)	1 (referent)	0.78 (0.04)[b]
medium (Q3 and Q4)	35.0–132.0	148	14		2.73 (1.16, 6.42)		2.41 (0.97, 5.94)	
high (Q5)	>133.0	74	22		4.83 (1.96, 11.9)		3.41 (1.27, 9.11)	
Urine NGAL								
low (Q1 and Q2)	0.82–20.7	150	12	0.056	1 (referent)	0.58 (0.06)	1 (referent)	0.79 (0.04)[b]
medium (Q3 and Q4)	20.8–134	151	6		0.46 (0.2, 1.07)		0.37 (0.15, 0.93)	
high (Q5)	>141	75	24		2.32 (1.12, 4.77)		2.02 (0.86, 4.73)	
Urine IL-18								
low (Q1 and Q2)	0.1–29.6	150	8	0.002	1 (referent)	0.63 (0.05)	1 (referent)	0.77 (0.04)[b]
medium (Q3 and Q4)	29.8–185	151	10		1.27 (0.57, 2.81)		1.06 (0.44, 2.55)	
high (Q5)	>185	75	24		3.63 (1.64, 8.03)		3.00 (1.25, 7.25)	
Plasma NGAL								
low (Q1 and Q2)	60–164.5	143	4	<0.0001	1 (referent)	0.74 (0.04)	1 (referent)	0.80 (0.04)[b]
medium (Q3 and Q4)	164.6–322.6	144	10		2.46 (0.92, 6.59)		2.53 (0.89, 7.18)	
high (Q5)	>322	71	34		11.66 (4.49, 30.27)		7.72 (2.65, 22.49)	

AKI progression is defined as worsening of AKIN stage (stage 1 to either stage 2 or 3 or from stage 2 to 3). Site is adjusted as a random effect. OR, odds ratio; CI, confidence interval; AUC, area under the curve; Q, quintile.
[a]Adjusted for age, sex, white race, CPB time>120 minutes, nonelective surgery, preoperative eGFR (<30, 30–60, and >60), diabetes, hypertension, intraoperative IABP, repeat cardiac surgery during the hospitalization, and percent change in postoperative serum creatinine from baseline at the time of AKI diagnosis.
[b]$P<0.05$ compared with the clinical model alone (clinical model AUC=0.75).

0.69 ($P<0.0001$) and IDI of 0.06 ($P<0.0001$) (Table 4). ACR also significantly improved the risk stratification for AKI progression with a category-free NRI of 0.42 ($P=0.008$). Figure 2 shows the change in risk stratification, among progressors and nonprogressors, that occurred with the addition of the individual biomarkers to the clinical model.

DISCUSSION

In this analysis of a prospective multicenter observational cohort study of adults undergoing cardiac surgery, we found that urine and plasma biomarkers at the time of creatinine-based diagnosis of AKI forecasted the progression to more severe AKI. Among the urinary biomarkers, ACR>133 mg/g at the time of AKI denoted a 3.4-fold odds of AKI progression compared with an ACR<35 mg/g, and urine IL-18 levels>185 showed a threefold risk of progression compared with lower levels in adjusted analysis. Urine NGAL levels were not significant after adjustment of clinical variables. A level of plasma NGAL of >323 ng/ml performed the best, conveying an over sevenfold risk of AKI progression compared with patients in the lowest two quintiles after controlling for clinical factors, including postoperative change in serum creatinine. All four biomarkers and serum creatinine improved the prediction for the diagnosis of AKI progression, as seen by the category-free NRI, when added to the clinical model.

