Results from the TRIBE-AKI Study found associations between post-operative blood biomarkers and risk of chronic kidney disease after cardiac surgery

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Patients undergoing cardiac surgery are placed under intense physiologic stress. Blood and urine biomarkers measured peri-operatively may help identify patients at higher risk for adverse long-term kidney outcomes.We sought to determine independent associations of various biomarkers with development or progression of chronic kidney disease (CKD) following cardiac surgery. In this sub-study of the prospective cohort –TRIBE-AKI Study, we evaluated 613 adult patients undergoing cardiac surgery in Canada in our primary analysis and tested the association of 40 blood and urinary biomarkers with the primary composite outcome of CKD incidence or progression. In those with baseline estimated glomerular filtration rate (eGFR) over 60 mL/min/1.73m^2, we defined CKD incidence as a 25% reduction in eGFR and an eGFR under 60. In those with baseline eGFR under 60 mL/min/1.73m^2, we defined CKD progression as a 50% reduction in eGFR or eGFR under 15. Results were evaluated in a replication cohort of 310 patients from one study site in the United States. Over a median follow-up of 5.6 years, 172 patients developed the primary outcome. Each log increase in basic fibroblast growth factor (adjusted hazard ratio 1.52 [95% confidence interval 1.19, 1.93]), Kidney Injury Molecule-1 (1.51 [0.98, 2.32]), N-terminal pro–B-type natriuretic peptide (1.19 [1.01, 1.41]), and tumor necrosis factor receptor 1 (1.75 [1.18, 2.59]) were associated with outcome after adjustment for demographic factors, serum creatinine, and albuminuria. Similar results were noted in the replication cohort. Although there was no interaction by acute kidney injury in continuous analysis, mortality was higher in the no acute kidney injury group by biomarker tertile. Thus, elevated post-operative levels of blood biomarkers following cardiac surgery were independently associated with the development of CKD. These biomarkers can provide additional value in evaluating CKD incidence and progression after cardiac surgery.

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More than 1 million cardiac surgeries are performed annually worldwide.[1,2] Patients receiving cardiac surgery undergo intense physiological stress and are at increased risk of adverse outcomes. Acute kidney injury (AKI) is a frequent complication after cardiac surgery, affecting up to 30% of patients.[1] It is well known that AKI is associated with an increased risk of all-cause mortality as well as adverse cardiovascular outcomes after cardiac surgery.[3–5] AKI has also been increasingly recognized as a major risk factor for chronic kidney disease (CKD).[6] Notably, however, only a fraction of patients who develop AKI progress to CKD whereas some patients who do not develop AKI subsequently develop CKD.[6] It remains unclear how to identify patients at the highest risk of CKD after surgery.

The use of serum creatinine–based definitions of AKI has a number of important limitations when evaluating a long-term outcome such as CKD, particularly in the inpatient setting. Acute changes in serum creatinine may not accurately reflect the severity or nature of kidney injury because of the influence of factors such as age, sex, muscle mass, nutritional

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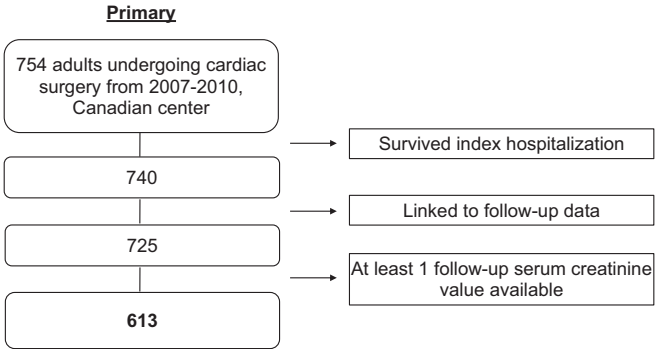

Figure 1 | Flowchart of the study population from the primary cohort.

status, medication effects on creatinine kinetics, i.v. fluid administration, and hemodynamic changes with subsequent oxygen supply-demand mismatch.[7] Therefore, serum creatinine measured in the hospital setting can vary significantly and unpredictably. Furthermore, elevations in serum creatinine are known to occur 48 to 72 hours after the episode of kidney injury.[8]

Previous research has demonstrated that patients with subclinical AKI, namely, those without AKI by serum creatinine but with elevated levels of kidney injury biomarkers, show clear structural kidney injury on histology.[9] Patients with subclinical AKI have higher long-term morbidity and mortality risk than do individuals with biomarker levels in the normal range.[4,10] A number of blood and urine biomarkers reflecting hemodynamic and cardiac function, as well as markers for structural injury, inflammation, and repair, have been investigated previously with long-term cardiovascular outcomes and mortality.[5]

