Relationship of Kidney Injury Biomarkers with Long-Term Cardiovascular Outcomes after Cardiac Surgery

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ABSTRACT
Clinical AKI, measured by serum creatinine elevation, is associated with long-term risks of adverse cardiovascular (CV) events and mortality in patients after cardiac surgery. To evaluate the relative contributions of urine kidney injury biomarkers and plasma cardiac injury biomarkers in adverse events, we conducted a multicenter prospective cohort study of 968 adults undergoing cardiac surgery. On postoperative days 1–3, we measured five urine biomarkers of kidney injury (IL-18, NGAL, KIM-1, L-FABP, and albumin) and five plasma biomarkers of cardiac injury (NT-proBNP, H-FABP, hs-cTnT, cTnI, and CK-MB). The primary outcome was a composite of long-term CV events or death, which was assessed via national health care databases. During a median 3.8 years of follow-up, 219 (22.6%) patients experienced the primary outcome (136 CV events and 83 additional deaths). Compared with patients without postsurgical AKI, patients who experienced AKI Network stage 2 or 3 had an adjusted hazard ratio for the primary composite outcome of 3.52 (95% confidence interval, 2.17 to 5.71). However, none of the five urinary kidney injury biomarkers were significantly associated with the primary outcome. In contrast, four out of five postoperative cardiac injury biomarkers (NT-proBNP, H-FABP, hs-cTnT, and cTnI) strongly associated with the primary outcome. Mediation analyses demonstrated that cardiac biomarkers explained 49% (95% confidence interval, 1% to 97%) of the association between AKI and the primary outcome. These results suggest that clinical AKI at the time of cardiac surgery is indicative of concurrent CV stress rather than an independent renal pathway for long-term adverse CV outcomes.


It is broadly accepted that clinical AKI, defined by an acute rise in the concentration of serum creatinine, is associated with increased risk for cardiovascular disease (CVD) and mortality.1,2 We have previously shown that kidney injury confers increased risk for long-term all-cause mortality among cardiac surgery recipients. In addition, the risk of long-term
mortality was seen even in those with “subclinical AKI,” who are patients without clinical AKI but have high levels of kidney injury biomarkers. Animal studies have suggested that AKI results in distant organ effects, including cardiac injury; however, it is unclear whether these processes occur in humans and directly link AKI to CVD.

The rise in serum creatinine indicating clinical AKI that occurs after cardiac surgery is either caused by structural kidney damage or hemodynamic derangements that reduce GFR without significant injury to the kidney. There are novel urine and plasma biomarkers that can specifically separate these two distinct processes. Urinary biomarkers such as IL-18, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), and liver-type fatty acid-binding protein (L-FABP) are associated with clinical AKI and specifically denote acute damage to the tubular cells. Plasma biomarkers including N-terminal pro-B-type natriuretic peptide (NT-proBNP), heart-type fatty acid–binding protein (H-FABP), high-sensitivity cardiac troponin T (hs-cTnT), cardiac troponin I (cTnI), and creatine kinase MB (CK-MB) are also associated with clinical AKI. These instead track cardiac injury and dysfunction leading to reduced perfusion and hemodynamic imbalance, which could lead to reduced glomerular filtration and increase in serum creatinine. It is unknown which of these two processes, or both, are direct causes of long-term cardiovascular events.

To explore these concepts, we analyzed data from a prospective cohort study of adults undergoing cardiac surgery and examined the relationships among serum creatinine–defined clinical AKI, urinary biomarkers specific for kidney injury, and plasma biomarkers specific for cardiac function. We quantified the independent associations of each individual biomarker with the long-term composite primary outcome of CV events and mortality, as well as with CV events alone. Additionally, we performed mediation analyses to assess the contribution of cardiac injury versus kidney injury on the relationship between clinical AKI and the primary outcome.

RESULTS

The final analytic cohort included 968 adults who underwent cardiac surgery between July of 2007 and December of 2009 (Figure 1). Baseline characteristics of patients stratified by serum creatinine–based AKI status are presented in Table 1. The average age was 73.4 years and 69% were men. Most surgeries were elective (81%) and used cardiopulmonary bypass (90%). The mean preoperative eGFR was 66.5 ml/min per 1.73 m². Three hundred forty eight (36%) patients developed serum creatinine–based AKI after surgery. Patients who developed AKI were more likely to have a history of diabetes, hypertension, and congestive heart failure. All results exhibited no sex-based differences.