These results augment the findings from our previous publication that showed a role for these same biomarkers in the diagnosis of early AKI.[17] Biomarkers measured in the first 6 postoperative hours were found to forecast the future rise in serum creatinine as well as adverse patient outcomes (increased length of ICU and hospital stay and mortality). In that study, the highest quintiles of urine IL-18 and plasma NGAL were strongly associated with a postoperative doubling of serum creatinine or the receipt of acute dialysis (6.8- and 5-fold adjusted odds, respectively). The current analyses show that serial daily monitoring of these same biomarkers as well as ACR may be equally beneficial in that they provide additional information with regards to the risk of AKI progression and other adverse clinical outcomes during the critical first few postoperative days. In our cohort, 95% of all cases of AKI were diagnosed within the first 72 postoperative hours, and the majority of those patients with progressive AKI declared themselves within these same 3 days. Thus, future investigation seeking to use adult cardiac surgery as a model for AKI should focus their efforts/recruitment on these first few critical days. These investigations should systematically pair novel biomarker measures with serum creatinine with sample collection every 24 hours to maximize renal event capture.

We showed that the ACR has use as a tool in determining AKI progression. Albuminuria, which was not different preoperatively between progressors and nonprogressors, is increasingly being recognized as an important risk factor for the development of AKI in a variety of clinical settings.[20–22] Recently, the work by Huang *et al.*[20] showed that, before coronary artery bypass grafting, the presence of mild and heavy proteinuria (defined by urinary dipstick) was associated with an increased odds of both developing postoperative AKI and requiring postoperative renal replacement therapy. Their study did not attempt to quantify the urinary proteinuria beyond dipstick or comment on the role of postoperative proteinuria in forecasting adverse patient outcomes. Several other studies have delineated that the presence of baseline proteinuria is associated with increased risk for the future development of AKI[21,23–25]; however, to our knowledge, our work represents the first documentation of a quantified postoperative protein concentration predicting the severity of AKI. This information elevates the use of ACR in the setting of AKI and also supports the role of proteinuria as a biomarker of AKI after cardiac surgery.[20,22]

Much of the previously published novel biomarker literature has focused on the ability of these new tests to predict the development of AKI earlier than serum creatinine.[7–10] However, in a recent pooled analysis of several prospective observational cohort studies, elevations of urine and plasma NGAL were shown to predispose patients for the need for renal replacement therapy, longer hospital stays, and increased mortality, even in the absence of a significant elevation in serum creatinine.[26] This ability to augment the diagnosis of AKI, beyond the constraints of serum creatinine, and forecast adverse patient outcomes represents an important paradigm shift. NGAL and other biomarkers of structural kidney injury may be viewed as tests that add value to sole assessment of kidney function by serum creatinine in the setting of AKI rather than replace it. Our data show and validate another example of this changing application of biomarkers; we found that, even after correction for clinical factors

Table 4. Net reclassification improvement and integrated discrimination improvement of including biomarkers in the risk factor model to predict AKI progression

	Category-Free NRI (SE), *P* Value	NRI in Progressors (SE)	NRI in Nonprogressors (SE)	IDI (SE), *P* Value
Serum creatinine	0.172 (0.159), 0.28	0.022 (0.149), 0.88	0.150 (0.055), 0.006	0.012 (0.008), 0.16
Urine ACR	0.423 (0.161), 0.008	0.500 (0.151), <0.0001	−0.077 (0.055), 0.17	0.021 (0.011), 0.06
Urine NGAL	0.541 (0.159), 0.0007	0.511 (0.149), 0.0006	0.030 (0.055), 0.58	0.042 (0.015), 0.004
Urine IL-18	0.442 (0.159), 0.005	−0.200 (0.149), 0.18	0.642 (0.055), <0.0001	0.019 (0.011), 0.09
Plasma NGAL	0.694 (0.161), <0.0001	0.455 (0.151), 0.003	0.240 (0.057), <0.0001	0.06 (0.016), <0.001

Clinical model contains age, sex, white race, CPB time>120 minutes, nonelective surgery, preoperative eGFR, diabetes, hypertension, intraoperative IABP, repeat cardiac surgery during the hospitalization, and percent change in postoperative serum creatinine.

