

Association of T Cell–Derived Inflammatory Cytokines With Acute Kidney Injury and Mortality After Cardiac Surgery



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Introduction: Animal models of renal ischemia-reperfusion injury (IRI) demonstrate that interferon (IFN)- γ producing T-helper (Th)-1 cells worsen acute kidney injury (AKI), whereas interleukin (IL)-4– and IL-13–producing Th2 cells lead to repair. We tested the association of these cytokines with AKI and mortality in patients who underwent cardiac surgery.

Methods: In 1444 participants of a multicenter, prospective, observational cohort, we measured 10 plasma biomarkers before and after cardiac surgery (IFN- γ , IL-4, IL-13, tumor necrosis factor [TNF]- α , IL-1 β , IL-2, IL-6, IL-8, IL-10, and IL-12p70) and combined these biomarkers using principal component analysis (PCA). We also tested independent associations of Th1 (IFN- γ) and Th2 (IL-4 and IL-13) biomarkers with clinical outcomes of postoperative AKI and 1-year mortality.

Results: AKI occurred in 492 participants (34%), and 1-year mortality occurred in 81 participants (6%). Within 6 hours after surgery, IFN- γ , IL-4, and IL-13 increased 2.1-, 6.0-, and 4.6-fold, respectively, from their preoperative levels. Patients with higher levels of IFN- γ had higher odds of AKI (adjusted odds ratio per log change, 1.35 [1.13, 1.6]) and mortality (1.51 [1.17, 1.94]). Patients with higher levels of IL-4 and IL-13 also had higher odds of AKI (1.26 [1.09, 1.46] and 1.4 [1.16, 1.69], respectively) and mortality (1.46 [1.18, 1.82] and 1.71 [1.27, 2.31], respectively). Adding biomarkers to the clinical variables through use of PCA improved the area under the curve by 0.01 for AKI and 0.04 for mortality, resulting in final areas under the curve of 0.85 (0.83–0.87) and 0.76 (0.70–0.81), respectively.

Conclusion: Both Th1 and Th2 cytokines increased immediately after cardiac surgery and were associated with AKI and 1-year mortality. Our findings indicate activation of both Th1 and Th2 pathways after cardiac surgery rather than predominance of either pathway.

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KEYWORDS: acute kidney injury; cardiac surgery; cardiopulmonary bypass; inflammation

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Systemic inflammatory response plays a critical role in the development of postoperative complications after cardiac surgery.¹ This inflammatory response is triggered by contact of blood components with the cardiopulmonary bypass circuit and is a result of tissue

ischemia and injury from hypoperfusion. In animal models of IRI, inflammatory mediators lead to increased kidney injury and mortality, and their inhibition ameliorates these adverse events.² Cardiac surgery performed without the use of cardiopulmonary bypass (CPB), which is associated with lower inflammatory response, was shown to be associated with lower AKI and ventilator use,^{3–5} although this approach is technically challenging and is associated with lower long-term bypass graft patency.^{6,7} Thus alternative approaches that decrease inflammation associated with

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cardiac surgery are needed to reduce postoperative complications, particularly in a subset of high-risk patients.^{8–10} The study of inflammatory mediators released during cardiac surgery in humans can identify such high-risk patients, guide biomarker-based enrollment of these patients in trials, and lead to development of targeted antiinflammatory therapies to reduce postoperative complications.

We hypothesized that subsets of Th cells mediate organ injury and repair through release of their characteristic inflammatory mediators in patients undergoing cardiac surgery. Specifically, we predicted that patients in whom IFN- γ -producing Th1-cells are activated will experience increased AKI and mortality, whereas those with IL-4- and IL-13-producing Th2-cell activation will have lower rates of these complications. In animal models of IRI, Th1 cells play a critical role in perpetuating organ injury and fibrosis, and inhibiting these cells leads to improvement in outcomes after IRI. On the other hand, Th2 cells are associated with improved repair after IRI to the kidneys.¹¹

