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Comparative Analysis of Urinary Biomarkers for Early Detection of Acute Kidney Injury Following Cardiopulmonary Bypass

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Abstract

The purpose of this study was to compare the performance of six candidate urinary biomarkers, kidney injury molecule 1 (KIM-1), N-acetyl-β-(D)-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), cystatin C, and α-1 microglobulin, measured 2 hours following cardiopulmonary bypass (CPB) for the early detection of acute kidney injury (AKI) in a prospective cohort of patients undergoing cardiac surgery. 103 subjects were enrolled, AKI developed in 13%. Urinary KIM-1 achieved the highest area under-the-receiver-operator-characteristic curve (AUC = 0.78, 95% CI 0.64-0.91), followed by IL-18 and NAG. Only urinary KIM-1 remained independently associated with AKI after adjustment for a preoperative AKI prediction score (Cleveland Clinic Foundation score; P= 0.02), or CPB perfusion time (P = 0.006). In this small pilot-cohort, KIM-1 performed best as an early biomarker for AKI. Larger studies are needed to further explore the role of biomarkers for early detection of AKI following cardiac surgery.

Keywords

Acute kidney injury; early detection; urinary biomarker; cardiac surgery

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DECLARATION OF INTEREST The authors alone are responsible for the content and writing of the paper. Dr. Bonventre reports that he is a co-inventor on KIM-1 patents (patent numbers 6,664,385 and 7,179,901) that are licensed to Johnson and Johnson.

INTRODUCTION

Acute kidney injury (AKI) is a common complication of cardiac surgery that employs cardiopulmonary bypass (CPB), and is associated with adverse clinical outcomes (Chertow et al., 1998, Bahar et al., 2005). Despite advances in the understanding of its pathophysiology, the clinical management of AKI remains largely supportive. An important reason for this discrepancy is the reliance on serial measurement of serum creatinine for the detection of AKI, which is an imperfect marker in the acute setting. This is related to several factors, including extracellular fluid volume expansion, muscle wasting, and malnutrition, to name a few (Moran and Myers, 1985, Star, 1998, Bonventre and Weinberg, 2003). Animal studies have identified several preventive and treatment strategies for AKI, which have previously been extensively reviewed (Schrier et al., 2004, Lameire et al., 2005, Bellomo et al., 2005). If instituted early in the disease process, well before serum creatinine changes, these novel treatment strategies may be effective. The lack of early markers of kidney injury remains a limiting step for translating these promising findings to human AKI.

In recent years, several urinary markers of tubular damage have been proposed as more accurate alternatives to serum creatinine for the early detection of AKI following cardiac surgery. These markers include neutrophil gelatinase-associated lipocalin (NGAL) (Mishra et al., 2005, Parikh et al., 2006, Bennett et al., 2008), interleukin-18 (IL-18) (Parikh et al., 2006), kidney injury molecule-1 (KIM-1) (Han et al., 2008), and cystatin C (Koyner et al., 2008). Unfortunately, most studies have focused on one or two markers, with variable performance for the early detection of AKI when measured and tested at different time points following CPB.

The objective of this pilot study was to compare the performance characteristics of six of these urinary biomarkers for the early detection of AKI among adults undergoing on-pump cardiac surgery, and explore whether combining such markers enhances their predictive value.

SUBJECTS AND METHODS

Study design and population

This ancillary prospective cohort study was conducted between January 2004 and May 2006 at two tertiary care hospitals located in Boston, Massachusetts, and is part of an ongoing parent study aimed at evaluating genetic risk markers for AKI following cardiac surgery. All consecutive adult subjects (age 18 years or greater) scheduled to undergo on-pump cardiac surgery CPB were eligible for enrollment. Exclusion criteria were age under 18 years, off-pump surgery, pregnancy, long-term or acute dialysis, and organ transplantation within the prior year. Written informed consent was obtained from all study participants or next of kin. The institutional review board of each participating center approved the study protocol, and the study was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki of 1975, as revised in 1983.