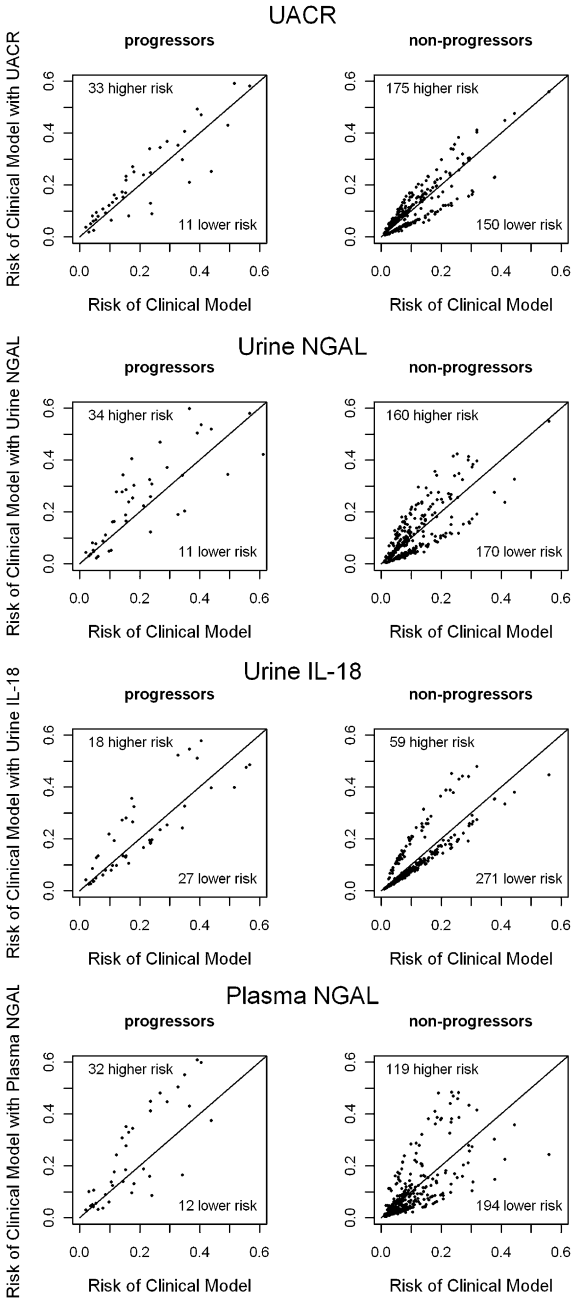

Figure 2. Improvements in predicted risk with the inclusion of biomarkers in the clinical model. The plots display the changes in risk stratification for (left) progressors and (right) nonprogressors with the addition of individual biomarkers urinary ACR (UACR), urine NGAL, urine IL-18, and plasma NGAL to the clinical model. The *x* axis for all plots is the predicted risk according to the clinical model alone, and the *y* axis is the clinical model plus the given biomarker. The diagonal line indicates the line of identity; for the points above this line, the predicted risk is higher in the new model, and for points below this line, the predicted risk is lower. The individual plots display the number of patients who were at increased and decreased risk of progressive AKI after the addition of a biomarker to the model. As an example, inclusion of plasma NGAL in the model led to increased predicted risk in 28 of 45 progressors (62%) and decreased predicted risk in 216 of 335 nonprogressors (66.4%). The clinical model contains age, sex, white race, CPB time>120 minutes, nonelective surgery, preoperative estimated GFR, diabetes, hypertension, intraoperative intra-aortic balloon pump, repeat cardiac surgery during the hospitalization, and percent change in postoperative serum creatinine.