In this study, we measured cytokines in the Th1 (IFN- γ) and Th2 (IL-4 and IL-13) pathways from plasma samples of all adult participants of the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) cohort. The TRIBE-AKI cohort is a multicenter, prospective, observational cohort of patients who underwent cardiac surgery. In the pediatric arm of the TRIBE-AKI study, we demonstrated that TNF- α was associated with prolonged ventilation.¹² In a subset of adult patients, we demonstrated that mediators of vascular inflammation (IL-6) and monocyte-macrophage pathway-1 were associated with a higher risk of postoperative AKI and mortality after cardiac surgery.^{13,14} Our objectives in this study were to describe the perioperative changes in Th1 and Th2 inflammatory cytokines after cardiac surgery and to test their association with postoperative complications of AKI and 1-year all-cause mortality. We predicted that Th1 biomarkers will be associated with higher AKI and mortality, whereas Th2 biomarkers will be associated lower rates of these complications. We also measured 7 other cytokines and chemokines that have been described in kidney inflammation (TNF- α , IL-1 β , IL-2, IL-6, IL-8, and IL-12p70) and its regulation (IL-10).¹⁵

METHODS

Participants and Setting

We previously published complete details regarding this study.^{16,17} Briefly, The TRIBE-AKI study is a prospective, multicenter, observational cohort of adult participants who underwent cardiac surgery. We

enrolled participants at 6 academic medical centers in North America. Participants with at least one risk factor for AKI from the following list were eligible for enrollment: emergency surgery, preoperative serum creatinine >2 mg/dl (>177 μ mol/L), ejection fraction $<35\%$ or grade 3 or 4 left ventricular dysfunction, age >70 years, diabetes mellitus, concomitant coronary artery bypass graft CABG and valve surgery, or repeat revascularization surgery. We excluded patients with evidence of AKI before surgery, prior kidney transplantation, preoperative serum creatinine >4.5 mg/dl, or end-stage renal disease. We obtained written informed consent from all participants. The study was approved by the Yale human investigation committee. We collected preoperative characteristics, operative details, and postoperative complications using definitions of the Society of Thoracic Surgeons.

Biomarkers

We measured 10 biomarkers (IFN- γ , IL-4, IL-13, TNF- α , IL-1 β , IL-2, IL-6, IL-8, IL-10, and IL-12p70) from plasma samples collected from study participants. We measured biomarker levels from plasma samples collected before, 0 to 6 hours after, and 24 hours after surgery. After one freeze-thaw cycle of plasma samples stored at -80 °C, we measured biomarkers using “V-PLEX Proinflammatory Panel 1” of the Mesoscale Discovery Platform, which measures multiple protein markers using a validated, sandwich electrochemiluminescence assay.¹⁸ The inter-assay coefficients of variation ranged from 4.5% to 7.7%. Inter-assay coefficients of variation and detection ranges are presented in [Supplementary Table S1](#).

Outcomes

We evaluated 2 outcomes in this study: in-hospital AKI and 1-year all-cause mortality. We defined AKI as serum creatinine rise of 0.3 mg/dl or $\geq 50\%$ from the preoperative level during hospitalization, which corresponds to AKI Network stage 1 AKI. We defined prolonged ventilation as >24 hours of mechanical ventilation, which corresponds to the definition of the Society of Thoracic Surgeons. We obtained mortality data after discharge through multiple references and cross-referenced the information when possible. For US participants, we telephoned patients' homes, searched the National Death Index, and obtained hospital records. For participants from Canada, we used telephone calls and data held at the Institute for Clinical Evaluative Sciences to acquire vital status. These datasets were linked using unique encoded identifiers and analyzed at the Institute for Clinical Evaluative Sciences. We ascertained the vital status of 1408 (98%) out

of 1444 participants, and these participants were included in the analysis for 1-year mortality.