Data collection

Medical records were reviewed prospectively to retrieve hospitalization data, including baseline demographic characteristics, pre-operative clinical and laboratory variables, intra-operative variables including surgery electivity and type, CPB perfusion time and aortic cross clamp time, and several postoperative variables including serial serum creatinine measurements. The Cleveland Clinic Foundation (CCF) score (Thakar et al., 2005), an established preoperative AKI risk score was determined for each patient. This score consists of 11 preoperative variables including gender, congestive heart failure, left ventricular ejection fraction, use of intra-aortic balloon counterpulsation, chronic lung disease, insulin-

requiring diabetes mellitus, previous cardiac surgery, emergency surgery, valve surgery, procedures other than coronary bypass or valve, and serum creatinine (Thakar et al., 2005). The Acute Physiology and Chronic Health Evaluation (APACHE) II score (Knaus et al., 1985) was determined the first day following surgery. The baseline estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study 4-variable equation (Levey, 2000).

Predictor variables

We selected six candidate urinary biomarkers, some of which had previously been tested in isolation, for the early detection of AKI. These included KIM-1, a tubular dedifferentiation marker (Ichimura et al., 1998, Vaidya et al., 2006); N-acetyl-β-(D)-glucosaminidase (NAG), a tubular brush border enzyme (Wellwood et al., 1975, Price, 1992); NGAL, an iron-siderophore binding protein (Mishra et al., 2003); IL-18, a marker of inflammation and potentially a mediator of tubular injury (Parikh et al., 2004); and cystatin C, and alpha-1 microglobulin, two markers of impaired tubular protein absorption (Herget-Rosenthal et al., 2004). Urinary biomarker measurements obtained 2 hours following CPB were used to predict AKI, and compared to the CCF score and CPB perfusion time, two known pre- and intra-operative risk factors for AKI.

Outcome measure

The outcome measure was AKI defined by an increase in serum creatinine by ≥ 50% in the first 72 hours following termination of CPB. We chose the 72-hour time point instead of the 48-hour time point that is currently recommended by the AKI Network definition (Mehta et al., 2007), to account for the hemodilution that occurs during CPB. The latter has previously been described in the literature (Rosner and Okusa, 2006). In a sensitivity analysis, *early* AKI was defined as a similar serum creatinine rise in the first 24 hours following CPB.

Urine collection, processing and storage

Urine samples were freshly collected prior to surgery, 2, 24 and 48 hours following discontinuation of CPB. Samples were kept on ice and centrifuged within 30 minutes to remove insoluble elements. The supernatant was treated with a protease inhibitor cocktail tablet (Complete Mini, Roche Applied Science, Indianapolis, IN) to prevent biomarker degradation by urinary proteases. Urinary aliquots were stored at -80°C until assayed. Urinary samples were frozen in small aliquots to prevent frequent freeze and thaw cycles during the measurement phase of the study.

Measurement of urinary biomarkers

Urinary KIM-1 was measured using a previously described sandwich ELISA (Han et al., 2002). In brief, the wells were incubated with 100 μl of urine at room temperature for 3 hours. After 4 washes with PBS-T, biotinylated AKG 7 antibody was added followed by horseradish peroxidase-conjugated strepavidin, and tetramethyl benzidine as substrate. The KIM-1 lower limit of detection was 0.05 ng/ml, and the intra- and inter-assay coefficient of variation (CV) was 9.4% and 12.9%, respectively. There was no interference of the protease inhibitor cocktail with this assay.

Urinary NAG activity was measured using a previously described colorimetric assay (Boehringer Mannheim, Germany) (Liangos et al., 2007). The NAG lower limit of detection was 3 mU/ml, and the inter- and intra-assay CV was 4.3% and 6.0%, respectively.

