Urinary Biomarkers in the Clinical Prognosis and Early Detection of Acute Kidney Injury

Jay L. Koyner,* Vishal S. Vaidya,† Michael R. Bennett,‡ Qing Ma,‡ Elaine Worcester,* Shahab A. Akhter,§ Jai Raman,§ Valluvan Jeevanandam,§ Micheal F. O'Connor,‖ Prasad Devarajan,‡ Joseph V. Bonventre,† and Patrick T. Murray¶

*Section of Nephrology, Department of Medicine, §Section of Cardiothoracic Surgery, Department of Surgery, and ‖Department of Anesthesia and Critical Care, University of Chicago, Chicago, Illinois; †Renal Division, Brigham's and Women's Hospital, Harvard Medical School, Boston Massachusetts; ‡Section of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, Ohio; and ¶Department of Medicine, University College Dublin School of Medicine and Medical Science, Dublin, Ireland

Background and objectives: Several novel urinary biomarkers have shown promise in the early detection and diagnostic evaluation of acute kidney injury (AKI). Clinicians have limited tools to determine which patients will progress to more severe forms of AKI at the time of serum creatinine increase. The diagnostic and prognostic utility of novel and traditional AKI biomarkers was evaluated during a prospective study of 123 adults undergoing cardiac surgery.

Design, setting, participants, & measurements: Urinary neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), kidney injury molecule-1 (KIM-1), hepatocyte growth factor (HGF), π-glutathione-S-transferase (π-GST), α-GST, and fractional excretions of sodium and urea were all measured at preoperative baseline, postoperatively, and at the time of the initial clinical diagnosis of AKI. Receiver operator characteristic curves were generated and the areas under the curve (AUCs) were compared.

Results: Forty-six (37.4%) subjects developed AKI Network stage 1 AKI; 9 (7.3%) of whom progressed to stage 3. Preoperative KIM-1 and α-GST were able to predict the future development of stage 1 and stage 3 AKI. Urine CyC at intensive care unit (ICU) arrival best detected early stage 1 AKI (AUC = 0.70, $P < 0.001$); the 6-hour ICU NGAL (AUC = 0.88; $P < 0.001$) best detected early stage 3 AKI. π-GST best predicted the progression to stage 3 AKI at the time of creatinine increase (AUC = 0.86; $P = 0.002$).

Conclusion: Urinary biomarkers may improve the ability to detect early AKI and determine the clinical prognosis of AKI at the time of diagnosis.

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Acute kidney injury (AKI) is a common and serious complication of cardiothoracic surgery (1); depending on the definition of AKI used, it may occur in over 40% of adults, with 1% to 5% requiring renal replacement therapy (RRT) (2-9). Recently, standardized clinical definitions of AKI have been implemented through the use of the RIFLE (Risk, Injury, Failure, Loss, and ESRD) and AKIN (Acute Kidney Injury Network) criteria (10,11). However, these criteria are still very much dependent on delayed serum creatinine elevations, the current gold standard for the diagnosis of AKI. Furthermore, as a functional marker of glomerular filtration, serum creatinine is not ideally suited to diagnose AKI caused by renal tubular injury, rather than reversible prerenal azotemia (10).

In recent years, several novel human biomarkers have been demonstrated to detect acute tubular injury and have shown promise in their ability to precede and/or complement serum creatinine in the diagnosis of AKI (12-15). Cardiac surgery has long been used to study AKI because of the ability to prospectively follow patients before and after a well timed renal insult; for this reason, several urinary proteins have been shown to serve as biomarkers of AKI after cardiac surgery, including neutrophil gelatinase-associated lipocalin (NGAL) (16-20), cystatin C (CyC) (19,21), kidney injury molecule-1 (KIM-1) (18,21), interleukin-18 (IL-18) (22), and α-glutathione-S-transferase (α-GST) (23,24). Limited data are available comparing the ability of these markers to predict renal outcomes at the time of AKI diagnosis. In fact, nephrologists have limited tools in their arsenal to assess the presence and severity of renal tubular injury at the time of AKI diagnosis. Although urinalysis with microscopy has been shown to be of some utility in the differential diagnosis of AKI in a generalized hospital-based cohort (25), data supporting its use in the specific setting of cardiac surgery are lacking (24). Similarly, diagnostic mainstays of AKI

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Correspondence: Dr. Patrick T. Murray, University College Dublin School of Medicine and Medical Science, Catherine McAuley Centre, Nelson Street, Dublin 7, Ireland. Phone: 00-353-1-7166319; Fax: 00-353-1-7166355; E-mail: patrick.murray@ucd.ie

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evaluation such as the fractional excretion of sodium (FENa) have long been shown to be suboptimal tools in the complex setting of cardiac surgery AKI (24), in which volume status, fluid responsiveness, and diuretic use confound inferences regarding the relationship between tubular function and injury (26,27). Additionally, although recent data support the utility of the fractional excretion of urea (FEUrea) as a diagnostic tool in AKI (28), not all data support its use (29). Furthermore, very little is known about the utility of FENa or FEUrea compared with the novel urinary biomarkers discussed above for the differential diagnosis and prognostic evaluation of AKI.

In this study, we assessed the diagnostic utility of urinary NGAL, CyC, KIM-1, hepatocyte growth factor (HGF), α-GST (a proximal tubular damage marker), π-GST (a marker specific to distal tubule damage), FENa, and FEUrea as biomarkers for the detection of early and severe AKI after adult cardiac surgery. These novel biomarkers can be thought of as falling into two categories: constitutive markers (proteins/enzymes that are normally present in renal tubular cells and not normally found in the urine in significant concentration but are released into the urine in direct response to cellular injury), and inducible biomarkers (proteins that are not normally found in high concentrations in renal tubular cells or urine until their production is directly upregulated in response to cellular injury). CyC, α-GST, and π-GST are constitutive proteins that are extruded into urine in the presence of site-specific renal tubular injury (CyC and α-GST are proximal and π-GST is distal); intracellular GSTs are released into urine by damaged tubular cells, whereas injured proximal tubules fail to reabsorb filtered CyC. In contrast, KIM-1 and NGAL are inducible biomarkers, gene products that are increased in direct response to nephron damage (30,31). We also evaluated the ability of these markers to predict the severity/stage of AKI at the time of clinical diagnosis by serum creatinine increase. We performed all of the above analyses for those subjects who developed AKI as defined by the AKIN (11). Recent data demonstrate that urine NGAL after cardiac surgery varies with baseline renal function (32); as such, a secondary analysis of baseline GFR was conducted for the aforementioned panel of biomarkers (32). Finally, we interpreted the data for all novel biomarker concentrations adjusted and unadjusted for dilution by indexing to urinary creatinine, but for brevity's sake, we only report the indexed values unless otherwise noted.

Materials and Methods

We screened all patients admitted to the University of Chicago Cardiac Surgery service for elective surgery between August 2005 and December 2008. All patients eligible for enrollment were approached. Exclusion criteria included (*1*) age <18 years old, (*2*) pre-existing end-stage kidney disease receiving RRT or postrenal transplantation, (*3*) emergent cardiac surgery, and (*4*) unstable renal function (change in serum creatinine of ≥0.2 mg/dl within the past month, or oliguria <400 ml/day). Written informed consent was obtained from all patients at the time of their enrollment. The Institutional Review Board of the University of Chicago approved the study.