Statistical Analysis

We reported continuous variables as mean (\pm SD) or median (interquartile range) and categorical variables as frequency (percentage). We compared continuous variables using the Wilcoxon rank sum test and categorical variables using the χ^2 test. We used linear regression models to test the association of CPB duration and log₂ transformed postoperative biomarkers. We imputed CPB duration of 0 for those who received off-pump surgery. We reported correlations between biomarkers at each time point using Spearman's correlation coefficient. We tested the association of biomarker tertiles with each outcome using multivariable logistic regression models that adjusted for key covariates including participant demographics, comorbidities, laboratory findings, and surgical characteristics consistent with prior publications from this cohort^{19,20} (see [Supplementary Table S2](#) for a complete list of covariates). Because of a high level of correlation between biomarkers, we used PCA to combine the 10 postoperative biomarkers. PCA with orthogonal rotation converts highly correlated variables into uncorrelated principal components.²¹ We retained components with eigenvalue >1 and proportion of variance >10%. We also performed the scree test to determine components to retain. We used SAS version 9.4 software (SAS Institute, Cary, NC) to perform the analyses.

RESULTS

Baseline Characteristics of Study Participants

Of the 1444 participants of the TRIBE-AKI study, 492 participants (34%) experienced AKI and 81 (6%) had died at 1 year of follow-up ([Supplementary Figure S1](#)). Baseline characteristics of participants by AKI status is presented in [Table 1](#). Patients who experienced postoperative AKI were more likely to have diabetes, hypertension, congestive heart failure, and chronic kidney disease. Patients who experienced AKI also had higher preoperative creatinine and albuminuria. Among participants who underwent CPB surgery, those with longer duration of bypass had higher AKI.

Perioperative Changes in Biomarkers

Both Th1 (IFN- γ) and Th2 (IL-4 and IL-13) cytokines increased at 6 hours after surgery; IFN- γ , IL-4, and IL-13 increased 2.1-, 6.0-, and 4.6-fold, respectively, from their preoperative levels ([Figure 1](#)). IFN- γ decreased to within 50% of preoperative value, whereas IL-4 and IL-13 remained 3.8- to 4.6-fold higher than

Table 1. Patient characteristics by acute kidney injury status

Characteristic	Acute kidney injury (0.3 mg/dl or 50% rise in serum creatinine)		P value ^a
	No (range or %) N = 952	Yes (range or %) N = 492	
Age, yr	74 (67, 78)	74 (66, 79)	0.78
Sex, female	310 (33)	137 (28)	0.07
White	895 (94)	463 (94)	0.94
Diabetes	340 (36)	214 (43)	0.004
Hypertension	739 (78)	412 (84)	0.006
Ejection fraction <35% or grade 3 or 4 left ventricular function	86 (9)	55 (11)	0.19
Myocardial infarction	246 (26)	126 (26)	0.89
Congestive heart failure	178 (19)	147 (30)	<0.001
Preoperative serum creatinine, mg/dl ^b	1 (0.8, 1.2)	1.1 (0.9, 1.3)	<0.001
Preoperative eGFR	70.1 (57.2, 84.3)	65.7 (49.4, 80.8)	<0.001
CKD stage 3 or higher	285 (30)	201 (41)	<0.001
Albuminuria (urine albumin-to-creatinine ratio >30 mg/g)	40 (4)	44 (9)	<0.001
Surgical characteristics			
Surgery type			0.003
CABG and valve	201 (21)	138 (28)	
CABG or valve	750 (79)	354 (72)	
Perfusion time, min	102 (79, 130)	121 (90, 164)	<0.001
Cardiopulmonary bypass (on pump)	852 (90)	452 (92)	0.13
CPB duration >120 min	285 (30)	242 (49)	<0.001
Cross-clamp time, min	70 (50, 96)	85 (58, 117)	<0.001
Postoperative complications			
Prolonged ventilation, >24 hr	51 (5)	91 (19)	<0.001
Length of ICU stay, d	2 (1, 2.5)	2 (1, 4)	<0.001
Length of hospital stay, d	6 (5, 7)	7 (6, 10)	<0.001
1-year mortality	32 (4)	49 (10)	<0.001

AKI, acute kidney injury; CABG, coronary artery bypass grafting; CKD, chronic kidney disease; CPB, cardiopulmonary bypass; eGFR, estimated glomerular filtration rate calculated using Chronic Kidney Disease Epidemiology Collaboration equation; ICU, intensive care unit.