Urinary NGAL was measured by a human Lipocalin-2/NGAL immunoassay (Quantikine®, R & D systems, Minneapolis, MN). According to the manufacturer, this assay does not cross-react with Lipocalin-1, MMP-9 or the MMP-9/NGAL complex. The NGAL lower

limit of detection was 0.012 pg/ml, and average inter- and intra-assay CV was 6.5% and 4.0%, respectively. 2-hour post-CPB urinary NGAL values that were undetectable were re-assayed after urinary aliquots were first concentrated using Micron centrifugal filter devices (Millipore Corp.), according to the manufacturers' instructions.

Urinary IL-18 was measured using a sandwich ELISA (MBL, Naka-ku Nagoya, Japan). The IL-18 lower limit of detection was 3.5 pg/ml, and the average intra-assay and inter assay CV was 5.0% and 7.5%, respectively.

Urinary cystatin C and α-1 microglobulin were measured by a particle enhanced nephelometric immunoassay using the Behring Nephelometer II System (Dade Behring, Deerfield, IL). The cystatin C lower limit of detection was 0.05 mg/L, and the average intra- and inter-assay CV was less than 2.5% and less than 3.5%, respectively. The α-1 microglobulin lower limit of detection was 5.6 mg/L, and the intra- and inter-assay CV was less than 4.1% and less than 10.3%, respectively.

All measurements were performed in duplicate by blinded investigators (W.K.H., A.K., M.C.P.) except for NAG, which was performed in single measurements. All time points were evaluated and assay protocols were strictly adhered to. For all the biomarker measurements, outliers, as defined by values that were 2 standard deviations above or below the mean, were re-assayed. All the results were normalized to urinary creatinine values and expressed in the respective biomarker unit per mg of creatinine.

Statistical analysis

Continuous variables were described as means (with standard deviation) or medians (with inter-quartile range), as appropriate, and categorical variables as percentages. The Wilcoxon rank sum test was used to compare urinary biomarker levels at each time point between patients with and without AKI.

Test performance characteristics for the early detection of AKI were evaluated for each biomarker using a receiver operator characteristic curve (ROC) analysis. In addition, biomarkers with the best area-under-the-ROC curve (AUC) were combined, biomarkers were compared with the CCF score, and biomarkers with or without the addition of the CCF score and CPB perfusion time were evaluated with the same method. 95% confidence interval for the AUC was calculated, and formal statistical testing was performed using the non-parametric method of DeLong (DeLong et al., 1988).

Optimal cut-off points for early detection of AKI were determined for each biomarker using the Youden index (Youden, 1950), based on which sensitivity, specificity, positive and negative predictive values were calculated. The Youden index is calculated by adding sensitivity and specificity and subtracting 1, which identifies the point on the ROC curve that has the maximum vertical distance to the diagonal or chance line. Logistic regression analysis was also used to examine the predictive value of each biomarker for the early detection of AKI, using the 2-hour post-CPB time point. For these analyses, all biomarkers were log transformed due to a skewed distribution. The analyses were adjusted for the CCF score or CPB perfusion time. Differences were considered statistically significant at a P value of less than 0.05. All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Characteristics of the cohort

A total of 103 study participants were enrolled, and their characteristics are displayed in Table 1. In brief, mean age was 68 years, 96% were white, 28% were women, pre-operative mean left ventricular ejection fraction was 51%, and pre-operative mean serum creatinine was 1.1 mg/dl and baseline estimated GFR 73 ml/min*1.73 m^2. Serial preoperative serum creatinine values remained stable and none of the enrolled subjects had preoperative AKI. Twenty-eight percent of patients underwent elective cardiac surgery, the remainder were categorized as urgent but none were emergent. Mean CPB perfusion and aortic cross clamp time was 111 and 83 minutes, respectively. In all participants, cardioplegia was achieved, after external aortic cross-clamping, through antegrade and retrograde cold blood diastolic cardioplegic arrest. Cardiac function was regained at the end of CPB either spontaneously by reperfusion with warm, circulating blood or through defibrillation with 20 joule. On day-1 following surgery, mean APACHE II score was 9, and postoperative AKI developed in 13 subjects (13%). Serum creatinine profiles throughout the observation time points are shown in Figure 1, indicating similar serum creatinine level prior to CPB as well as excellent and sustained separation between the AKI and non-AKI group following CPB. Preoperative radiocontrast exposure was not associated with development of AKI. Patients who developed AKI had a higher preoperative CCF score, a higher prevalence of pre-existing chronic lung disease and congestive heart failure, and were also less likely to have 3-vessel coronary artery disease but more likely to undergo valvular surgery. In addition, CPB perfusion time was longer and post-operatively, the AKI group had a higher APACHE II score at day-1 and required prolonged assisted mechanical ventilation. Renal replacement therapy was required in only one case, which also fulfilled the serum creatinine criteria for AKI.