All patients were prospectively followed from the time of their enrollment. Blood and urine samples were collected simultaneously at predetermined timepoints: at the time of study enrollment, on the day of the surgery preoperatively (immediately postanesthesia induction), postoperatively (after coming off cardiopulmonary bypass [CPB]), upon arrival in the intensive care unit (ICU), 6 hours after arriving in the ICU, daily for a minimum of the next 2 days (days 1 to 2), and up to a maximum of 5 days. Patients were considered to have completed the study at the time of their hospital discharge. Baseline renal function was defined by the value calculated on the morning of the surgery at the preoperative timepoint.

As described previously, blood and urine samples were centrifuged at 4000 rpm for 10 minutes and the plasma was stored at −80°C. Urine supernatants were aliquoted and stored at −80°C (17). Sample analysis was batched and deferred until after the time of a subject's hospital discharge.

Preoperative patient characteristics, significant intraoperative risk factors, and peri- and postoperative complications were recorded on all patients. All therapeutic and clinical care decisions were made by the primary cardiac surgery service. Additionally, the decision to initiate RRT or make other renal interventions was made by the primary service and the Nephrology Consult service, without involvement of the study investigators.

The primary endpoint of the study was the development of AKI, defined by stage 1 AKI per the AKIN classification (11). Secondary endpoints included stage 2 and 3 AKI, need for RRT, in-hospital mortality, and length of ICU and hospital stays. Other variables recorded included age, sex, ethnicity, CPB time, crossclamp time, past medical history (hypertension, congestive heart failure, diabetes, *etc.*), previous cardiothoracic surgery, preoperative estimated GFR (eGFR; Modification of Diet in Renal Disease equation) (33), serial plasma and urine creatinine, sodium, and urea nitrogen.

Biomarker Measurements

As described previously, the levels of CyC in urine were measured using ELISA assay (human CyC ELISA kits: BioVendor, LLC, Candler, NC) (19). The urinary NGAL was measured via an ELISA (antibody HYB211-05, Antibody Shop, Genotfte, Denmark), as previously published (17,19). α-GST and π-GST were measured by enzyme immunoassay (Argutus Medical, Ltd., Dublin, Ireland) as described previously (34). KIM-1 and HGF protein were measured using Microsphere-based Luminex xMAP technology, as reported previously (13). Plasma and urine creatinine were measured by the alkaline picrate reaction, plasma and urine urea nitrogen were measured by the urease method, and plasma and urine sodium were measured with an ion-selective electrode (Beckman Unicell DxC 600, Beckman Coulter, Fullerton, CA). FENa and FEUrea were calculated as follows: $FENa\ (\%) = 100 \times (Urine_{Na} \times Plasma_{Cr}/Plasma_{Na} \times Urine_{Cr})$, and $FEUrea\ (\%) = 100 \times (Urine_{urea} \times Plasma_{Cr}/BUN \times U_{Cr})$, where *BUN* is the blood urea nitrogen. To correct for variations in urine flow, enzyme activities were normalized to urinary creatinine and reported in their adjusted and unadjusted forms. All laboratory investigators were blinded to the sample source and clinical outcomes and all measurements were made in duplicate or triplicate.

Statistics

Results are expressed as mean ± SEM or median and interquartile range (IQR; 25% to 75%). Sigma Stat 3.5 (Systat Software Inc., San Jose, CA) or Stata Version 11 (StataCorp, College Station, TX) were used for analyses. For continuous variables, a *t* test or a Mann–Whitney U test, as appropriate, was performed for comparison between two groups. When comparing more than two groups, ANOVA or the Kruskal–Wallis test was used. Spearman rank correlation coefficients were utilized for further assessment of the correlation between marker levels

and severity of AKI. For an overall comparison of change over time between groups, a repeated measures ANOVA was performed with particular interest in the interaction between group and time. A χ^2 or Fisher's exact test was used for analysis of categorical variables. $P < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) curves were generated for the various markers at the ICU admit and at 6-hour ICU timepoints, with the maximum value obtained within the first 6 postoperative hours (referred to as the 'early max timepoint') and at the time of AKI diagnosis. The area under the ROC curve (AUC) was calculated as a measure of the utility of the biomarker concentrations as early markers of AKI. An AUC of 0.5 indicates a marker with accuracy no better than expected by chance, whereas a value of 1.0 signifies a biomarker with perfect diagnostic accuracy. The AUCs were compared using methods described by DeLong *et al.* (35), and, when appropriate, a Bonferroni adjustment was made to account for multiple comparisons.

Results

Clinical Characteristics

The clinical and demographic characteristics of the 123 adult subjects are reported in Table 1; note that one patient who died intraoperatively, before any postoperative sample collection, was completely excluded from the analysis. Forty-six (37.4% of total) patients developed postoperative AKI, of whom 36 (29.3%) developed only stage 1 AKI. One subject (0.8%) developed AKIN stage 2, whereas nine subjects (7.3%) developed AKIN stage 3; eight of these nine required RRT. Seventy-seven subjects (62.6%) did not develop any AKI at all.

There was a significant increase in length of ICU stay ($P < 0.05$) and in-hospital mortality ($P < 0.05$) in those with AKI compared with those without AKI. Additionally, those who went on to develop stage 3 AKI had higher baseline serum creatinine ($P < 0.05$) but not statistically lower eGFRs. They also had longer CPB ($P < 0.01$) and crossclamp times ($P < 0.05$), longer hospital and ICU stays ($P < 0.01$ for both), and increased mortality ($P = 0.001$) compared with those without stage 3 AKI.

ROC Analysis for Early Diagnosis of Stage 1 AKI

Figure 1 demonstrates the values for NGAL (ng/mg creatinine), urine CyC (mg/g creatinine), KIM-1 (ng/mg creatinine), HGF (ng/mg creatinine), α-GST (ng/mg creatinine), and π-GST (ng/mg creatinine) in the early perioperative period for those without AKI ($n = 77$), those with stage 1 and 2 AKI ($n = 37$), and those with stage 3 AKI ($n = 9$). The corresponding AUCs for all biomarkers to predict stage 1 AKI at select pre- and postoperative timepoints as well as the early max can be found in Table 2. For the early diagnosis of AKI, urine CyC performed best at the ICU arrival timepoint with an AUC of 0.72 (0.61 to 0.83; $P < 0.001$); at the subsequent timepoint, the 6-hour ICU value for NGAL was identical 0.72 (0.61 to 0.83; $P < 0.001$). Additionally, the analysis demonstrated that various early postoperative timepoints for CyC, NGAL, KIM-1, HGF, and α-GST all had the ability to predict the future development stage 1 AKI. The preoperative KIM-1 was significantly different in those who did and did not go on to develop stage 1 AKI; KIM-1 provided an AUC of 0.67 (0.57 to 0.77; $P = 0.01$), whereas the KIM-1 early max performed even better (0.69 [0.58 to 0.80]; $P = 0.002$). The α-GST preoperative baseline values were statistically different for the entire cohort ($n = 123$), with those without AKI having higher values than those who went on to develop stage 1 AKI (0.37 [0.24 to 0.48], $P = 0.03$). AUC for α-GST (μg/L) at the post-CPB timepoint was 0.64 (0.52 to 0.76; $P = 0.03$), with higher levels being found in those who went on to develop AKI. Individual timepoints for α-GST demonstrated no statistical differences when looking at the cohort stratified by baseline GFR and AKI status. However, the 11 subjects who underwent a cardiac catheterization within 72 hours of their operation had statistically higher α-GSTs (ng/mg, 21.0 [4.4 to 28.2]) compared with the rest of the cohort ($n = 112$) (3.4 [1.2 to 7.0], $P = 0.02$). Ten of these 11 subjects did not go on to develop postoperative AKI, so it follows that those without AKI actually had higher baseline α-GST values. No other biomarker (including KIM-1) had statistical differences in the baseline preoperative biomarker concentrations between those with and without a cardiac catheterization.