and change in serum creatinine, plasma NGAL, urine IL-18, and ACR at the time of creatinine-based diagnosis of AKI can help predict the progression of AKI. This ability to distinguish those patients at highest risk for the worst patient outcomes will be crucial as nephrologists and intensivists conduct more clinical trials for the treatment of AKI and its most severe form of acute tubular necrosis.[27] The incidence of AKI after cardiac surgery is high (>30%)[10,28]; however, as in our study, the vast majority of those patients with AKI do not progress, and they improve within 48–72 hours. Thus, the number of patients needed to show a positive result in a randomized controlled trial for AKI would be very large, because the majority of patients in the placebo and treatment group will spontaneously resolve. However, plasma and urine biomarkers will allow future clinical trials of AKI interventions to be targeted to those patients at highest risk of progression, which would be far more efficient in terms of costs and resources. Focusing future trials and interventions on those patients with the most severe forms of AKI should not undermine the increased risk of post-AKI CKD, morbidity, and mortality experienced by those patients with AKIN stage 1-limited AKI compared with those patients without AKI.

We evaluated the ability of several biomarkers to improve risk stratification for worsening of AKI. The category-free NRI values for all four biomarkers and serum creatinine show that each assay has the ability to improve on the risk assessment provided by the clinical model alone. This effect is also shown by the statistically significant increase in the adjusted area under the curve for all four biomarkers compared with the clinical model alone (area under the curve=0.75) (Table 3). The NRI and IDI represent modern, more sensitive statistical methods to quantify model improvement of the addition of a biomarker (or new variable) to an existing clinical model. The popular method of examining the increase in the area under the curve is problematic, because it is difficult to interpret the clinical significance of a small change in area under the curve (*i.e.*, 0.05 in plasma NGAL), and the biomarker (or new variable) must have exceptional discriminatory ability to increase the area under the curve after it reaches a certain level.[29] The NRI does not directly depend on the performance of the baseline model, and by definition, it considers an improvement in reclassification as any increase in model-based predicted probabilities after the addition of the biomarker for events (AKI progression) and a decrease in probabilities for nonevents. Similarly, a worsening in reclassification is defined by a decrease in probabilities for events and an increase in probabilities for nonevents.

According to the work by Pencina *et al.*,[29] medium effect sizes have an NRI of 0.4–0.6 (urine NGAL, IL-18, and ACR), and large effect sizes have an NRI greater than 0.6 (plasma NGAL). The IDI can be defined as the difference in discrimination slopes between the biomarker-adjusted and -unadjusted models, with large effect sizes having an IDI roughly greater than or equal to 0.10 (none of our biomarkers) and medium effect sizes having values around 0.05 (plasma NGAL).[29] Our data suggest that ACR improves the NRI of progressors, whereas IL-18 predominantly improves stratification for the nonprogressors. Plasma NGAL showed the unique ability to improve the NRI for both progressors and nonprogressors; thus, if validated in future studies, plasma NGAL may serve as an ideal biomarker to help select subjects for future AKI trials. All of these biomarkers show promise in that they have both an elevated NRI (>0.40) as well as a statistically increased area under the curve compared with the clinical model alone.

After adjustment for clinical variables, urinary IL-18 showed promise in its ability to predict the worsening of AKI at the time of creatinine-based diagnosis of AKI. IL-18 is specific to the renal tubules and is known to be up-regulated in response to ischemia reperfusion injury.[30] However, initial creatinine elevations after ischemic injury often do not occur until 24–48 hours after an insult (68.4% of our cohort developed AKI on or after day 2).[31] Thus, creatinine kinetics supply ample time for urinary IL-18 concentrations to dissipate, and this time lag may explain why other markers displayed more robust results. Despite this limitation, the area under the curve for predicting progression of AKI using urinary IL-18 (0.79) was statistically greater compared with the clinical model alone (0.75) and was associated with a threefold increase in risk of worsening AKI. These data, along with its ability to predict a sixfold increased risk for a postoperative doubling of serum creatinine or receipt of acute dialysis in the early postoperative period,[17] support urine IL-18's potential role in a biomarker panel to predict severity of AKI at a variety of time points after adult cardiac surgery. Despite prior reports showing that urine NGAL concentrations may predict progression through the RIFLE or AKIN criteria,[10,32,33] our investigation did not find benefit to urine NGAL measurement after adjusting for variables known to impact AKI risk. It is important to note that these prior publications were not specific to cardiac surgery[32,33] and were limited by small sample sizes[10]; additionally, the aforementioned creatinine kinetics may play a role in our negative urine NGAL findings.