^aWilcoxon rank sum or χ^2 test.

^bTo convert serum creatinine value from mg/dl to μ mol/L, multiple by 88.4.

preoperative levels 24 hours after surgery. Biomarkers were higher in patients who later experienced AKI than in those who did not experience AKI. We also noted an association between postoperative biomarker level and duration of CPB such that participants with longer duration of CPB had higher postoperative biomarker levels ([Supplementary Figure S2](#)).

Association of Biomarkers With AKI

AKI occurred in 492 (34%) of the 1444 study participants. For postoperative IFN- γ measured 6 six hours after surgery, we noted higher adjusted odds of AKI with each log increase in biomarker level (adjusted odds ratio, 1.35 [1.13, 1.6]), as well as in patients in the highest biomarker tertile (1.87 [1.28, 2.72]) in multivariable analyses ([Table 2](#) and [Figure 2](#)). Similarly, postoperative IL-4 and IL-13 measured 6 hours after surgery were associated with postoperative AKI with each log increase in biomarker level (1.26 [1.09, 1.46])

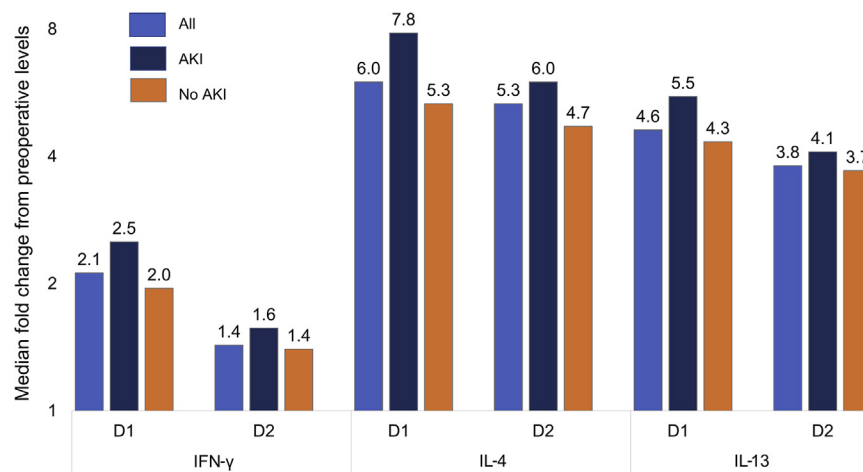


Figure 1. Fold change in biomarker levels from preoperative to postoperative time points. AKI, acute kidney injury; D1, day 1 (0–6 hours after surgery); D2, day 2 (24 hours after surgery); IFN, interferon; IL, interleukin. For AKI versus no AKI comparisons, *P* < 0.001 at all time points.

and 1.4 [1.16, 1.69], respectively) and for the highest tertile (1.73 [1.2, 2.5] and 1.69 [1.13, 2.53]). We did not note any consistent association of preoperative plasma cytokines with AKI (Supplementary Table S3).

Association of Biomarkers With 1-Year Mortality

After 1 year of follow-up, 81 participants (6%) had died. We noted an increased risk of 1-year mortality with each log increase in IFN-γ levels (1.51 [1.17, 1.94]) but not with highest tertile of the biomarker (1.77 [0.88, 3.54]) in multivariable analyses (Table 3). Both IL-4 and IL-13 were associated with 1-year mortality with each log increase (1.46 [1.18, 1.82] and 1.71 [1.27, 2.31], respectively) and for the highest tertile (2.92 [1.35, 6.29] and 2.57 [1.2, 5.49]). We did not note any

consistent association of preoperative cytokines with 1-year mortality (Supplementary Table S4).