Neither the FENa nor the FEUrea (including the early max and early minimum, defined as the lowest value obtained within the first 6 postoperative hours) demonstrated the ability to detect early AKI. There was only one timepoint that demonstrated statistically different AUCs for the adjusted and unadjusted values. The creatinine-indexed early max value for HGF (ng/mg) (0.62 [0.52 to 0.73]; $P = 0.03$) was different from the unadjusted HGF value (pg/ml) (0.53 [0.42 to 0.64] P = NS) χ^2 comparison ($P = 0.04$).

Further analysis of the cohort with respect to biomarker performance stratified by baseline eGFR was conducted with stage 1 AKI as the endpoint (Table 2). There were 49 subjects who had baseline eGFRs <60 ml/min (19 of whom developed AKI and 30 of whom had no AKI). There were 74 subjects who had baseline eGFRs >60 ml/min, of whom 27 developed postoperative AKI and 47 did not. With the exception of CyC, the novel biomarkers did not display the ability to predict the future development of AKI in those with an eGFR <60 ml/min. However, for those with an eGFR >60 ml/min, CyC, NGAL, and KIM-1 all consistently displayed AUCs between 0.68 and 0.81 at various early postoperative timepoints.

ROC Analysis for Early Diagnosis of Stage 3 AKI

Table 3 demonstrates the AUCs for the novel urinary biomarkers in the entire cohort ($n = 123$) adjusted and unadjusted for urine creatinine to determine the future development of stage 3 AKI ($n = 9$). At various early postoperative timepoints, CyC, NGAL, KIM-1, α-GST, and π-GST all demonstrated the capability to diagnose the subsequent development of stage 3 AKI. The timepoint with the best overall performance was the unadjusted NGAL at the 6-hour ICU timepoint with an AUC of 0.88 (0.73 to 0.99; $P < 0.001$). The KIM-1 early max and the preoperative π-GST were the only timepoints that demonstrated statistically different AUCs for the adjusted and unadjusted values. Neither set of the π-GST values (adjusted or unadjusted) or unadjusted KIM-1 was useful for early diagnosis of stage 3 AKI; however, the adjusted KIM-1 value set was.

We have examined the role of baseline eGFR on the biomarker performance in predicting the development of stage

Table 1. Clinical characteristics of University of Chicago cardiac surgery study subjects ($n = 123$)

Characteristic	No AKI ($n = 77$)	AKIN Stage 1 to 2 ($n = 37$)	AKIN Stage 3 ($n = 9$)
Age			
median years	68	72	72
IQR	56.5 to 77	61 to 81	59 to 79.3
Gender, male/female (%)	66.2/33.8	62.2/37.8	66.7/33.3
Race			
Caucasian	54	32	6
African American	18	4	3
other	5	1	0
Hispanic, *n* (%)	5 (6.5)	2 (5.4)	1 (11.1)
Baseline GFR (MDRD), ml/min per 1.73 m^2 (mean ± SEM)	72.3 ± 3.2	73.1 ± 5.4	64.4 ± 9.4
Baseline creatinine[b]			
median mg/dl	1.04	1.09	1.40
IQR	0.87 to 1.33	0.90 to 1.20	1.20 to 1.67[b]
Diabetes mellitus, *n* (%)	21 (27.2)	10 (27.0)	3 (33.3)
COPD, *n* (%)	19 (27.5)	7 (18.9)	3 (33.3)
Ejection fraction			
n	59	29	5
mean ± SEM	4.4 ± 2.2	49.5 ± 3.2	51.0 ± 4.0
Preoperative medications, *n* (%)			
beta blockers	32 (41.6)	9 (24.3)	5 (55.5)
ACEI/ARBs	23 (29.9)	11 (29.7)	4 (44.4)
calcium channel blockers	12 (15.6)	4 (14.8)	2 (22.2)
diuretics	28 (36.4)	8 (21.6)	6 (66.6)
statins	27 (35.1)	11 (29.7)	5 (55.5)
Operative factors			
surgery type			
CABG alone	24	8	2
valve alone	10	11	1
CABG + valve	20	11	5
CABG + other	3	0	0
valve + other	17	5	1
other	3	2	0
previous CT surgery, *n* (%)	18 (23.4)	5 (13.5)	2 (22.2)
CPB time[c]			
median minutes	162	160.5	250.5
IQR	126 to 213	128 to 214	182 to 345.5
off-pump surgery, *n* (%)	8 (10.4)	3 (8.1)	1 (11.1)
crossclamp time, minutes (mean ± SEM)[b]	126.2 ± 5.6	129.3 ± 10.9	156.1 ± 16.6
no crossclamp, *n* (%)	14 (18.2)	3 (8.1)	1 (11.1)
Outcomes			
ICU days, median (IQR)[a,d]	3 (2 to 5)	3 (2 to 5)	9 (5.75 to 16)[d]
postoperative hospital days, mean ± SEM[c]	7 (6 to 9.3)	7 (6 to 10.3)	19 (8.5 to 30.3)[c]
in-hospital mortality, *n*[a,d]	0	1	4

COPD, chronic obstructive pulmonary disease; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CABG, coronary artery bypass graft; MDRD, Modification of Diet in Renal Disease.

No AKI *versus* AKI [a]$P < 0.05$; stage 3 *versus* all others [b]$P < 0.05$, [c]$P < 0.01$, and [d]$P < 0.001$; all other P = NS.