To our knowledge, no studies have investigated the relationship between blood and urine biomarkers of injury, inflammation, or repair with CKD independent of serum creatinine in the setting of cardiac surgery. Therefore, in this study, we aimed to examine the independent associations of biomarkers specific for structural injury, inflammation, and repair with long-term CKD in patients after either coronary artery bypass grafting or valvular cardiac surgery. We hypothesized that kidney injury and repair biomarkers would be associated with either incident CKD in patients with estimated glomerular filtration rate (eGFR) $\geq$60 ml/min per 1.73 m^2 or progression of CKD in patients with GFR $<$60 ml/min per 1.73 m^2 whereas cardiac biomarkers would not have any significant independent associations with the primary outcome.

RESULTS

Study population

After excluding 127 participants with missing data during follow-up or unable to link to follow-up data (17.2%), the analytical population consisted of 613 patients in the primary cohort (Figure 1). There were no significant differences in baseline characteristics in those with versus without follow-up serum creatinine values available (Supplementary Table S1). Table 1 outlines the baseline characteristics of participants by composite primary outcome in the primary cohort.[11] The mean age of patients at the time of surgery was 71.0 $\pm$ 8.8 years, and 168 patients (27%) were female. The mean baseline eGFR was 71.0 $\pm$ 18.2 ml/min per 1.73 m^2, and there were no significant differences in baseline eGFR between participants who developed the primary outcome and those who did not. Similarly, there were no significant differences in baseline hypertension, congestive heart failure, and prior myocardial infarction or differences in surgery type and indication.

Results from the primary cohort

Over a median follow-up of 5.6 years (interquartile range, 4.3–8.6 years), 172 patients (28%) developed the primary outcome of CKD incidence or progression at a rate of 53.2 per 1000 person-years on the basis of at least 1 follow-up serum creatinine measurement. We noted a higher rate of the primary outcome in patients with increasing AKI stage, from 96 patients without inhospital AKI developing the primary outcome (23.8%) to 24 patients with stage 2 or 3 AKI developing the primary outcome (50%).

Of the 172 patients who developed the primary outcome, 144 patients (84%) had at least 2 serum creatinine measurements during follow-up spaced $\geq$90 days apart. In total, a median of 21 (interquartile range, 12–34) serum creatinine values were measured per patient over the course of follow-up, with those who developed AKI stages 2 or 3 having notably more follow-up creatinine values (31; interquartile range, 13–34).

Forty blood and urine biomarkers were analyzed for the association with the primary outcome. In unadjusted analyses after natural log transformation, higher postoperative values of blood basic fibroblast growth factor (bFGF), interleukin-2, interleukin-10, kidney injury molecule-1 (KIM-1), N-terminal pro–B-type natriuretic peptide (NT-proBNP), tumor necrosis factor receptor 1 (TNF-r1), vascular endothelial growth factor receptor 1, and YKL-40 were significantly associated with the primary outcome (Table 2). After adjustment for age, sex, AKI stage, preoperative albuminuria, preoperative serum creatinine, and discharge serum creatinine, the levels of biomarkers bFGF, NT pro-BNP, and TNF-r1 remained significantly associated with an increased risk of the CKD incidence or progression (Table 2). Additionally, KIM-1 was associated with an increased risk of CKD incidence or progression that was approaching statistical significance (adjusted hazard ratio, 1.51; 95% confidence interval, 0.98–2.32; $P = 0.07$). The association of these 4 postoperative biomarkers with the primary outcome was then evaluated by tertiles, with the first tertile serving as the reference group. In categorical analysis, only participants in the highest tertile of bFGF had a significantly higher risk of the primary outcome than did those in the lowest tertile (hazard ratio, 1.89; 95% confidence interval, 1.26–2.82) (Supplementary Table S2). Adjustment for preoperative biomarker levels yielded similar point estimates for bFGF, KIM-1, NT pro-BNP, and TNF-r1, but with wider confidence intervals such that NT-proBNP no