Postoperative Creatinine-Based AKI and Risk of CV Events and Mortality

During a median follow-up of 3.8 (3.1–4.6) years, 219 (22.6%) patients experienced the primary composite outcome of CV events or death. Of these, 136 (14.1%) experienced a CV event, and 83 (8.6%) died without a CV event. Figure 2A shows the event rates of the primary composite outcome, after patients were stratified according to AKI stage. AKI Network stages were independently associated with the primary composite outcome. Compared with patients without AKI, the adjusted hazard ratios were 1.99 (95% confidence interval [95% CI],
null
Figure 2. Increased Risk for CV events or death by increasing AKI stage or duration of AKI. Differently dashed lines indicate different AKI stages or duration, as explained in the figure.
plasma cardiac markers had a significant association with the CV outcome (Supplemental Table 3).

**DISCUSSION**

In this established multicenter cohort study of adults undergoing cardiac surgery, we found that severity and duration of postoperative creatinine-defined AKI were strongly associated with CV events and mortality; however, peak postoperative elevations in urinary kidney injury biomarkers were not significantly associated with increased risk after adjusting for confounders. In contrast, peak plasma biomarkers of cardiac injury were indeed associated with the primary outcome, even after adjustment for the same confounders. Mediation analyses estimated that the peak cardiac injury biomarkers explained approximately half of the association between clinical AKI and the primary composite outcome of CV events and mortality, whereas the peak kidney injury biomarkers did not contribute to this relationship (Figure 5). Results were virtually identical when CV events were considered as the sole end point. These results indicate that intrinsic kidney damage, as indicated by specific urine biomarkers, does not appear to be causally related to CV outcomes; rather, our data suggest that the association between serum creatinine–defined AKI and CV events is more likely a result of hemodynamic changes or underlying cardiac injury and dysfunction.

Our findings challenge the prevalent view that AKI itself is causally linked to CVD. A growing number of clinical studies have shown that AKI is associated with long-term cardiovascular outcomes after discharge. Preclinical models have shown that AKI can initiate a systemic inflammatory response and activation of the renin-angiotensin system, which may produce distant organ effects including cardiac cell apoptosis, leukocyte infiltration, myocardial fibrosis, and ultimately, cardiac dysfunction. However, establishing causality in observational studies is difficult, and some have argued that the majority of the observed strong associations between clinical AKI and poor outcomes are caused by residual confounding. This argument is made on the basis of the fact that the kidney is an excellent barometer of cardiac and vascular function. As serum creatinine is currently the routine clinical marker of kidney function, previous studies have typically used acute changes in creatinine to define AKI. These prior studies linking AKI with CVD generally used large registries, and were not able to distinguish the reasons for the change in serum creatinine (prerenal/hemodynamic versus intrinsic AKI). Serum creatinine is routinely measured almost daily in hospitalized patients, and the peak rise in creatinine during a patient’s hospital stay has been consistently associated with long-term outcomes. Without further exploration using biomarkers, acutely impaired kidney health has appeared to be a causal risk factor for CVD. Thus, it is vitally important to utilize more biologic data to parse out this relationship in the clinical arena. Biomarkers of kidney damage are more sensitive for detecting kidney injury and are preferred for prospective analyses. Our study examined the effect of kidney injury on CVD through the measurement of both serum creatinine and novel biomarkers. Our results indicate that the associations between serum creatinine–defined AKI and cardiovascular and mortality risk may not be directly causal, at least in the setting of cardiac surgery.

Our study has several strengths. It is a large, multicenter cohort utilizing several key kidney and cardiovascular biomarkers that were measured from samples collected, stored, and analyzed under standardized protocols. Our findings were consistent across multiple biomarkers and remained consistent after adjustment for multiple covariates, which supports their validity. However, there are also several limitations of our study that merit discussion. We only included patients undergoing cardiac surgery, and thus the relationship between the biomarkers and outcomes may not generalize to patients without a high burden of baseline CV disease or other AKI-prone conditions. Second, because our study was observational, we are not able to draw conclusions about how therapies for kidney injury may affect CVD. Third, the severe AKI defined here by serum creatinine and kidney injury biomarkers may have been less severe than that seen in other cohorts and other settings, and there were fewer numbers of patients with severe AKI.
Despite this, even stage 1 AKI was strongly associated with the primary outcome (adjusted hazard ratio of 2.0; 95% CI, 1.5 to 2.7) and can potentially be used for clinical prediction of future CV disease. Finally, although we adjusted for several potential confounders, our results may have been affected by residual confounding.

In this study, we have analyzed data from the Translational Research Investigating Biomarker Endpoints for AKI (TRIBE-AKI) cohort to demonstrate that the increased risk for CV events and death that is associated with clinically defined AKI does not seem to be mediated by biomarkers of kidney injury, but rather by biomarkers of cardiac injury. Thus, the association of creatinine-defined AKI with cardiovascular risk is more likely explained by underlying cardiac damage and subsequent hemodynamic derangement rather than directly by kidney tubular injury. These findings also suggest that treatments that solely target kidney injury may not improve subsequent cardiovascular prognoses.