Creatinine kinetics, in part, allow plasma NGAL to outperform the urinary biomarkers. Plasma NGAL is generated outside of the kidney, and owing to the drop in GFR in AKI, it accumulates in the blood over the first several postoperative hours to days. These high concentrations at the delayed clinical time point interrogated in our investigation create the ideal pairing of plasma NGAL and the progression of AKI. Additionally, plasma NGAL did not correlate highly with the urinary biomarkers, making it an ideal partner for biomarker pairings to improve the prediction of progressive AKI (Supplemental Table 3A). This finding is in sharp contrast to several of the urinary biomarkers, which were highly covariant (Supplemental Table 2). The correlation between IL-18 and ACR reached nearly 0.7. Thus, given the similar statistical performance and widespread availability of ACR, as well as the decreased assay cost, the routine measurement of ACR at the time of AKI after cardiac surgery seems clinically appropriate.

Our study has a number of strengths; our data, samples, and measurement were all performed as part of a large prospective, multicenter international investigation. Additionally, we relied on standardized modern AKI staging criteria (AKIN) that are currently employed by the international community and are readily duplicated in follow-up investigations. However, we did not measure traditional urine indices to which biomarker results could be compared, such as fractional excretion of sodium and urea, or microscopy data. However, these clinical tools have been shown to be nondiagnostic in the postcardiac surgery setting, where volume status, fluid responsiveness, and diuretic use confound the relationship between tubular function and injury.[10,12–16]

In summary, urinary IL-18, ACR, and plasma NGAL measurement at the time of clinical creatinine increase (AKI diagnosis) forecasted the progression of AKI in adults after cardiac surgery. Given the strong correlation between urinary biomarkers, routine measurement of ACR and plasma NGAL may identify a subpopulation that is at the highest risk for the most adverse of patient outcomes. Improving risk prediction may improve monitoring and care of postoperative patients, guide patient counseling and decision-making, and facilitate participation in interventional trials of AKI.

CONCISE METHODS

Study Sample

As previously described, we prospectively enrolled 1219 adults undergoing cardiac surgery (coronary artery bypass grafting or valve surgery) who were at high risk for AKI at six academic medical centers in North America between July of 2007 and December of 2009.[17,28] All participants provided written informed consent, and the study was approved by each institution's research ethics board. Only patients who developed at least AKIN stage 1 were included in this analysis; however, those patients whose initial AKI diagnosis was stage 3 (n=3, 0.7% of those patients with AKI) were excluded from the analysis, because they could not progress further.[4] Patients were also excluded if they did not have blood and urine samples collected on the day of initial AKI diagnosis. This clinical study was registered at Clinicaltrials.gov as NCT00774137.

Study Protocol

We collected urine and plasma specimens preoperatively and daily for up to 5 postoperative days. The first postoperative samples were collected soon after admission to the ICU. For the first 24 hours postoperatively, urine samples were collected every 6 hours. The remaining daily blood and urine samples were obtained at the time of routine morning blood collection conducted for clinical care. Specimen collection was stopped on postoperative day 3 in patients

who had no evidence of an increase in serum creatinine. Sample collection and processing have been previously described.[17,28]