3 AKI (Table 3). For those with eGFRs <60 ml/min ($n = 49$), there were 5 subjects who developed stage 3 AKI compared with 44 who did not progress to stage 3. In the 74 subjects with eGFRs >60 ml/min, there were 4 subjects who developed stage 3 AKI, compared with 70 who did not progress to stage 3 AKI. No single novel biomarker was consistently able to predict the future development of stage 3 AKI in those with eGFRs <60 ml/min, although several baseline levels (NGAL, KIM-1, and α-GST) were predictive. In those with eGFR >60 ml/min, CyC, NGAL, and π-GST all consistently

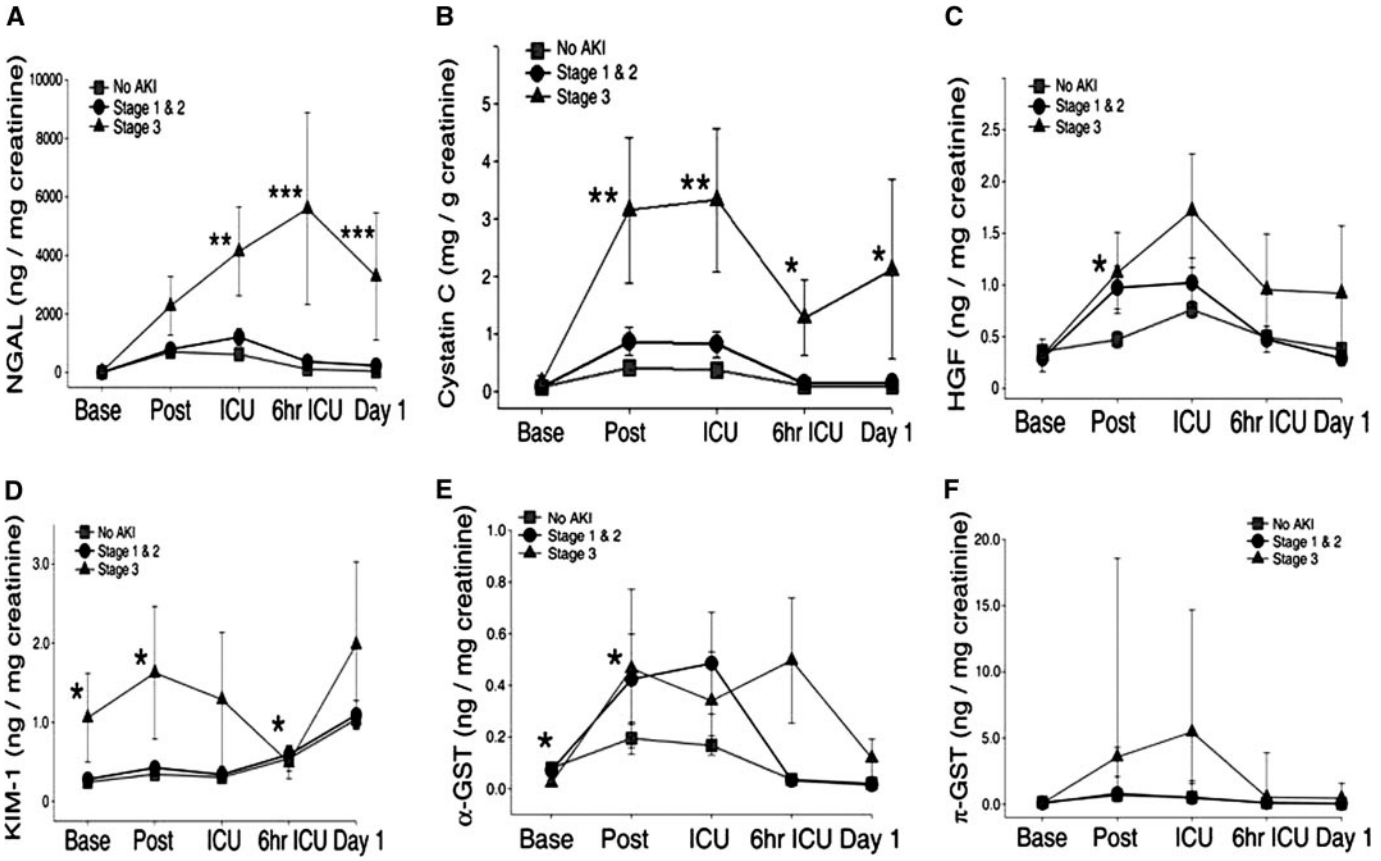

Figure 1. Urinary biomarkers during the perioperative period for those subjects without AKI, those with stage 1 and stage 2 AKI, and those with stage 3 AKI. Stage 1 AKI was defined as a ≥50% relative increase or 0.3-mg/dl absolute increase in plasma creatinine from preoperative baseline or within the first 72 postoperative hours. (A) NGAL (ng/mg creatinine; mean ± SEM), (B) CyC (mg/g creatinine; mean ± SEM), (C) HGF (ng/mg creatinine; mean ± SEM), (D) KIM-1 (ng/mg creatinine; mean ± SEM), (E) α-GST (ng/mg creatinine; median [IQR 25% to 75%]) (F) π-GST (ng/mg creatinine; median [IQR 25% to 75%]). Note that the No AKI plot (red) is obscured from view by the plot for those with stage 1 and 2 AKI (blue). *$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$.

detected stage 3 AKI at several early postoperative timepoints.

ROC Analysis of Biomarkers for Prediction of AKI Severity at the Time of Clinical Diagnosis by Serum Creatinine Elevation

Table 4 demonstrates the median values with 25% to 75% IQR values and AUCs for the absolute and percentage change in plasma creatinine (from baseline) as well as the novel urinary biomarker concentrations (adjusted and unadjusted) to predict those who will progress to stage 3 AKI ($n = 9$) at the time of AKI diagnosis, compared with those who remain at stage 1 or 2 ($n = 37$). No biomarker had a statistically significant difference when comparing the AUC/ROC curves for their adjusted and unadjusted biomarker values (data not shown). At the time of clinical creatinine increase, CyC, NGAL, and π-GST all demonstrated the ability to determine the progression to stage 3 AKI. The biomarker that performed the best was π-GST, with an AUC of 0.86 (0.74 to 0.99; $P = 0.002$).

Discussion

Several recent studies have presented data on various novel urinary biomarkers of AKI and their ability to detect injury before serum creatinine (12,14,16-19,21,30,36-40). In this study, we investigated six such novel urinary biomarkers in the setting of adult cardiac surgery and demonstrate that

- Some urinary biomarkers (π-GST, NGAL, and CyC) are potentially valuable tools in determining the prognosis and severity of AKI at the time of initial creatinine-based diagnosis, providing a *de facto* diagnosis of the clinical syndrome of acute tubular necrosis (*versus* prerenal azotemia, or other rapidly reversible causes).
- Several individual biomarkers are useful in the diagnosis of early stage 1 and early stage 3 AKI after adult cardiac surgery.
- The diagnostic utility of urinary AKI biomarkers may be affected by such factors as baseline eGFR and preoperative cardiac catheterization, and further exploration of these factors is required to ensure appropriate use of these novel tools.