Peri-operative urinary biomarker profiles

Figure 2 displays the peri-operative profile of the six urinary biomarkers stratified by AKI. In brief, most urinary biomarkers reached higher median post-operative levels in the AKI group compared to the non-AKI group. However, statistical significance was reached only for KIM-1 and NAG, at the 2-hour ($P = 0.001$) and 48-hour ($P = 0.02$) time point, respectively. There was also a non-significant trend toward higher urinary IL-18 at the 2-hour mark ($P = 0.07$) in the AKI group. In addition, 2-hour urinary IL-18 and cystatin C were negatively correlated with postoperative urine output and positively correlated with postoperative total furosemide dose within the first 24 hours following CPB. 2-hour urinary KIM-1 was also associated with furosemide dose but not with urine output, whereas α-1 microglobulin was correlated with urine output but not with furosemide dose (data not shown).

Urinary biomarker diagnostic performance

Results of the ROC analysis are displayed in Table 2. In brief, among the urinary biomarkers, the 2-hour post-CPB urinary KIM-1 level displayed the highest AUC of 0.78 (95% CI 0.64, 0.91; $P < 0.01$), followed by IL-18, NAG and α-1 microglobulin, which were not significant. When combined, the three best performing biomarkers KIM-1, IL-18 and NAG were only marginally and not significantly better than KIM-1 alone (AUC 0.78; 95% CI 0.62, 0.94; $P < 0.01$). The AUC of the CPB perfusion time (AUC 0.67; 95% CI 0.52, 0.82; $P = 0.02$) was comparable to urinary IL-18, but was lower than that of KIM-1. The CCF score was highly predictive of AKI (AUC 0.83; 95% CI 0.74, 0.91, $P < 0.01$) but did not perform significantly better than KIM-1 ($P = 0.54$). When the CPB perfusion time was combined with the 2-hour post-CPB urinary KIM-1 level, the performance was not better than KIM-1 alone ($P = 0.79$). When the CCF score was combined with KIM-1, the

performance improved substantially over that of KIM-1 alone, reaching statistical significance (P = 0.02).

Table 3 displays the optimal biomarker cut-off values and their corresponding sensitivities, specificities, positive and negative predictive values at the 2-hour post-CPB time point. At a cut-off value of 0.42 ng/mg, urinary KIM-1 displayed the best sensitivity and specificity of 92% and 58%.

In a sensitivity analysis restricted to *early* AKI, as defined by a steeper rise in serum creatinine of equal or greater than 50% within the first 24 hours, the diagnostic performance of the 2-hour post-CPB urinary KIM-1 strengthened with an AUC of 0.91 (95% CI 0.80, 1.00; P < 0.01). Using the urinary KIM-1 optimal cut-off value of 0.95 ng/mg, as determined by the Youden index, the corresponding sensitivity and specificity of this biomarker for detection of *early* AKI improved to 100% and 76%, respectively. Of note, the diagnostic performance of the other 5 urinary biomarkers or of the CCF score, as measured by the AUC, did not significantly change when tested in this sensitivity analysis.

Logistic regression analyses

Results of the logistic regression analyses are displayed in Table 4. On univariate analysis, each two-fold increase in 2-hour post-CPB urinary KIM-1 and IL-18 increased the odds of developing AKI by 1.96-fold (P < 0.01), and 1.38-fold (P = 0.04), respectively. The other urinary biomarkers were not significantly associated with development of AKI. Following adjustment for either the CCF score or the CPB perfusion time, only KIM-1 remained independently associated with development of AKI (Table 4).