Principal Component Analysis

We noted a high correlation between the 10 proteins in the multiplex panel (Figure 3a). Therefore, we performed a PCA to include these biomarkers into a single model. The principal components 1 and 2 had eigenvalues >1 (component 1: 6.2; component 2: 1.2) and explained 78% of the variance in the 10 biomarkers (Figure 3b and c). Component 1 included cytokines that are traditionally considered either “inflammatory” or “antiinflammatory,” whereas component 2 contained IL-10, which is traditionally considered “regulatory” (Figure 3d). Compared with the clinical model, addition

Table 2. Association of postoperative biomarkers with acute kidney injury after cardiac surgery

Biomarker	Category	n (%)	Odds ratio (95% confidence interval)		
			Model 1	Model 2	Model 3
Th1 cytokine					
IFN-γ	Per log change		1.6 (1.41, 1.82)	1.42 (1.22, 1.65)	1.35 (1.13, 1.6)
	T1 (1.3–5.8)	118 (25)	1 (referent)	1 (referent)	1 (referent)
	T2 (5.8–11.0)	152 (32)	1.42 (1.07, 1.88)	1.29 (0.95, 1.76)	1.25 (0.87, 1.79)
	T3 (11.0–664.6)	222 (46)	2.64 (2, 3.47)	2.09 (1.51, 2.89)	1.87 (1.28, 2.72)
Th2 cytokines					
IL-4	Per log change		1.51 (1.36, 1.69)	1.32 (1.16, 1.5)	1.26 (1.09, 1.46)
	T1 (0.03–0.15)	110 (23)	1 (referent)	1 (referent)	1 (referent)
	T2 (0.16–0.32)	157 (32)	1.58 (1.19, 2.1)	1.27 (0.93, 1.74)	1.11 (0.77, 1.6)
	T3 (0.33–37.95)	225 (46)	2.84 (2.15, 3.76)	2.04 (1.49, 2.8)	1.73 (1.2, 2.5)
IL-13	Per log change		1.51 (1.33, 1.71)	1.35 (1.15, 1.59)	1.4 (1.16, 1.69)
	T1 (0.5–2.9)	117 (24)	1 (referent)	1 (referent)	1 (referent)
	T2 (2.9–6.7)	183 (38)	1.91 (1.45, 2.52)	1.58 (1.16, 2.15)	1.41 (0.98, 2.03)
	T3 (6.7–185.5)	192 (40)	2.06 (1.56, 2.72)	1.5 (1.06, 2.13)	1.69 (1.13, 2.53)

IFN, interferon; IL, interleukin; T1, T2, T3, lowest, middle and highest tertile of biomarker, respectively.

Values in parenthesis indicate biomarker levels in pg/ml within each tertile.

Model 1: unadjusted.

Model 2: adjusted for age, sex, race, nonelective surgery, surgery type, preoperative estimated glomerular filtration rate, diabetes, hypertension, congestive heart failure, myocardial infarction, urine albumin-to-creatinine ratio, site, cardiopulmonary bypass time, and preoperative biomarker level.

Model 3: adjusted for all variables in Model 2 and change in creatinine from preoperative level.

Excludes 37 (2.6%) participants due to missing covariates for final sample sizes of 1407. AKI event rate, 480 (34%).