Table 1 | Baseline demographic characteristics of the primary cohort

Variable	All (N = 613)	No primary outcome (n = 441)	Developed primary outcome (n = 172)	P
Age at the time of surgery, yr	70.97 ± 8.84	70.68 ± 9.14	71.70 ± 8.01	0.20
Sex, female	168 (27.4)	113 (25.6)	55 (32.0)	0.11
Race, White	589 (96.1)	427 (96.8)	162 (94.2)	0.13
Diabetes	256 (41.8)	173 (39.2)	83 (48.3)	0.03
Hypertension	484 (79.0)	345 (78.2)	139 (80.8)	0.20
Congestive heart failure	62 (10.1)	40 (9.1)	22 (12.8)	0.11
Left ventricular ejection fraction <40%	48 (7.8)	29 (6.6)	19 (11.0)	0.048
Previous myocardial infarction	173 (28.2)	123 (27.9)	50 (29.1)	0.26
eGFR, ml/min per 1.73 m^2	71.00 ± 18.23	70.79 ± 19.32	71.55 ± 15.12	0.64
eGFR, ml/min per 1.73 m^2				
>60	447 (72.9)	301 (68.3)	146 (84.9)	<0.001
<60	165 (26.9)	140 (31.7)	25 (14.4)	
Serum creatinine, mg/dl	1.03 ± 0.31	1.04 ± 0.31	1.01 ± 0.32	0.25
Urine microalbumin, preoperative, >30 mg/g	365 (59.5)	248 (56.2)	117 (68.0)	0.027
STS score[11]	9.08 ± 3.49	8.99 ± 3.52	9.33 ± 3.40	0.27
Elective surgery	561 (91.5)	404 (91.6)	157 (91.3)	0.28
Isolated CABG or valve surgery	480 (78.3)	347 (78.7)	133 (77.3)	0.75
Off-pump	69 (11.3)	53 (12.0)	16 (9.3)	0.20
Reoperation	6 (1.0)	—	—	—
Perfusion time, min	107.12 ± 57.93	106.93 ± 57.57	107.63 ± 59.02	0.89
Cross-clamp time, min	71.59 ± 43.86	71.89 ± 43.48	70.80 ± 44.97	0.78
AKIN stage				
0	403 (65.7)	307 (69.6)	96 (55.8)	0.001
1	186 (30.3)	122 (27.7)	64 (37.2)	
2 or 3	24 (3.9)	12 (2.7)	12 (7.0)	
AKI duration, d				
1–2	143 (23.3)	94 (21.3)	49 (28.5)	0.015
3–6	50 (8.2)	31 (7.0)	19 (11.0)	
>6	16 (2.6)	9 (2.0)	7 (4.1)	
Last serum creatinine before discharge	1.03 ± 0.45	1.01 ± 0.31	1.07 ± 0.67	0.15

AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; CABG, coronary artery bypass graft; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; STS, Society of Thoracic Surgeons.
Data are expressed as mean ± SD and n (%) for the continuous and categorical variables, respectively.
Dash indicates small cell counts that cannot be presented because of privacy concerns.
The primary outcome was a composite of CKD incidence or progression: CKD incidence (preoperative eGFR ≥60 ml/min per 1.73 m^2): 25% reduction in eGFR and a fall below 60 ml/min per 1.73 m^2; CKD progression (preoperative eGFR <60 ml/min per 1.73 m^2): 50% reduction in eGFR or a fall below 15 ml/min per 1.73 m^2.

longer reached statistical significance (Supplementary Table S3).

Results from the replication cohort

Supplementary Table S4 lists the baseline characteristics of the replication cohort, on the basis of data from the largest US center, in the Translational Research Investigating Biomarker Endpoints in Acute Kidney Injury cohort. Over a median follow-up of 6.5 years (interquartile range, 4.2–8.6 years), 60 patients (19%) developed the primary outcome of CKD incidence or progression at a rate of 61.3 per 1000 person-years on the basis of at least 1 follow-up serum creatinine measurement. In addition to bFGF, NT pro-BNP, and TNF-r1, we evaluated the associations between KIM-1 and our primary outcome in the replication cohort, given its established association with kidney injury from prior literature and the strength of association relative to other biomarkers. Similar to the primary cohort, higher postoperative blood levels of bFGF, KIM-1, and NT pro-BNP remained significantly associated with the primary kidney outcome after adjustment in the replication cohort (Supplementary Table S5). Increases in postoperative TNF-r1 concentration were not significantly associated with an increased risk of CKD incidence or progression (hazard ratio, 1.73; 95% confidence interval, 0.94–3.18). Patients in the third tertile of all 3 biomarkers had a significantly higher risk than did those in the first tertile (Supplementary Table S5).