DISCUSSION

In this prospective pilot study of adults undergoing on-pump cardiac surgery, we compared the predictive value of 6 candidate urinary biomarkers measured 2 hours following CPB, for the early detection of AKI. Urinary KIM-1 displayed the best diagnostic performance. A type 1 trans-membrane protein, KIM-1 is a tubular dedifferentiation marker that is upregulated in proximal tubules following ischemic and toxic injury, and is detected in human AKI (Ichimura et al., 1998, Han et al., 2002). Shedding of its ecto-domain results in the appearance of a 90 kDa, soluble form of KIM-1 in the urine (Bailly et al., 2002), which has been proposed to be sensitive and highly specific for the detection of AKI in humans (Han et al., 2002, Han et al., 2008).

Other urinary biomarkers tested in the present study performed less well, notably urinary NGAL, which had previously been shown to have an excellent diagnostic performance, particularly among children undergoing cardiac surgery (Mishra et al., 2005, Parikh et al., 2006). More recent studies on the use of urinary NGAL in an adult cardiac surgical population have been less impressive (Wagener et al., 2006, Wagener et al., 2008). The apparent variability in the discriminatory value of urinary biomarkers between children and adults might in part be due to differences in patient characteristics including the burden of co-morbidities in adults undergoing cardiac surgery, especially cardiovascular and chronic kidney disease. Other reasons for discrepancies amongst the urinary NGAL diagnostic studies might be related to differences in the measurement methods employed, including western blots and research-based commercially available ELISA kits, as well as differences in storage times of urine samples.

Interleukin-18 has previously been shown to mediate ischemic injury through activation of apoptosis in renal tubular epithelial cells (Melnikov et al., 2001, Melnikov et al., 2002). Urinary IL-18 performed better in our adult cohort than in a previously reported pediatric

cohort undergoing on-pump cardiac surgery, despite similar peri-operative urinary levels, and a similarly adopted AKI definition (Parikh et al., 2006). Urinary cystatin C and α-1 microglobulin, two tubular protein reabsorption markers that have previously been tested for prediction of dialysis requirement among patients with established non-oliguric AKI (Herget-Rosenthal et al., 2004), did not perform well enough in our study to serve as reliable markers for early detection of AKI. These results contrast with a recent report demonstrating that urinary cystatin C, when measured 6 hours after CPB, is most useful for predicting AKI (Koyner et al., 2008).

Lastly, urinary NAG, a proximal tubular epithelial cell glycosidase that is considered a *legacy* biomarker for detection of kidney injury (Wellwood et al., 1975) has previously been shown to increase in response to cardiac surgery (Gormley et al., 2000), and is a good predictor of adverse outcomes in patients with established AKI (Liangos et al., 2007). Unfortunately, in the present study, similarly to four of the markers, the utility of NAG as an early detection marker was limited, although it strengthened over the observation time interval and displayed fair performance 48 hours post-CPB (data not shown).

There are several strengths and limitations of this study that should be noted. To our knowledge, this is the first study to compare the prediction value of 6 candidate urinary biomarkers for the early detection of AKI. Although our study cohort was relatively small, the sample size is consistent with the majority of the previously published reports of prospective studies of urinary AKI biomarkers (Mishra et al., 2005, Wagener et al., 2006, Parikh et al., 2006). Since our study was ancillary to a parent study not originally designed to examine the predictive value urinary biomarkers for early detection of AKI, only a single early post-CPB time point was available for evaluation. This is an important shortcoming and represents a limitation of our study and contrasts with previous studies that performed more frequent measurements in the first 12 hours following surgery and found better predictive performance of markers at later time points. On the other hand, focusing on a very early post-operative period is relevant, since potentially successful therapeutic interventions for AKI are likely to be beneficial if instituted in this very early postoperative period.