Table 2. Biomarker performance for stage 1 AKI—entire cohort and stratified by eGFR

Stage 1 AKI	Entire Cohort (n = 123)	eGFR <60 ml/min (n = 49)	eGFR >60 ml/min (n = 74)
CyC (mg/L)			
baseline	0.50 (0.37 to 0.62)	0.48 (0.29 to 0.67)	0.52 (0.37 to 0.67)
ICU arrival	0.72 (0.61 to 0.83)[b]	0.70 (0.53 to 0.87)[a]	0.70 (0.56 to 0.83)[a]
6-hour ICU	0.68 (0.56 to 0.80)[a]	0.58 (0.38 to 0.77)	0.74 (0.59 to 0.89)[a]
early max	0.71 (0.61 to 0.81)[b]	0.70 (0.54 to 0.86)[a]	0.73 (0.60 to 0.86)[a]
CyC (mg/g)			
baseline	0.49 (0.38 to 0.61)	0.45 (0.26 to 0.64)	0.56 (0.40 to 0.71)
ICU arrival	0.66 (0.55 to 0.77)[a]	0.67 (0.50 to 0.84)[a]	0.66 (0.51 to 0.80)[a]
6-hour ICU	0.64 (0.53to 0.75)[a]	0.53 (0.33 to 0.72)	0.71 (0.56 to 0.86)[a]
early max	0.68 (0.58 to 0.79)[b]	0.69 (0.52 to 0.85)[a]	0.67 (0.53 to 0.82)[a]
NGAL (pg/ml)			
baseline	0.44 (0.32 to 0.57)	0.52 (0.30 to 0.73)	0.44 (0.28 to 0.60)
ICU arrival	0.69 (0.57 to 0.80)[a]	0.62 (0.44 to 0.81)	0.71 (0.57 to 0.85)[a]
6-hour ICU	0.72 (0.61 to 0.83)[b]	0.58 (0.37 to 0.79)	0.81 (0.70 to 0.92)[a]
early max	0.68 (0.57 to 0.79)[a]	0.61 (0.42 to 0.80)	0.71 (0.57 to 0.85)[a]
NGAL (ng/mg)			
baseline	0.49 (0.37 to 0.61)	0.58 (0.38 to 0.78)	0.47 (0.31 to 0.63)
ICU arrival	0.69 (0.58 to 0.80)[a]	0.65 (0.47 to 0.84)	0.71 (0.56 to 0.85)[a]
6-hour ICU	0.71 (0.60 to 0.82)[b]	0.61 (0.41 to 0.81)	0.80 (0.68 to 0.92)[a]
early max	0.68 (0.56 to 0.79)[a]	0.63 (0.44 to 0.82)	0.69 (0.55 to 0.84)[a]
KIM-1 (pg/ml)			
baseline	0.67 (0.57 to 0.77)[a]	0.64 (0.47 to 0.82)	0.68 (0.55 to 0.81)[a]
ICU arrival	0.56 (0.45 to 0.67)	0.59 (0.40 to 0.77)	0.55 (0.40 to 0.70)
6-hour ICU	0.64 (0.52 to 0.74)[a]	0.52 (0.33 to 0.72)	0.70 (0.56 to 0.83)[a]
early max	0.62 (0.52 to 0.73)[a]	0.56 (0.39 to 0.74)	0.67 (0.53 to 0.80)[a]
KIM-1 (ng/mg)			
baseline	0.67 (0.57 to 0.77)[a]	0.62 (0.44 to 0.79)	0.70 (0.57 to 0.82)[a]
ICU arrival	0.59 (0.48 to 0.69)	0.64 (0.46 to 0.82)	0.57 (0.42 to 0.70)
6-hour ICU	0.66 (0.56 to 0.77)[a]	0.51 (0.31 to 0.70)	0.76 (0.64 to 0.88)[a]
early max	0.69 (0.58 to 0.80)[a]	0.63 (0.46 to 0.80)	0.73 (0.62 to 0.85)[a]
HGF (pg/ml)			
baseline	0.47 (0.35 to 0.58)	0.50 (0.31 to 0.69)	0.43 (0.29 to 0.58)
ICU arrival	0.50 (0.39 to 0.61)	0.55 (0.36 to 0.73)	0.46 (0.31 to 0.61)
6-hour ICU	0.45 (0.33 to 0.57)	0.47 (0.26 to 0.67)	0.44 (0.29 to 0.59)
early max	0.53 (0.42 to 0.64)	0.54 (0.37 to 0.71)	0.52 (0.37 to 0.66)
HGF (ng/mg)			
baseline	0.45 (0.34 to 0.56)	0.47 (0.28 to 0.66)	0.43 (0.29 to 0.57)
ICU arrival	0.53 (0.41 to 0.64)	0.60 (0.42 to 0.78)	0.45 (0.31 to 0.60)
6-hour ICU	0.46 (0.34 to 0.57)	0.41 (0.22 to 0.61)	0.47 (0.33 to 0.63)
early max	0.62 (0.52 to 0.73)[a]	0.68 (0.52 to 0.84)[a]	0.58 (0.44 to 0.72)
a-GST (μg/L)			
baseline	0.36 (0.25 to 0.47)[a]	0.37 (0.18 to 0.55)	0.38 (0.24 to 0.53)
ICU arrival	0.59 (0.47 to 0.71)	0.52 (0.33 to 0.72)	0.63 (0.48 to 0.79)
6-hour ICU	0.48 (0.36 to 0.61)	0.54 (0.32 to 0.76)	0.44 (0.29 to 0.60)
early max	0.62 (0.51 to 0.73)[a]	0.57 (0.39 to 0.76)	0.66 (0.51 to 0.81)[a]
α-GST (ng/mg)			
baseline	0.37 (0.24 to 0.48)[a]	0.34 (0.15 to 0.54)	0.39 (0.24 to 0.53)
ICU arrival	0.60 (0.48 to 0.72)	0.59 (0.39 to 0.78)	0.61 (0.45 to 0.75)
6-hour ICU	0.48 (0.36 to 0.61)	0.51 (0.31 to 0.72)	0.47 (0.30 to 0.63)
early max	0.64 (0.52 to 0.76)[a]	0.63 (0.45 to 0.80)	0.66 (0.51 to 0.81)[a]
π-GST (μg/L)			
baseline	0.44 (0.33 to 0.56)	0.51 (0.30 to 0.71)	0.41 (0.27 to 0.55)
ICU arrival	0.54 (0.42 to 0.66)	0.56 (0.37 to 0.75)	0.52 (0.36 to 0.68)

Table 2. (Continued)

Stage 1 AKI	Entire Cohort (n = 123)	eGFR <60 ml/min (n = 49)	eGFR >60 ml/min (n = 74)
6-hour ICU	0.50 (0.38 to 0.63)	0.54 (0.34 to 0.75)	0.47 (0.31 to 0.63)
early max	0.60 (0.49 to 0.72)	0.56 (0.38 to 0.75)	0.61 (0.47 to 0.76)
π-GST (ng/mg)			
baseline	0.43 (0.32 to 0.55)	0.49 (0.28 to 0.69)	0.40 (0.27 to 0.54)
ICU arrival	0.55 (0.43 to 0.67)	0.59 (0.40 to 0.77)	0.52 (0.36 to 0.68)
6-hour ICU	0.52 (0.40 to 0.65)	0.54 (0.34 to 0.74)	0.51 (0.34 to 0.67)
early max	0.60 (0.48 to 0.72)	0.60 (0.41 to 0.78)	0.60 (0.44 to 0.75)
FENa (%)			
baseline	0.49 (0.36 to 0.61)	0.46 (0.26 to 0.67)	0.50 (0.34 to 0.67)
ICU arrival	0.46 (0.35 to 0.57)	0.46 (0.28 to 0.65)	0.45 (0.30 to 0.60)
6-hour ICU	0.48 (0.36 to 0.59)	0.42 (0.23 to 0.61)	0.49 (0.33 to 0.66)
early max	0.49 (0.38 to 0.61)	0.46 (0.28 to 0.64)	0.50 (035 to 0.65)
early min	0.46 (0.35 to 0.57)	0.44 (0.27 to 0.61)	0.45 (0.30 to 0.60)
FEUrea (%)			
baseline	0.59 (0.45 to 0.73)	0.51 (0.30 to 0.71)	0.52 (0.36 to 0.69)
ICU arrival	0.46 (0.33 to 0.59)	0.36 (0.18 to 0.54)	0.51 (0.35 to 0.67)
6-hour ICU	0.50 (0.37 to 0.63)	0.45 (0.24 to 0.66)	0.48 (0.32 to 0.63)
early max	0.53 (0.41 to 0.65)	0.35 (0.18 to 0.53)	0.58 (0.43 to 0.73)
early min	0.49 (0.37 to 0.60)	0.45 (0.27 to 0.63)	0.43 (0.28 to 0.58)

[a]$P < 0.05$, [b]$P < 0.001$.