Table 2 | Risk of CKD incidence or progression by postoperative biomarker level

Blood biomarkers (natural log transformed)	n	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
bFGF	612	1.50 (1.20–1.87)	1.52 (1.19–1.93)[a]
KIM-1	612	1.55 (1.14–2.10)	1.51 (0.98–2.32)[b]
NT-pro-BNP	387	1.21 (1.05–1.39)	1.19 (1.01–1.41)[a]
TNF-r1	612	1.77 (1.26–1.49)	1.75 (1.18–2.59)[a]
IL-10	612	1.14 (1.01–1.29)	1.11 (0.98–1.27)
IL-2	612	1.19 (1.01–1.40)	1.08 (0.91–1.28)
VEGFr1	612	1.31 (1.08–1.59)	1.21 (0.98–1.49)
YKL-40	612	1.27 (1.03–1.56)	1.10 (0.88–1.38)

bFGF, basic fibroblast growth factor; CI, confidence interval; CKD, chronic kidney disease; HR, hazard ratio; IL-2, interleukin-2; IL-10, interleukin-10; KIM-1, kidney injury molecule-1; NT-proBNP, N-terminal pro–B-type natriuretic peptide; TNF-r1, tumor necrosis factor receptor 1; VEGFr1, vascular endothelial growth factor receptor 1.
Adjusted for age, sex, acute chronic injury stage, preoperative albuminuria, preoperative serum creatinine, and discharge serum creatinine.
[a]$P < 0.05$.
[b]$P \leq 0.07$.

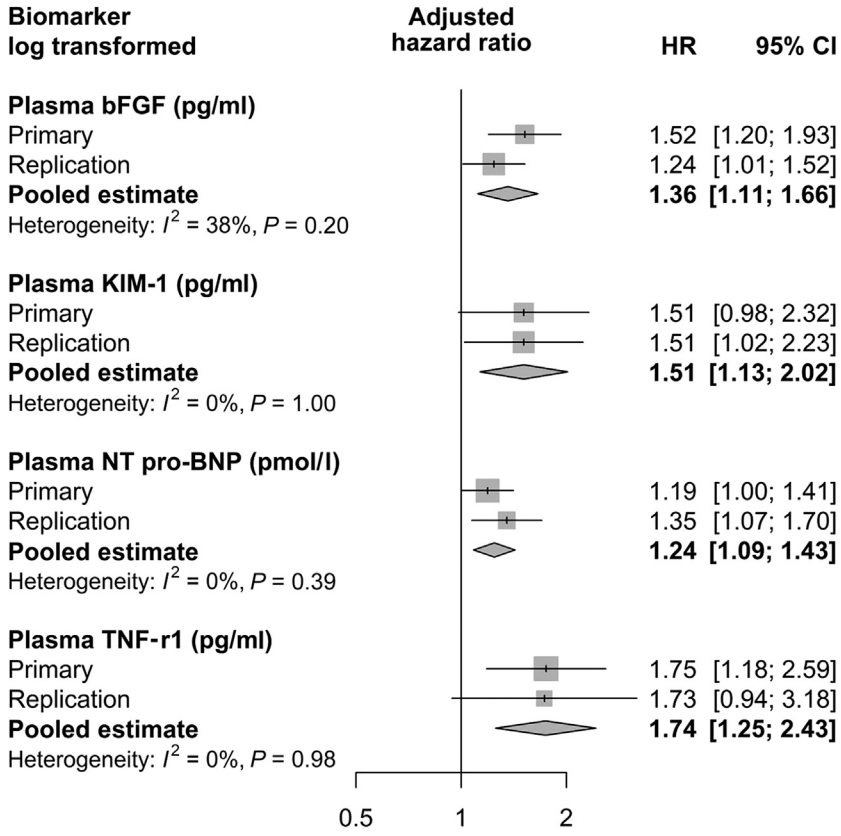

Figure 2 | Forest plot with the pooled hazard ratio of chronic kidney disease incidence or progression by postoperative biomarker level in primary and replication cohorts. bFGF, basic fibroblast growth factor; CI, confidence interval; HR, hazard ratio; KIM-1, kidney injury molecule-1; NT-proBNP, N-terminal pro–B-type natriuretic peptide; TNF-r1, tumor necrosis factor receptor 1.

In a meta-analysis of both cohorts, after natural log transformation, the pooled hazard ratios for all 4 biomarkers were significantly associated with our primary outcome (Figure 2).