With a total number of 103 patients included in the study and an AKI frequency of 13%, the study is underpowered to provide definitive conclusions on urine biomarker performance. Nevertheless, one of the biomarkers, KIM-1, performed fairly well, even after adjustment for important preoperative variables, and appeared better in direct comparison with the other markers tested. In the present study, we normalized the urinary biomarker levels to urinary creatinine in order to account for differences in relative amounts of water extracted along the nephron. Although the results were attenuated somewhat when absolute urinary biomarker values were used in the analysis, urinary KIM-1 maintained its statistical significance as well as its superiority compared with the other markers without normalization for urinary creatinine (data not shown). The clinical diagnosis of AKI rests on acute changes in serum creatinine, which can be affected by hemodynamic alterations of glomerular filtration rate in the acute postoperative period, and thereby attenuate the relationship of urinary biomarker levels and subsequent change in serum creatinine. Potential discrepancies between presence of kidney injury and changes in serum creatinine are further underscored in our study by the association of urinary biomarkers not predictive of serum creatinine changes, with decreased urine output and requirement for diuretic therapy, both indicators of kidney dysfunction or injury. Nevertheless, serum creatinine remains an accepted and most widely used method for the detection of AKI.

We used a clinical definition that has been widely utilized in previous reports (Mishra et al., 2005, Wagener et al., 2006, Parikh et al., 2006, Bennett et al., 2008). Although patient population heterogeneity could be an important cause for discrepancies between our results

and those published by others in the literature, the current lack of a common validated measurement platform for the tested biomarkers with resulting variable measurement methods, and differences in the definition of AKI are also likely to introduce an additional element of heterogeneity when comparing results across studies. We can only speculate as to whether these methodological heterogeneities might explain the differences in results observed for some of the tested urinary biomarkers between this and other previously published studies. We attempted to optimize the urinary samples through removal of cellular elements, addition of protease inhibitors and storage at -80°C to minimize biomarker degradation, concentration of diluted samples using centrifugal filter devices, and normalizing the biomarker values to urinary creatinine. In addition, all urinary samples were frozen in multiple separate small aliquots to prevent frequent freeze and thaw cycles and subsequent biomarker degradation.

The performance of the CCF score in our cohort merits further discussion. This clinical score predicted AKI very well and although it lacked statistical superiority over KIM-1, it could be viewed as a useful substitute for a biomarker-based method. Its advantages are early availability and low cost, however, its disadvantages include the need to collect 11 variables and calculating a score, which could be cumbersome. In addition, the score is specific for preoperative cardiac surgical patients, whereas biomarkers have the potential to be used in other clinical settings, although this cannot be concluded from the present study.

In conclusion, in the present pilot study, we tested 6 distinct urinary biomarker candidates for their performance characteristics as early detection markers for AKI among adults undergoing on-pump cardiac surgery. When measured 2 hours post-CPB, urinary KIM-1 displayed the best diagnostic performance for detection of AKI, and was followed by IL-18. Urinary NGAL, cystatin C, α-1 microglobulin, and NAG also did not adequately predict AKI at the 2-hour post-CPB time point. A large multi-center cohort study of adults undergoing on-pump cardiac surgery is required to identify the best single or combination of biomarkers, using numerous time points to further refine the optimal timing for early detection of AKI.

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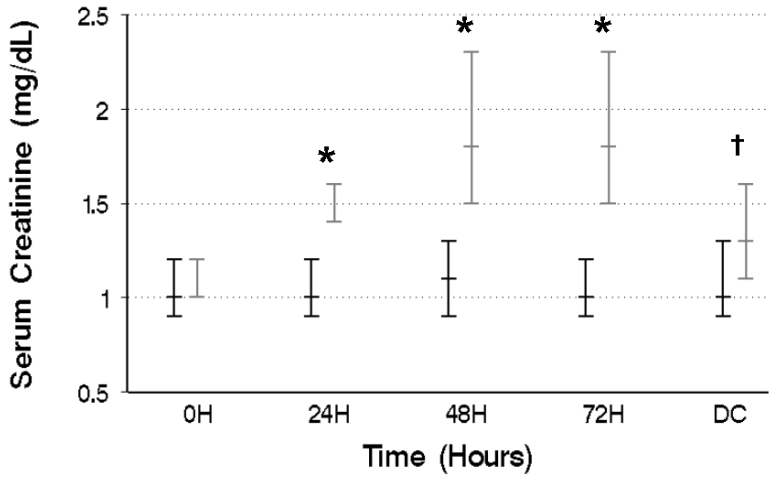