Several studies have evaluated the performance of biomarkers of AKI after cardiac surgery. In our study, we found that several biomarkers are capable of predicting the future development of stage 1 AKI in the early postoperative period. The performances of NGAL, CyC, and KIM-1 (normalized and unnormalized) are on par with AUCs that have been previously reported after adult cardiac surgery ranging from 0.62 to 0.72 (16,18-20,23). These are much lower AUCs than reported in the seminal NGAL study first performed in pediatric cardiac surgery (17) and no doubt relate to the nature of underlying renal disease and other comorbidities in an adult population undergoing cardiac surgery. To this end, similar to the recent publication of McIlroy *et al.* (32), our exploratory analysis demonstrates that urine NGAL may have diminished ability to detect AKI in those with pre-existing renal disease but performs better in those with baseline eGFRs >60 ml/min. Subsequent studies of NGAL and other urinary biomarkers need to account for the role that pre-existing tubular damage (from chronic kidney disease) may play in determining biomarker performance. Our data, which demonstrate a similar eGFR-dependent effect for KIM-1 and an eGFR independent effect for CyC (for early detection of stage 1 AKI), need to be verified in larger multicenter trials. Additionally, this is the first study to show that α-GST may be useful as a potential marker of AKI after adult cardiac surgery. Previous work has demonstrated that α-GST may be useful in other settings of AKI (34), but it is clear that further work must be done to further validate these markers in the setting of cardiac surgery.

We investigated the performance of several biomarkers at detecting stage 1 AKI and found that NGAL, CyC, KIM-1, and α-GST all displayed the ability to detect stage 1 AKI. Perhaps more rigorous definitions of AKI, which somehow account for the duration of AKI, should be utilized in defining a future clinical role for these biomarkers (41). KIM-1 and α-GST demonstrated the ability to predict AKI at the preoperative timepoint, before the initiation of surgery. This is remarkable and may speak to the marker's ability to measure subclinical proximal tubular injury, undetected by an insensitive serum creatinine, in the preoperative period. It should be noted that KIM-1 and α-GST have been demonstrated to be expressed in very high levels in proximal tubule epithelial cells (30,42), and in our study have similar AUC profiles. Given these similarities, we speculate that these markers may detect a specific form and severity of AKI different from those detected by NGAL, CyC, and π-GST and as a result may be useful in preoperative risk stratification models of adult cardiac surgery. Potential sources of this preoperative elevation include exposure to radiocontrast via preoperative cardiac catheterization, hypotensive events, or pre-existing CKD. Very little is known about the nature of these biomarkers in the setting of pre-existing CKD. More specifically, exploration of the effect that contrast media and cardiac catheterization have on α-GST needs to be explored because in our extremely small sample size (n = 11), concentrations were significantly elevated in this cohort, even in those who do not go on to develop AKI.

Several biomarkers demonstrated the ability to detect stage 3 AKI at early postoperative timepoints, including CyC, NGAL, KIM-1, and π-GST. Although the numbers of those progressing to this severe form of AKI remain low (n = 9), our data and previous reports (19,43,44) support a role for these urinary biomarkers in detecting early severe AKI. In this most severe form of AKI, one would postulate that there is diffuse renal

Table 3. Biomarker performance for stage 3 AKI—entire cohort and stratified by eGFR