Additional analyses

There were a total of 78 deaths (12.7%) in the primary cohort and 31 deaths (10%) in the replication cohort. We performed a competing risk analysis of death in the primary cohort. There was no significant difference in the primary outcome after accounting for competing risk of death using the Fine and Gray subdistribution model (Supplementary Table S6). We performed interaction testing to evaluate the effect of clinical AKI. There was no significant interaction between the biomarker level and our primary outcome by AKI status in continuous analysis. However, in categorical analysis, patients without clinical AKI in the highest tertile of bFGF, KIM-1, and TNF-r1 had similar or higher rates of the primary outcome than did patients with clinical AKI in the lowest tertiles (Figure 3). We additionally evaluated for interaction by preoperative CKD status and did not find any significant difference in outcome for any of the 4 biomarkers (Supplementary Table S7). Notably, the analysis of TNF-r1 produced wide confidence intervals, though the interaction P value was not significant, likely as a result of limited power for this analysis. Within each cohort, we examined model performance by calculating the net reclassification index for models with biomarker measurements added to a base model with clinical parameters alone (Supplementary Table S8). In sensitivity analysis, we additionally examined the association between the peak postoperative biomarker level (Supplementary Table S9) and the mean postoperative biomarker level (Supplementary Table S10) with CKD incidence or progression. We found generally similar results to our primary analysis using first postoperative biomarker levels.

DISCUSSION

In this prospective cohort study of adults undergoing cardiac surgery, we evaluated 40 blood and urine biomarkers and found that elevated postoperative levels of blood bFGF, KIM-1, NT pro-BNP, and TNF-r1 were independently associated with an increased risk of CKD incidence or progression regardless of AKI status after surgery.

It is well established that patients who develop AKI are at a higher risk of long-term adverse outcomes including mortality and cardiovascular disease than those who do not.[6,12,13] In recent years, several studies have evaluated the risk of

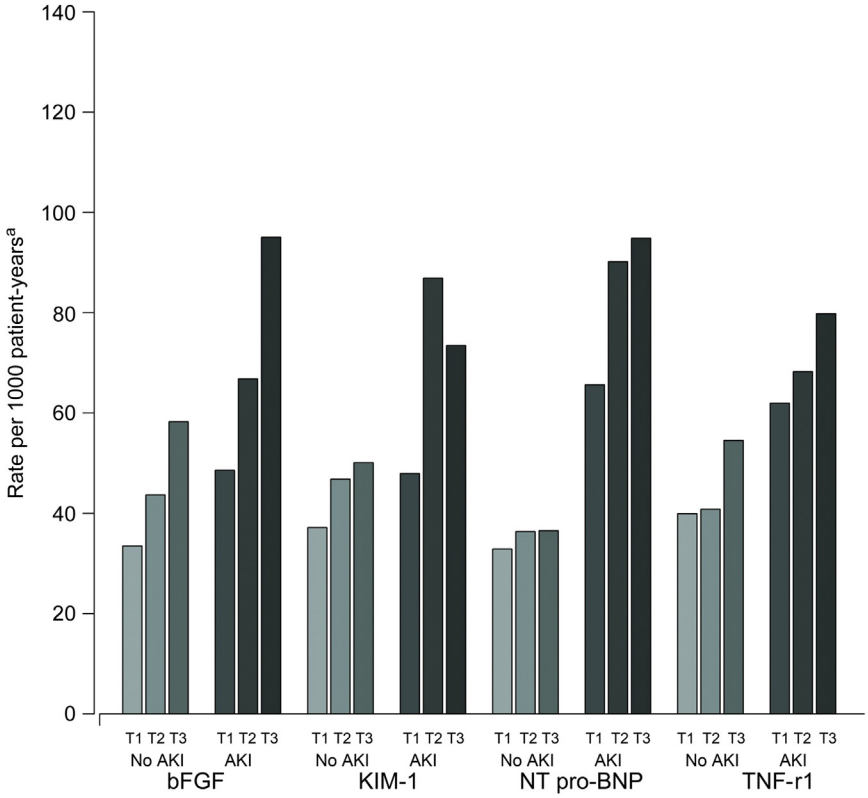

Figure 3 | Chronic kidney disease incidence or progression event rates per 1000 patient-years by biomarker tertile and clinical acute kidney injury (AKI) status. [a]The event rates in the highest tertile of the injury biomarkers (basic fibroblast growth factor [bFGF], kidney injury molecule-1 [KIM-1], and tumor necrosis factor receptor 1 [TNF-r1]) in patients without clinical AKI are similar to the event rates in the lowest tertile of the biomarker in those with clinical AKI. The event rates for all tertiles of N-terminal pro–B-type natriuretic peptide (NT-proBNP) are lower in patients without clinical AKI than in those with clinical AKI. T1, tertile 1; T2, tertile 2; T3, tertile 3.

cardiovascular outcomes after subclinical AKI, with elevated levels of kidney injury or repair biomarkers in the setting of normal serum creatinine.[9,10] Elevations in biomarkers of injury, inflammation, and repair have been associated with the long-term risk of overall mortality and cardiovascular disease.[3,5,14,15] This is the first study to evaluate a large number of blood and urine biomarkers establish their association with the increased risk of CKD incidence or progression in a cardiac surgery cohort.