Figure 1.

Peri-operative median serum creatinine profiles stratified by presence or absence of acute kidney injury (AKI). * $P < 0.001$ vs. no AKI; † $P = 0.004$ vs. no AKI. The AKI group is displayed in gray and the non-AKI group in black. Error bars represent 25^{th}, and 75^{th} percentile values. DC denotes the day of hospital discharge.

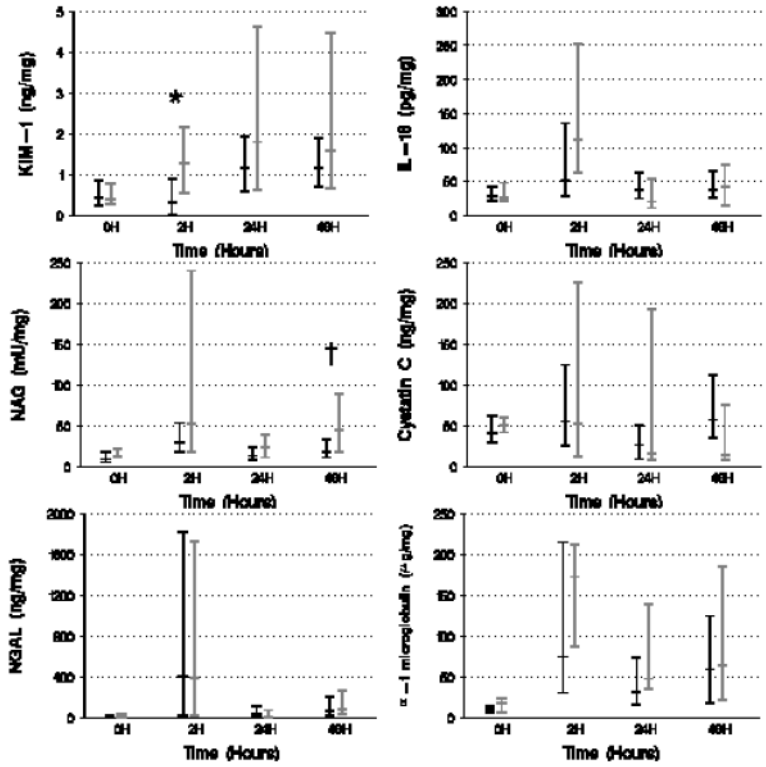

Figure 2.

Peri-operative median urinary biomarker profiles stratified by presence or absence of acute kidney injury (AKI). * P = 0.001 vs. no AKI; † P = 0.02 vs. no AKI. The AKI group is displayed in gray and the non-AKI group in black. Error bars represent 25th, and 75th percentile values.