### CONCISE METHODS

#### Study Population
The detailed methods of the TRIBE-AKI cohort have been described previously. Between July of 2007 and December of 2009, we prospectively enrolled 1219 adults undergoing cardiac surgery (cardiopulmonary bypass grafting or valve surgery) who were at high risk for AKI at six academic medical centers in North America. The study was approved by the institutional review board of each participating site, and written informed consent was obtained from all participants. Patients who died during hospitalization (n=20) were excluded from the analyses.

#### Sample Collection and Biomarker Assays
Sample collection and processing were performed as described previously. In brief, we collected urine and blood samples preoperatively and then daily, for up to 5 days after surgery. The first postoperative sample was collected within 6 hours of the end of surgery. For the first 24 hours after surgery, urine samples were collected every 6 hours. The remaining daily urine and blood samples were obtained at the time of routine morning blood collection. We stopped sample collection on day 3 in patients who did not experience a significant increase in serum creatinine. Blood samples were collected in EDTA tubes, centrifuged to separate plasma, and subsequently stored at −80°C.

We measured urinary IL-18, NGAL, KIM-1, L-FABP, and albumin as previously described. In plasma, we measured NT-proBNP, H-FABP, hs-cTnT, cTnI, and CK-MB. Peak biomarker values were defined as the highest observed biomarker value within the first 3 days after surgery.

#### Study Variables
Using preoperative creatinine as the baseline creatinine, AKI was defined as a ≥50% or 0.3 mg/dl increase in postoperative serum creatinine, within 7 days of surgery. Severity of AKI was classified by the AKI Network staging criteria on the basis of the peak serum creatinine within 7 days of surgery. We also evaluated duration of AKI, which was defined by the number of days AKI was present after surgery, during hospitalization. Duration of AKI was also stratified into three categories, as published in prior reports.

### Adjusted Hazard Ratios per log-unit increase (95% CI)

**A** Urine Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>1.10 (0.94, 1.28)</td>
</tr>
<tr>
<td>IL-18</td>
<td>1.04 (0.89, 1.21)</td>
</tr>
<tr>
<td>KIM-1</td>
<td>1.19 (0.99, 1.43)</td>
</tr>
<tr>
<td>L-FABP</td>
<td>1.06 (0.95, 1.17)</td>
</tr>
<tr>
<td>NGAL</td>
<td>1.01 (0.92, 1.12)</td>
</tr>
</tbody>
</table>

**B** Plasma Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td>1.11 (0.88, 1.42)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>1.78 (1.49, 2.13)</td>
</tr>
<tr>
<td>hs-cTnT</td>
<td>1.34 (1.09, 1.64)</td>
</tr>
<tr>
<td>cTnI</td>
<td>1.20, (1.02, 1.41)</td>
</tr>
<tr>
<td>H-FABP</td>
<td>1.44 (1.12, 1.86)</td>
</tr>
</tbody>
</table>

Adjusted for STS Score, sex, cardiopulmonary bypass time>120min, non–elective surgery, hypertension, congestive heart failure, preoperative urine albumin-to-creatinine ratio, and cardiac catheterization in the 72 hours before surgery.

**Figure 4.** Peak post-operative plasma biomarkers are independently associated with CV events and mortality, while peak post-operative urine biomarkers are not independently associated with the outcome. Adjusted hazard ratios are displayed here by log-transformed urine biomarkers (A) and plasma biomarkers (B). 95% CIs are shown, with vertical dashed gray lines indicating hazard ratios of 1. n represents the number of patients.
The primary outcome of this study was a composite of cardiovascular events and death. We obtained vital status after discharge using several methods, and cross-referenced when possible. For patients living in the United States, we called patients’ homes, reviewed hospital records, and searched the National Death Index. For Canadian participants, we called patients’ homes and utilized data from the Institute for Clinical Evaluative Sciences. There was complete ascertainment of vital status in the cohort.

CV events were defined as major adverse cardiovascular events: hospitalization for acute coronary syndrome, myocardial infarction, congestive heart failure, coronary bypass, and percutaneous coronary intervention. For patients living in the United States, information about CV events was obtained through linkages with Center for Medicare and Medicaid Services (CMS) databases. Variables used in the probabilistic matching to CMS data included surgery site, surgery date, age, sex, race, admission date, discharge date, death date, and zip code. For Canadian participants, information was obtained from data held at the Institute for Clinical Evaluative Sciences (ICES). These datasets were linked using unique, encoded identifiers and analyzed at ICES. We identified patients with CV events using International Classification of Diseases, 9th and 10th revisions, Ontario Health Insurance Plan, and Canadian Classification of Health Interventions codes (Supplemental Table 4).20–28 These codes have been shown to be highly sensitive, with excellent positive predictive value.29–31 Patients who could not be linked were excluded from the analyses (Figure 1).