There are a number of plausible mechanisms by which levels of bFGF, KIM-1, NT pro-BNP, and TNF-r1 can associate with CKD incidence or progression. bFGF, also referred to as FGF2, is a member of the fibroblast growth factor family distinct from FGF-21 and FGF-23. Similar to other members of the FGF family, bFGF serves multiple roles in cellular differentiation and function through various signaling pathways, several of which include inhibition of bone mineralization, angiogenesis, and cell proliferation.[16] bFGF has been implicated in the response to inflammation by upregulation of endothelial cell adhesion molecules as well, with chronic inflammation being a known factor in CKD progression.[17]

KIM-1 is a transmembrane protein located in the proximal tubule of the kidney, and its expression is significantly upregulated in the setting of proximal tubular injury.[18,19] Urinary KIM-1 has been shown to be a robust marker for acute tubular injury, with localization to the proximal tubule, in both animal and human studies.[18–20] However, elevated blood levels of KIM-1 have been associated with the progression to CKD in patients with type 1 diabetes mellitus and even in healthy adults.[21,22]

NT pro-BNP has also been independently associated with the progression of CKD.[23] Elevations in NT pro-BNP may help distinguish patients at increased risk of a cardiorenal phenotype of CKD.[24] One study in patients with type 2 diabetes demonstrated the association between both TNF-r1 and NT pro-BNP with the progression of CKD.[25] These findings are consistent with these prior studies supporting the association between elevated levels of these markers with kidney function decline.

TNF-r1 serves as a cell membrane receptor that binds TNF-α and accentuates endothelial inflammation. TNF-r1 has been associated with the progression to end-stage kidney disease and mortality in patients with type 1 and type 2 diabetes mellitus beyond established risk factors including proteinuria.[26,27] Elevated levels of TNF-r1 have been implicated in other kidney diseases including various glomerulonephritides, obstructive kidney injury, and

kidney transplant rejection.[26,27] The presence of higher circulating TNF-r1 levels is an acute and sensitive marker for inflammation and is upregulated in the setting of elevated TNF-α. We did see a loss of significance evaluating the risk of the primary outcome by TNF-r1 level in the replication cohort after full adjustment. However, the direction of the association was similar to what was observed in the primary cohort, and the loss of significance may be due to sample size limitations.

These findings suggest that these 4 biomarkers are associated with CKD incidence or progression even after adjustment for serum creatinine and AKI stage. Moreover, our subgroup analysis evaluating patients by clinical AKI status indicated that even without clinical AKI, patients in the highest tertile of bFGF, KIM-1, and TNF-r1 had a similar or higher risk of the primary outcome than did those with clinical AKI who had lower postoperative biomarker levels. These results further highlight the importance of using biomarkers to detect subclinical AKI, given its association with CKD incidence or progression. The results of this research may have practical applications in follow-up care after hospital discharge. Patients at a higher risk of CKD incidence or progression could be seen sooner and, more frequently, with greater emphasis on reducing other known risk factors for CKD. Furthermore, these patients represent a unique subset of individuals with a known high risk of CKD and could be enrolled in clinical trials to evaluate interventions to reduce or prevent CKD.

This study has a number of strengths. With the use of 2 cohorts with unique follow-up after discharge from surgery, we were able to replicate findings from the primary cohort. We were also able to evaluate a large number of biomarkers. One strength of this study is the measurement of 40 blood and urine biomarkers at multiple time points postoperatively. We were therefore able to perform additional analyses evaluating the association between peak and mean biomarker levels with CKD incidence or progression, finding generally similar results to our primary analysis and demonstrating that biomarkers measured immediately after cardiac surgery are equally informative. This study benefits from the use of high-quality data, sample collection, processing, and retention beyond hospital discharge and short-term follow-up.

There are a number of limitations in this study. The replication cohort had a relatively small final analytical population because of challenges in obtaining postdischarge laboratory data across multiple centers. This loss to follow-up may not be at random and could have biased our cohort toward inclusion of those at a higher risk of CKD. Similarly, we were able to evaluate only our primary outcome as a composite of CKD incidence or progression, and not as individual outcomes, because of concerns with adequate power. Our clinical AKI definition incorporated serum creatinine at baseline but did not account for urine output over a 48-hour window. Most participants in the Translational Research Investigating Biomarker Endpoints in Acute Kidney Injury Study were male and White, limiting the generalizability of our findings. Also, on average, participants in both cohorts were relatively older, with a mean age above 70 years. Furthermore, we did not have information to ascertain the phenotype of CKD over the course of follow-up in those who had a decline in kidney function. The definition of incident CKD was based on the first serum creatinine value that satisfied the end point. We additionally do not have information on the etiology of CKD in patients on follow-up. Although we were able to obtain serum creatinine levels over the course of follow-up, we do not have information on biomarker levels throughout follow-up to evaluate how these may have changed over time. Finally, because serum creatinine values were primarily followed clinically and not in a protocolized fashion across centers, there is some concern for ascertainment bias.