AKI Biomarker Measurements

For the purposes of this study, postoperative biomarkers were measured on the initial day that the serum creatinine first crossed the AKIN stage 1 threshold (rise of ≥50% or 0.3 mg/dl from baseline value). Urine albumin was reported as the ratio to urinary creatinine (ACR) and was measured by immunoturbidimetry on a Siemens Dimension Plus with HM clinical analyzer per the manufacturer's instructions. Urine creatinine was measured by the modified Jaffe reaction. Urine IL-18 and NGAL were measured with the ARCHITECT assay (Abbott Diagnostics, Abbott Park, IL). The coefficient of variation for the urine creatinine assay was 5%, whereas the coefficient of variation for the NGAL and IL-18 assays were 5% and 8%, respectively. Plasma NGAL was measured through the Triage NGAL immunoassay in conjunction with the Triage Meter (Biosite Inc., San Diego, CA). The Triage assay has a detection range of 60–1300 ng/ml with a coefficient of variation of 10–15%. Personnel performing the biomarker measurements were blinded to each patient's clinical information. All biomarkers were measured from frozen aliquots that did not undergo any additional freeze–thaw cycles.[34,35] Additional details on repeat biomarker measurements and assay performance and stability have been previously described.[17]

Outcome Definitions

The primary outcome was the progression of AKI, defined by worsening of AKIN stage (from stage 1 to either stage 2 or 3 or from stage 2 to 3). Patients treated with acute dialysis at any point during hospitalization were classified as stage 3. All preoperative creatinine values were measured within 2 months before surgery (median=4 days, mean=8 days). Pre- and postoperative serum creatinine levels were measured in the same clinical laboratory for each patient at all sites. Serum creatinine values were recorded for every patient throughout the hospital stay. Additional clinical outcomes were need for acute dialysis, in-hospital mortality, and length of stay in hospital and ICU.

Variable Definitions

We collected preoperative characteristics, operative details, and postoperative complications using definitions of the Society of Thoracic Surgeons (http://www.ctsnet.org/file/rptDataSpecifications252_1_ForVendorsPGS.pdf). We recorded whether the patient had received cardiac catheterization within 72 hours before surgery. We estimated preoperative GFR using the CKD epidemiology collaboration equation equation.[36]

Statistical Analyses

We categorized into quintiles the values of urine IL-18, NGAL, and ACR and plasma NGAL at the time of AKI clinical diagnosis. Because of the small number of progressors, we combined quintiles 1 and 2 (low risk) and quintiles 3 and 4 (intermediate risk), and we defined quintile 5 as high risk.[37] We assessed unadjusted linear trends by the Cochran–Armitage test for dichotomous outcomes and the Jonckheere–Terpstra test for continuous outcomes. Continuous variables were compared between progressors and nonprogressors with a two-sample *t* test or Wilcoxon rank sum test, and dichotomous variables were compared with the chi-squared or Fisher exact test. We determined the adjusted odds ratios of AKI progression with mixed logistic regression with random intercepts for each center. We adjusted for important covariates described in the STS model to predict AKI in the cardiac surgery setting: patient demographics (age, sex, and white race), clinical risk factors (baseline estimated GFR, hypertension, and diabetes), and operative characteristics (elective or urgent procedure and cardiopulmonary bypass time>120) as well as the postoperative percent change in serum creatinine from baseline to the time of AKI diagnosis.[18] We used area under the receiver operating characteristic curve to determine the ability of the biomarkers to discriminate between patients with AKI that did and did not progress. We compared areas under the curve using the test developed by DeLong *et al.*[38] Areas under the curve for biomarker pairings were calculated by logistic regression with and without the clinical model as noted. We quantified the improvement of biomarkers on progressive AKI risk prediction with the category-free NRI and IDI indices.[39] For the primary analyses, urinary biomarkers were not corrected for urine creatinine, with the exception of the ACR in the analyses. We performed the analyses in SAS version 9.2 (SAS Institute, Cary, NC, USA) and R 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria).

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DISCLOSURES

S.G.C. and Dr. Devarajan are consultants to Abbott Diagnostics. Dr. Charles Edelstein and C.R.P. are named coinventors on the IL-18 patent. Dr. Devarajan is the coinventor on the NGAL patents. Dr. Devarajan is a consultant to Biosite, Inc. There are no conflicts for the other authors.

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