Stage 3 AKI	Entire Cohort (n = 123)	eGFR <60 ml/min (n = 49)	eGFR >60 ml/min (n = 74)
CyC (mg/L)			
baseline	0.41 (0.16 to 0.66)	0.44 (0.09 to 0.80)	0.40 (0.16 to 0.85)
ICU arrival	0.84 (0.68 to 0.99)[b]	0.74 (0.43 to 0.98)	0.93 (0.84 to 0.99)[a]
6-hour ICU	0.78 (0.85 to 0.99)[a]	0.51 (0.04 to 0.98)	0.98 (0.94 to 0.99)[a]
early max	0.85 (0.69 to 0.99)[b]	0.81 (0.52 to 0.98)[a]	0.93 (0.83 to 0.99)[a]
CyC (mg/g)			
baseline	0.35 (0.09 to 0.61)	0.43 (0.06 to 0.80)	0.45 (0.30 to 0.83)
ICU arrival	0.82 (0.67 to 0.97)[a]	0.69 (0.40 to 0.98)	0.93 (0.86 to 0.99)[a]
6-hour ICU	0.77 (0.52 to 0.99)[a]	0.47 (0.01 to 092)	0.98 (0.96 to 0.99)[a]
early max	0.85 (0.71 to 0.98)[b]	0.77 (0.50 to 0.98)[a]	0.91 (0.82 to 0.99)[a]
NGAL (pg/ml)			
baseline	0.73 (0.56 to 0.89)[a]	0.81 (0.59 to 0.98)[a]	0.59 (0.42 to 0.75)
ICU arrival	0.79 (0.65 to 0.94)[a]	0.68 (0.41 to 0.94)	0.92 (0.78 to 0.98)[a]
6-hour ICU	0.88 (0.73 to 0.99)[b]	0.73 (0.34 to 0.97)	0.97 (0.90 to 0.99)[a]
early max	0.82 (0.66 to 0.97)[a]	0.73 (0.44 to 0.97)	0.92 (0.75 to 0.98)[a]
NGAL (ng/mg)			
baseline	0.71 (0.51 to 0.91)[a]	0.85 (0.67 to 0.99)[a]	0.48 (0.26 to 0.70)
ICU arrival	0.80 (−0.66 to 0.94)[a]	0.65 (0.41 to 0.89)	0.92 (0.77 to 0.99)[a]
6-hour ICU	0.88 (0.73 to 0.99)[b]	0.69 (0.30 to 0.90)	0.98 (0.92 to 0.99)[a]
early max	0.82 (0.66 to 0.98)[a]	071 (0.41 to 0.95)	0.92 (0.74 to 0.99)[a]
KIM-1 (pg/ml)			
baseline	0.82 (0.68 to 0.95)[a]	0.82 (0.65 to 0.99)[a]	0.80 (0.53 to 0.99)[a]
ICU arrival	0.69 (0.44 to 0.93)	0.69 (0.17 to 0.90)	0.70 (0.37 to 0.90)
6-hour ICU	0.45 (0.17 to 0.73)	0.52 (0.15 to 0.85)	0.47 (0.14 to 0.81)
early max	0.58 (0.37 to 0.79)	0.65 (0.36 to 0.94)	0.48 (0.14 to 0.82)
KIM-1 (ng/mg)			
baseline	0.76 (0.57 to 0.94)[a]	0.77 (0.52 to 0.98)[a]	0.67 (0.52 to 0.83)[a]
ICU arrival	0.68 (0.38 to 0.97)	0.65 (0.20 to 0.87)	0.73 (0.36 to 0.95)
6-hour ICU	0.54 (0.27 to 0.82)	0.55 (0.19 to 0.87)	0.67 (0.42 to 0.92)
early max	0.72 (0.51 to 0.93)[a]	0.80 (0.49 to 0.98)	0.55 (0.37 to 0.91)
HGF (pg/ml)			
baseline	0.44 (0.23 to 0.65)	0.51 (0.24 to 0.76)	0.23 (0.04 to 0.44)[a]
ICU arrival	0.57 (0.35 to 0.78)	0.46 (0.22 to 0.71)	0.65 (0.25 to 0.98)
6-hour ICU	0.65 (0.45 to 0.86)	0.44 (0.22 to 0.85)	0.73 (0.50 to 0.98)[a]
early max	0.54 (0.35 to 0.74)	0.40 (0.21 to 0.60)	0.65 (0.30 to 0.95)
HGF (ng/mg)			
baseline	0.43 (0.17 to 0.63)	0.51 (0.16 to 0.85)	0.16 (0.02 to 0.38)[a]
ICU arrival	0.64 (0.38 to 0.89)	0.57 (0.21 to 0.92)	0.68 (0.25 to 0.98)
6-hour ICU	0.67 (0.45 to 0.90)	0.52 (0.12 to 0.95)	0.84 (0.67 to 0.98)[a]
early max	0.67 (0.45 to 0.88)	0.60 (0.32 to 0.87)	0.69 (0.30 to 0.99)
α-GST (μg/L)			
baseline	0.23 (0.01 to 0.37)[a]	0.29 (0.08 to 0.50)[a]	0.19 (0.07 to 0.30)[a]
ICU arrival	0.58 (0.31 to 0.85)	0.41 (0.07 to 0.76)	0.77 (0.35 to 0.99)
6-hour ICU	0.68 (0.35 to 0.99)	0.61 (0.07 to 0.99)	0.75 (0.27 to 0.95)
early max	0.58 (0.30 to 0.87)	0. 46 (0.10 to 0.82)	0.74 (0.27 to 0.99)
α-GST (ng/mg)			
baseline	0.24 (0.06 to 0.42)[a]	0.30 (0.03 to 0.58)	0.13 (0.03 to 0.23)[a]
ICU arrival	0.60 (0.33 to 0.86)	0.44 (0.09 to 0.80)	0.77 (0.35 to 0.98)
6-hour ICU	0.67 (0.35 to 0.99)	0.53 (0.03 to 0.98)	0.77 (0.32 to 0.99)
early max	0.63 (0.38 to 0.88)	0.51 (0.18 to 0.83)	0.76 (0.33 to 0.98)
π-GST (μg/L)			
baseline	0.53 (0.32 to 0.73)	0.53 (0.27 to 0.78)	0.51 (0.17 to 0.85)
ICU arrival	0.70 (0.50 to 0.90)[a]	0.61 (0.34 to 0.89)	0.81 (0.50 to 0.99)[a]

Table 3. (Continued)

Stage 3 AKI	Entire Cohort (n = 123)	eGFR <60 ml/min (n = 49)	eGFR >60 ml/min (n = 74)
6-hour ICU	0.78 (0.56 to 0.99)[a]	0.56 (0.18 to 0.93)	0.93 (0.79 to 0.99)[a]
early max	0.72 (0.51 to 0.93)[a]	0.54 (0.22 to 0.86)	0.92 (0.82 to 0.99)[a]
π-GST (ng/mg)			
baseline	0.43 (0.20 to 0.66)	0.43 (0.09 to 0.77)	0.37 (0.10 to 0.73)
ICU arrival	0.69 (0.47 to 0.92)	0.59 (0.27 to 0.91)	0.81 (0.50 to 0.98)[a]
6-hour ICU	0.76 (0.54 to 0.99)[a]	0.46 (0.06 to 0.86)	0.93 (0.80 to 0.98)[a]
early max	0.74 (0.54 to 0.94)[a]	0.58 (0.28 to 0.89)	0.91 (0.83 to 0.99)[a]
FENa (%)			
baseline	0.63 (0.46 to 0.76)	0.61 (0.37 to 0.84)	0.52 (0.38 to 0.67)
ICU arrival	0.56 (0.41 to 0.70)	0.53 (0.24 to 0.83)	0.59 (0.45 to 0.73)
6-hour ICU	0.62 (0.39 to 0.84)	0.29 (0.02 to 0.65)	0.84 (0.71 to 0.97)[a]
early max	0.65 (0.51 to 0.79)	0.63 (0.38 to 0.89)	0.66 (0.49 to 0.82)
early min	0.64 (0.45 to 0.83)	0.50 (0.15 to 0.86)	0.81 (0.68 to 0.92)[a]
FEUrea (%)			
baseline	0.38 (0.17 to 0.59)	0.51 (0.30 to 0.71)	0.39 (0.12 to 0.65)
ICU arrival	0.47 (0.25 to 0.69)	0.36 (0.18 to 0.54)	0.47 (0.16 to 0.83)
6-hour ICU	0.53 (0.28 to 0.77)	0.45 (0.24 to 0.66)	0.56 (0.21 to 0.90)
early max	0.57 (0.36 to 0.74)	0.35 (0.18 to 0.52)	0.60 (0.39 to 0.82)

[a]$P < 0.05$, [b]$P < 0.001$.

tubular damage (necrosis) and that markers that detect distal tubular damage (NGAL and π-GST) may be optimal for detecting this type of injury. This concept can also be applied to the data at the time of the clinical creatinine increase, when π-GST and NGAL outperformed the other markers, possibly in part because of their ability to detect distal tubular damage.

It is important to note that although we present the data adjusted and unadjusted for urine creatinine, there is no clear guidance in terms of whether or not to normalize such data sets. Some believe that normalization is less than ideal, because implicit in this normalization is the idea of steady state for creatinine, which is clearly not the case for patients with AKI. However, in looking at those biomarkers that can detect a difference at the time of clinical creatinine increase (CyC, NGAL, and π-GST), we can see that there is a consistent trend toward increased AUC values and greater statistical significance with the adjustment for urine creatinine (Table 4). This is potentially interesting because we are looking at values from a timepoint where there is active tubular damage and dysfunction, and we would anticipate impaired urinary concentrating capacity. With urinary creatinine excretion impaired in the setting of severe tubular injury, normalizing the biomarker values likely only gives us a partial correction for the ongoing renal dysfunction, as opposed to correction in those with stage 1 AKI with intact tubular function. Our data suggest that correction may be of greater value in the presence of clinical AKI than at earlier timepoints, although the bulk of our data are consistent with limited data from several groups that have reported that there was no difference between the normalized and non-normalized values (37,45). Similarly, our data from pre-AKI timepoints demonstrate no difference in these data in 77 of 80 comparisons across various definitions of AKI and various biomarkers. Larger multicenter studies are needed to verify the need for creatinine adjustment because the normalization may be unnecessary or misleading in some clinical settings and may in fact be hindering the clinical development of urinary AKI biomarkers.