Table 1

Characteristics of the study cohort

Variable	All patients (N = 103)	No AKI (N = 90)	AKI (N = 13)	P-value
Pre-operative variables				
Age, years	68 (11)	67 (12)	73 (9)	0.15
Women, %	28	28	31	1.00
White ethnicity, %	96	96	100	1.00
Hypertension, %	81	81	77	0.71
Diabetes mellitus, %	29	29	31	1.00
Baseline serum creatinine, mg/dl	1.1 (0.3)	1.1 (0.3)	1.1 (0.2)	0.43
Baseline eGFR, ml/min*1.73m^2	73 (18)	73 (18)	68 (15)	0.32
Chronic lung disease, %	21	18	46	0.02
Congestive heart failure, %	23	18	62	0.002
Peripheral vascular disease, %	20	19	31	0.46
Cerebrovascular disease, %	11	10	15	0.63
3-vessel coronary artery disease, %	52	57	23	0.02
Left main coronary artery disease, %	32	31	39	0.75
Left ventricular ejection fraction, %	51 (12)	52 (12)	47 (16)	0.22
Previous cardiac surgery, %	19	20	15	0.63
Preoperative radiocontrast administration, %	57	57	54	1.00
Preoperative CCF score	2.7 (2.0)	2.5 (1.9)	4.5 (1.2)	< 0.001
Operative variables				
Elective surgery, %	28	28	31	0.82
Valvular surgery, %	40	33	85	0.001
CPB perfusion time, min	111 (44)	107 (41)	137 (52)	0.05
Aortic cross clamp time, min	83 (34)	81 (33)	99 (34)	0.07
Post-operative variables				
Assisted mechanical ventilation, hours	31 (111)	22 (95)	90 (181)	0.01
APACHE II score at day-1	9 (4)	8 (3)	15 (5)	< 0.001

Data are shown are mean (SD) or %, P-value by Wilcoxon rank sum or Fisher exact test, as appropriate; eGFR denotes estimated glomerular filtration rate calculated by the 4-variable Modification of Diet in Renal Disease equation (Levey, 2000), and CCF, Cleveland Clinic Foundation.

Table 2

Area-under-the-receiver-operator-characteristic curve (AUC) of the 2-hour post-CPB urinary biomarker levels for early detection of AKI

Predictor variable	AUC	95% CI
KIM-1	0.78	0.64-0.91
NAG	0.62	0.41-0.83
NGAL	0.50	0.33-0.68
IL-18	0.66	0.49-0.83
Cystatin C	0.50	0.27-0.72
α-1 microglobulin	0.62	0.47-0.76
KIM-1, NAG and IL-18	0.78	0.62-0.94
KIM-1 and CPB perfusion time	0.78	0.64-0.94
KIM-1 and preoperative CCF score	0.88	0.81-0.96

AUC denotes area under curve; CI, confidence interval; CPB, cardiopulmonary bypass; and CCF, Cleveland Clinic Foundation.

Table 3

Urinary biomarker test characteristics based on optimal cut-off value for the 2-hour post-CPB t point for detection of AKI

Predictor variable	Optimal cut-off point *	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
KIM-1 (ng/mg)	0.42	92	58	24	98
NAG (mU/mg)	66	50	79	26	91
NGAL (ng/mg)	166	67	11	15	90
IL-18 (pg/mg)	92	75	66	25	95
Cystatin C (ng/mg)	192	42	86	31	91
α-1 microglobulin (μg/mg)	115	75	58	21	94

* The optimal cut-off point value was generated using the Youden index; CPB denotes cardiopulmonary bypass

Table 4

Association of 2-hour post-CPB urinary biomarker level with development of AKI

Predictor variable	OR	95% CI	P value
Unadjusted			
KIM-1	1.96	1.26, 3.05	0.003
NAG	1.40	0.96, 2.06	0.08
NGAL	1.01	0.87, 1.16	0.93
IL-18	1.38	1.02, 1.88	0.04
Cystatin C	1.02	0.75, 1.39	0.88
α-1 microglobulin	1.33	0.88, 2.01	0.17
Adjusted for preoperative CCF score			
KIM-1	1.69	1.10, 2.59	0.02
NAG	1.25	0.83 1.90	0.29
IL-18	1.29	0.92, 1.82	0.14
Adjusted for intraoperative CPB perfusion time			
KIM-1	1.86	1.20, 2.89	0.006
NAG	1.27	0.84, 1.91	0.25
IL-18	1.28	0.91, 1.78	0.15

Logistic regression models were generated to indicate odds ratios per doubling in the urinary biomarker level; OR denotes odds ratio; CI confidence interval; CPB, cardiopulmonary bypass; and CCF, Cleveland Clinic Foundation.