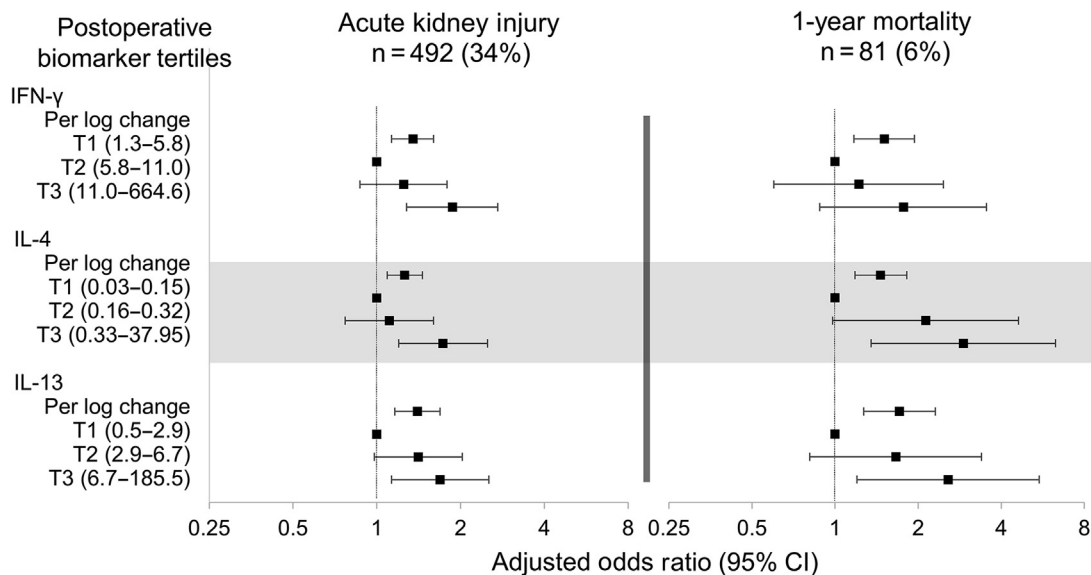


Figure 2. Association of postoperative biomarkers with postoperative outcomes. Multivariable analysis with lowest tertile of biomarkers as reference group. All analyses are adjusted for the following covariates: age, sex, race, nonelective surgery, surgery type, preoperative estimated glomerular filtration rate, diabetes, hypertension, congestive heart failure, myocardial infarction, urine albumin-to-creatinine ratio, site, cardiopulmonary bypass time, preoperative biomarker level, and change in creatinine from preoperative level. CI, confidence interval; IFN, interferon; IL, interleukin; T1, T2, T3 = lowest, middle, and highest tertile of biomarker, respectively.

of the 2 principal components significantly improved the AUC for AKI by 0.01 ($P = 0.04$) and mortality by 0.04 ($P = 0.03$; Figure 3e).

DISCUSSION

Cytokines in the Th1 and Th2 pathways released during cardiac surgery are thought to contribute to postoperative complications based on data from animal models. Specifically, Th1 pathway cytokines increase injury, whereas Th2 cytokines are involved in repair.

In this large, multicenter study, we demonstrated that plasma levels of Th1 cytokines, IFN- γ , and Th2 cytokines, IL-4 and IL-13, increased immediately after cardiac surgery, remained elevated for at least 24 hours after surgery, and were highly correlated with each other postoperatively. Moreover, not only IFN- γ but also IL-4 and IL-13 were associated with postoperative AKI and 1-year mortality after cardiac surgery.

Animal models of IRI demonstrate that T cells infiltrate the mouse kidneys within a few hours after

Table 3. Association of postoperative biomarkers with mortality after cardiac surgery