In our evaluation of the prognostic data at the time of clinical diagnosis of AKI, we found that some of the traditional tools that nephrologists use to determine the location and nature of tubular injury (FENa and FEUrea) are unable to detect a difference in AKI course, severity, and outcomes. It is notable that these same tests were equally unable to predict stage 1 or stage 3 AKI at the early perioperative timepoints. Although there is some literature supporting the use of FENa as a diagnostic tool 48 to 72 hours after surgery (24), most recent biomarker papers have not reported on the performance of FENa or FEUrea (which remain in common clinical use).

In the future, multibiomarker panels will likely aid in the diagnosis of various types of AKI, but one must recognize the different inherent performance characteristics of the individual biomarkers and the simple fact that AKI is a heterogeneous syndrome. The biomarker profile of cardiac surgery-induced AKI is likely to be entirely different from that of septic AKI, which will be different from causes such as toxin-induced or obstructive AKI. Therefore, determining profiles that perform well to detect cardiac surgery-related AKI does not necessarily preclude poorly performing biomarkers from being useful in other clinical settings. Additionally, although our study constitutes one of the larger publications to date in terms of the number of biomarkers measured frequently and serially (pre- and postinsult) in a cohort with a common AKI source, it is limited in being a single-center study with a relatively low

Table 4. Biomarker performance at the time of clinical serum creatinine increase

	Stage 1 or 2[a] ($n = 37$)	Stage 3[a] ($n = 9$)	AUC (95% confidence interval)	*P*
Plasma creatinine				
absolute change (mg/dl)	0.360 (0.30 to 0.58)	0.396 (0.28 to 0.52)	0.68 (0.42 to 0.94)	NS
% change from baseline	39.6 (28 to 52)	50.0 (24 to 114)	0.60 (0.33 to 0.87)	NS
CyC				
absolute (mg/L)	0.114 (0.027 to 0.23)	0.272 (0.22 to 0.72)	0.74 (0.53 to 0.96)	0.02
normalized[b] (mg/g)	0.101 (0.040 to 0.32)	0.725 (0.51 to 1.17)	0.77 (0.55 to 0.98)	0.01
NGAL				
absolute (pg/ml)	98.3 (17.0 to 324)	461 (231 to 2047)	0.78 (0.61 to 0.96)	0.02
normalized[b] (ng/mg)	79.7 (14.6 to 37.6)	571 (332 to 6273)	0.78 (0.59 to 0.98)	0.01
KIM-1				
absolute (pg/ml)	672.9 (407 to 2492)	743.4 (576 to 939)	0.49 (0.29 to 0.69)	NS
normalized[b] (ng/mg)	0.987 (0.480 to 1.74)	1.59 (1.02 to 1.83)	0.65 (0.45 to 0.86)	NS
HGF				
absolute (pg/ml)	164.3 (56.2 to 524.5)	209.8 (123.7 to 1344)	0.66 (0.45 to 0.86)	NS
normalized[b] (ng/mg)	0.148 (0.062 to 0.763)	0.601 (0.161 to 3.675)	0.68 (0.47 to 0.89)	NS
α-GST				
absolute (μg/L)	1.29 (0.79 to 2.82)	2.18 (0.38 to 20.9)	0.54 (0.25 to 0.84)	NS
normalized[b] (ng/mg)	1.45 (0.89 to 4.41)	2.93 (0.33 to 88.5)	0.54 (0.26 to 0.82)	NS
π-GST				
absolute (μg/L)	3.4 (3.4 to 10.9)	46.5 (13.5 to 286.3)	0.84 (0.69 to 0.99)	0.003
normalized[b] (ng/mg)	3.69 (2.35 to 11.5)	54.8 (17.0 to 1175.2)	0.86 (0.74 to 0.99)	0.002
FENa (%)	0.312 (0.010 to 0.869)	2.12 (0.23 to 2.395)	0.67 (0.47 to 0.87)	NS
FEUrea (%)	34.5 (14.7 to 38.4)	33.4 (19.2 to 60.8)	0.51 (0.25 to 0.77)	NS

[a]Values presented as median (IQR).
[b]Urine excretion normalized to urinary creatinine concentration.

number of event rates for stage 1 and stage 3 AKI. This issue is only further magnified when making considerations for GFR-stratified biomarker assessments. As such, all of these data need to be further validated in larger multicenter prospective studies.

Our study is further limited by the fact that several of the biomarkers were measured in various research laboratories. Furthermore, although research endeavors such as ours require the utilization of frozen samples and some tests have already compared the performance of a limited number of biomarker results on fresh and frozen samples (18), widespread validation of this process is still needed; of note, all of our samples were stored at −80°C, which decreases the potential for sample degradation (46).

Although we have no question about the validity of the assay measurements themselves, several of the assays in their current forms require several hours to complete, and this is impractical if we are attempting to minimize the time between renal insult and potential therapeutic intervention. Several of the biomarkers in development are measurable by point-of-care devices or standard automated clinical laboratory platforms, but not all of them. As these biomarkers are further validated in larger multicenter studies, we anticipate that more timely and improved assays will be developed.

Despite these limitations, several of the biomarkers investigated in our study (NGAL, CyC, and KIM-1) have repeatedly been shown to predict AKI and patient outcomes in various settings, including postcardiac surgery. Our data further strengthen the case for the utilization of these biomarkers, as well as the continued investigation of the utility of α-GST and π-GST as AKI biomarkers in various clinical settings. Additionally, our data demonstrate the impressive ability of these biomarkers to predict the progression of AKI at the time of clinical creatinine increase, which is the timepoint when the bulk of clinical laboratory testing, differential diagnostic assessment, and management plan formulation currently occurs in practice. Although these data clearly need further validation in various clinical settings, the use of such AKI biomarkers is a potentially valuable clinical strategy that may allow clinicians to aggressively and appropriately treat and triage those patients with impending severe AKI. These biomarkers may further serve to inform clinical decision-making (or as the entry criteria into clinical trials) regarding one of the greatest interdisciplinary challenges in clinical nephrology, "When to initiate RRT?" by correctly identifying those patients with AKI who have severe tubular injury and will require renal support pending recovery. Although our data are consistent with the literature in demonstrating that urinary AKI biomarkers are not the panacea that will correctly identify 100% of all AKI before the current standard serum creatinine, it is becoming increasingly evident that several of these individual biomarkers hold particular promise

to facilitate improved clinical care in patients at risk of or developing AKI.

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Disclosures

J.V.B. is co-inventor on KIM-1 patents that are licensed by Partners Healthcare to Genzyme Corporation, Johnson and Johnson, and Biogen Idec. P.D. is the co-inventor on patent applications covering the use of NGAL as a biomarker of acute and chronic kidney diseases. P.T.M. and J.L.K. are listed on patents for π-GST as a biomarker of AKI severity with Argutus Medical.

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