Statistical Analyses

Descriptive statistics were reported as mean (SD) or median (interquartile range) for continuous variables, and as frequency (percentage) for categorical variables. Continuous variables were compared with Wilcoxon rank sum tests or Kruskal–Wallis tests, and categorical variables were compared via chi-squared or Fisher exact tests as appropriate.

We used Cox regression models to estimate the associations between AKI, urinary kidney biomarkers, and plasma cardiac biomarkers with the primary composite outcome. We used Schoenfeld residuals to evaluate the proportional hazards assumption. Peak postoperative biomarker concentrations from days 1–3 were modeled as log-transformed (base e) continuous variables. The statistical model adjusted for the following variables: Society of Thoracic Surgery score,32 sex, cardiopulmonary bypass time, nonelective surgery, hypertension, congestive heart failure, preoperative urine albumin-to-creatinine ratio, and cardiac catheterization in the 72 hours before surgery. The STS score is a previously published score to estimate the risk of postoperative dialysis in patients undergoing cardiac surgery and is comprised of preoperative serum creatinine, age, surgery type, diabetes, chronic lung disease, recent myocardial infarction, race, reoperation, New York Heart Association class, and cardiogenic shock. Chronic lung disease was not captured in the TRIBE-AKI Study, thus in calculating the STS score, we assumed that none of the patients had this condition.

Table 2. Mediation analysis using univariable Cox proportional hazards models with the primary composite outcome of cardiovascular events and death

<table>
<thead>
<tr>
<th>Source</th>
<th>Log-Transformed Biomarker</th>
<th>Proportion of Effect Explained (95% CI), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>CK-MB</td>
<td>12 (–17 to 41)</td>
</tr>
<tr>
<td></td>
<td>NT-proBNP</td>
<td>34 (–2 to 70)</td>
</tr>
<tr>
<td></td>
<td>hs-cTnT</td>
<td>21 (–11 to 53)</td>
</tr>
<tr>
<td></td>
<td>cTnI</td>
<td>16 (–14 to 46)</td>
</tr>
<tr>
<td></td>
<td>H-FABP</td>
<td>37 (–3 to 77)</td>
</tr>
<tr>
<td></td>
<td>All plasma</td>
<td>49 (1 to 97)</td>
</tr>
<tr>
<td>Urine</td>
<td>Albumin</td>
<td>8 (–3 to 18)</td>
</tr>
<tr>
<td></td>
<td>IL-18</td>
<td>–3 (–19 to 13)</td>
</tr>
<tr>
<td></td>
<td>KIM-1</td>
<td>0 (–12 to 13)</td>
</tr>
<tr>
<td></td>
<td>L-FABP</td>
<td>12 (–3 to 27)</td>
</tr>
<tr>
<td></td>
<td>NGAL</td>
<td>1 (–12 to 14)</td>
</tr>
<tr>
<td></td>
<td>All urine</td>
<td>1 (–18 to 19)</td>
</tr>
</tbody>
</table>

Figure 5. Schematic displaying that CVD risk is not significantly associated with urine biomarkers but is significantly associated with plasma biomarkers. Solid lines represent associations presented in Figure 3 (AKI to composite outcome of CV events and death) and Figure 3 (biomarkers to composite outcome of CV events and death). Dashed lines represent models explored in mediation analyses and reported in Table 2. CV, cardiovascular; Cr, creatinine.
Mediation analysis was conducted to investigate the strength of evidence that the association between clinical AKI and the composite outcome was a causal effect resulting from direct kidney injury. Using the SAS MEDIATE macro, Cox proportional hazards regression models were fit for the composite outcome with clinical AKI alone and with clinical AKI plus each biomarker. The proportion of effect explained by each biomarker was calculated on the basis of the change to the AKI regression coefficient after adding the biomarker to the Cox regression model, and the variance was derived using the delta method (see Lin et al., Equation 5, page 1519). We performed mediation analyses for each biomarker separately and by sample type (all urine and all plasma), and we used models both with and without covariate adjustment.

Analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC) and R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). All tests of significance were two-sided, with P<0.05 considered significant. Small cell counts are only presented for data collected by the TRIBE-AKI Study, as in order to comply with privacy regulations at the Institute for Clinical Evaluative Sciences for minimizing the chance of identification of a study participant, data numbers of participants are suppressed in the case of five or fewer participants (reported as five or fewer).

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REFERENCES


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