Biomarker	Tertile	n (%)	Odds ratio (95% confidence interval)		
			Model 1	Model 2	Model 3
Th1 cytokine					
IFN- γ	Per log change		1.73 (1.4, 2.12)	1.54 (1.2, 1.98)	1.51 (1.17, 1.94)
	T1 (1.3–5.8)	15 (3)	1 (referent)	1 (referent)	1 (referent)
	T2 (5.8–11.0)	21 (5)	1.62 (0.84, 3.13)	1.26 (0.62, 2.53)	1.22 (0.6, 2.47)
	T3 (11.0–664.6)	41 (9)	2.97 (1.62, 5.44)	1.89 (0.95, 3.75)	1.77 (0.88, 3.54)
Th2 cytokines					
IL-4	Per log change		1.61 (1.35, 1.92)	1.45 (1.17, 1.79)	1.46 (1.18, 1.82)
	T1 (0.03–0.15)	11 (2)	1 (referent)	1 (referent)	1 (referent)
	T2 (0.16–0.32)	24 (5)	2.01 (0.99, 4.06)	1.87 (0.88, 3.96)	2.13 (0.98, 4.62)
T3 (0.33–37.95)	42 (9)	3.96 (2.07, 7.59)	2.67 (1.27, 5.59)	2.92 (1.35, 6.29)	
IL-13	Per log change		1.57 (1.25, 1.98)	1.68 (1.25, 2.25)	1.71 (1.27, 2.31)
	T1 (0.5–2.9)	14 (3)	1 (referent)	1 (referent)	1 (referent)
	T2 (2.9–6.7)	27 (6)	1.79 (0.96, 3.36)	1.52 (0.76, 3.06)	1.66 (0.81, 3.39)
T3 (6.7–185.5)	36 (8)	2.42 (1.33, 4.42)	2.41 (1.15, 5.04)	2.57 (1.2, 5.49)	

IFN, interferon; IL, interleukin; T1, T2, T3, lowest, middle and highest tertile of biomarker, respectively.

Values in parenthesis indicate biomarker levels in pg/ml within each tertile.

Model 1: univariable analyses.

Model 2: adjusted for age, sex, race, nonelective surgery, surgery type, preoperative estimated glomerular filtration rate, diabetes, hypertension, congestive heart failure, myocardial infarction, urine albumin-to-creatinine ratio, site, cardiopulmonary bypass time, and preoperative biomarker level.

Model 3: adjusted for all variables in Model 2 and change in creatinine from preoperative level.

Excludes 31 (2.2%) due to missing covariates for final sample sizes of 1377. Mortality event rate, 77 (5.6%).