In summary, we provide evidence for an association between postoperative biomarker levels of blood bFGF, KIM-1, NT pro-BNP, and TNF-r1 with CKD incidence or progression, independent of serum creatinine or AKI stage during hospitalization. In the future, measurement of these biomarkers in the immediate postoperative setting may help guide management of patients after cardiac surgery and other procedures who are at a higher risk of CKD outcomes and who could benefit from closer outpatient follow-up.

METHODS

Study design and data sources

We conducted a substudy of the Translational Research Investigating Biomarker Endpoints in Acute Kidney Injury Study, a longitudinal prospective cohort study of adults who underwent cardiac surgery in academic centers in North America. The detailed study enrollment methods have been described previously.[14] The study was approved by the institutional review board of each participating site, and written informed consent was obtained from all participants.

Population

Adults undergoing cardiac surgery, either coronary artery bypass grafting or valvular surgery, at a high risk of AKI were prospectively enrolled between July 2007 and December 2010.[14,28] Data from the largest Translational Research Investigating Biomarker Endpoints–affiliated academic medical center in Canada were used for our primary analysis, given the relatively larger sample size and near complete ascertainment of postdischarge laboratory data compared to other centers. Patients enrolled in the Canadian academic medical center who had consented to linkage with administrative data for long-term follow-up and had at least 1 follow-up serum creatinine measurement after discharge were included in the primary cohort. This patient cohort had rigorous follow-up of most patients because of its universal publicly funded health care system. Data from 1 US center was additionally included with available postdischarge laboratory data for replication.

Sample collection and biomarker measurement

Details of sample collection and processing have been described previously.[14] Urine and blood samples were collected preoperatively and then daily for up to 5 days after cardiac surgery. Immediate postoperative urine and blood samples were collected within 6 hours of the conclusion of the patient's surgery. Blood samples were

collected in ethylenediamine tetraacetic acid tubes, centrifuged to separate plasma, and subsequently stored at −80 °C. Seven urine biomarkers and 33 blood biomarkers were measured as previously described (Supplementary Table S11).[14,28–30] All analyses for the present study use the immediate postoperative biomarker measurements to minimize missingness across biomarkers that were present in subsequent sample collections. In the primary cohort, AKI stage was not available for 1 participant, preoperative albuminuria measurement for 2 participants, and discharge serum creatinine for 8 participants.

Covariate measurement

Clinical AKI was defined as an increase of ≥50% or 0.3 mg/dl in serum creatinine within the index hospitalization, with preoperative serum creatinine serving as baseline. All preoperative serum creatinine values were measured within 2 months before surgery. Serum creatinine values were obtained during routine clinical care for every patient throughout the hospitalization. eGFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration Equation.[31] The severity of AKI was classified using the Acute Kidney Injury Network staging criteria on the basis of the peak serum creatinine within the index hospitalization.[32] The Society of Thoracic Surgeons risk scores were calculated on the basis of pre- and postoperative details including demographic characteristics, surgical details, and postoperative complications based on prior literature.[11]

Outcomes

The primary outcome of the study was a composite of CKD incidence or progression. In those individuals with eGFR ≥60 ml/min per 1.73 m^2 preoperatively, *CKD incidence* was defined as a 25% reduction in eGFR and a fall below 60 ml/min per 1.73 m^2. In those individuals with eGFR <60 ml/min per 1.73 m^2 preoperatively, *CKD progression* was defined as a 50% reduction in eGFR or a fall below 15 ml/min per 1.73 m^2. These definitions were based on established cutoffs as outlined by the multicenter Assessment, Serial Evaluation, and Subsequent Sequelae of Acute Kidney Injury Study.[33,34]

In the primary cohort, vital status was obtained for all patients from the Registered Persons Database. Follow-up serum creatinine values were obtained using the Ontario Laboratories Information System, a province-wide integrated laboratory database incorporating outpatient and inpatient test results, with serum creatinine values available from 2007 to 2015. These data sets were linked using unique encoded identifiers and analyzed at ICES. Participants still alive on September 30, 2015, without the primary outcome were censored because of the end of data availability.

For patients in the replication cohort, follow-up creatinine measurements were ascertained through the Yale Joint Data Analytics Team's Helix data repository, which includes data from all Yale-New Haven Health–affiliated hospitals and outpatient practices, with serum creatinine values available from 2012 to 2018. Participants were censored at the time of their last serum creatinine measurement. In a subset of all participants (Canadian and American), home visits were done in the first year after discharge and samples for serum creatinine were collected.

Statistical analysis

Descriptive characteristics were reported using mean ± SD or median (interquartile range) for continuous variables and frequency (percentage) for categorical variables. First postoperative biomarker levels were modeled continuously (after natural log transformation) in unadjusted analysis, and those that were statistically significant were also modeled categorically as tertiles, with the first tertile serving as a reference group. Tertiles were defined on the basis of biomarker levels within each cohort. Cubic spline plots were used to explore the functional relationship of the biomarker with the outcome (Supplementary Figure S1). Models were adjusted for age, sex, AKI stage, preoperative albuminuria, preoperative serum creatinine, and discharge serum creatinine. The selection of these variables was based on the related work investigating progression to CKD after an episode of AKI, which showed strong predictive ability using those 6 variables alone.[35] Cox proportional hazards regression was used to examine the cause-specific association between the postoperative biomarker levels immediately after surgery and the primary outcome. Kolmogorov-type supremum tests were used to evaluate proportional hazards assumptions for all models. The subset of biomarkers with statistical significance after adjustment and those with the strongest point estimates were examined in the US replication cohort. This strategy facilitated selection of the most promising biomarkers among the 40 candidate blood and urine biomarkers measured postoperatively, thereby reducing resubstitution and model selection biases, largely addressing the concern of multiple testing.[36] We additionally estimated subdistribution hazard ratios using the Fine and Gray subdistribution hazard model, accounting for the competing risk of death. We performed additional analysis evaluating the interaction between AKI and our primary outcome as well as between preoperative CKD and our primary outcome.

We combined the results of the 2 cohorts and used the I^2 test statistic to quantify the magnitude of heterogeneity. A pooled estimate was used for all comparisons where the Q test was not statistically significant. All pooled hazard ratio estimates were calculated using the random effects meta-analysis method. All analyses were performed with SAS (version 9.4, SAS Institute, Cary, NC), Stata (version 14, StataCorp LLC, College Station, TX), and R (version 3.1.2, R foundation for Statistical Computing, Vienna, Austria). All tests of statistical significance were 2-sided, with $P < 0.05$ considered significant.

DISCLOSURE

MGS has received grant support from Cricket Health, has received consultancy fees from the University of Washington, and has equity in TAI Diagnostics and Cricket Health. SGC and CRP are members of the advisory board of Renalytix AI and own equity in the same. In the past 3 years, SGC has received consulting fees from Goldfinch Bio, CHF Solutions, Quark Biopharma, Janssen Pharmaceuticals, Takeda Pharmaceuticals, and Relypsa. JLK has received research fees from Bioporto and Astute Medical and consulting fees from Baxter, Astute Medical, and SphingoTec. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

SM and CRP led all stages of the work in collaboration with DGM, AXG, HT-P, EM, YJ, CL, WO, SGM, JLK, MGS, FPW, and SGC. AXG, SGC, and CRP were responsible for the study design. HT-P, EM, YJ, and CL contributed significantly to data analysis and preparation of figures. All authors contributed to drafting and critical revision of the manuscript for intellectual content before approving the final version.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Comparison of baseline demographics in patients with vs. without follow-up serum creatinine measurements.
Table S2. Risk of CKD incidence or progression by post-operative biomarker tertiles.
Table S3. Risk of CKD incidence or progression by post-operative biomarker level, adjusted for pre-operative level.
Table S4. Baseline demographics, replication cohort.
Table S5. Risk of CKD incidence or progression by post-operative biomarker level, replication cohort.
Table S6. Risk of CKD incidence or progression by post-operative biomarker level using sub-distribution hazard model, primary cohort.
Table S7. Evaluation for interactions between biomarker level and pre-operative CKD on the risk of CKD incidence or progression.
Table S8. Model performance using net reclassification index.
Table S9. Risk of CKD incidence or progression by peak biomarker level, primary cohort.
Table S10. Risk of CKD incidence or progression by mean biomarker level, primary cohort.
Table S11. Biomarker measurement details.
Figure S1. Post-operative biomarker spline plots using 3 cubic spline knots.

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