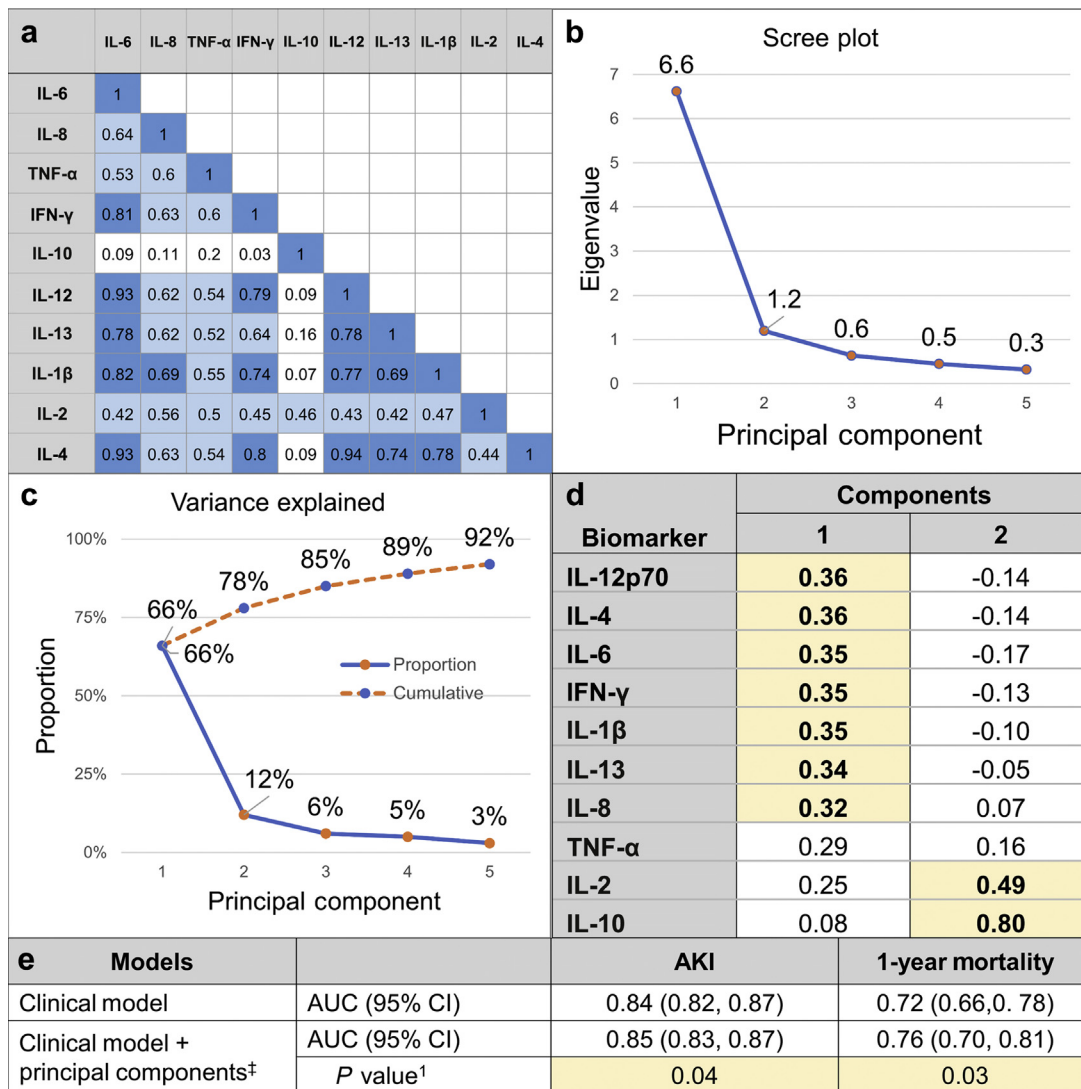


Figure 3. Principal component analysis of biomarkers. (a) Spearman correlation coefficients between biomarkers. Correlation coefficients ≤ 0.33 filled in white, between 0.33 and 0.65 filled in light blue, and ≥ 0.66 filled in dark blue. All P values < 0.05 . (b) Scree plot showing eigenvalues of each component. (c) Variance explained by each principal component. (d) First 2 components of the principal component analysis that were included in the final analysis. Numbers represent weights of each biomarker for the respective components. Values greater than 0.30 are highlighted in yellow. (e) Comparison of area under the curve (AUC) of clinical model with principal components. ¹Likelihood ratio test comparing clinical model + principal components to clinical model. [‡]Principal components 1 and 2 included in the model derived from the 10 postoperative biomarkers. Clinical model includes age, sex, race, nonelective surgery, surgery type, preoperative estimated glomerular filtration rate, diabetes, hypertension, congestive heart failure, myocardial infarction, urine albumin-to-creatinine ratio, site, cardiopulmonary bypass time, and change in creatinine from preoperative level. AKI, acute kidney injury; CI, confidence interval; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

injury and produce the characteristic cytokines in the T-cell pathway.²² These cytokines cause differentiation of naïve CD4⁺ T cells into its subtypes and increase production of other inflammatory mediators. Specifically, naïve CD4⁺ T cells can differentiate into Th1 type to produce IFN- γ or into Th2 type to produce IL-4 and IL-13.²³ Th1 cytokines cause increased kidney injury after ischemia-reperfusion.^{24,25} On the other hand, Th2 cytokines are involved in tissue repair and limit tissue injury; however, dysregulated Th2 responses can lead to tissue fibrosis.^{26–28}

Preliminary human studies have demonstrated that inflammatory cytokines are elevated after cardiac surgery. In a study of 20 male patients, levels of TNF- α increased after coronary artery bypass graft.²⁹ In a study of 82 neonates, IFN- γ and IL-13 increased after cardiac surgery.³⁰ Another study showed that cytokines including IL-1 β , IL-2, IL-4, IL-6, and TNF- α increased in open but not laparoscopic abdominal surgery.³¹ Consistent with these studies, we found that IFN- γ , IL-4, and IL-13 increased from their preoperative levels 6 and 24 hours after surgery. We also noted that Th2 cytokines IL-4 and IL-13 but not Th1 cytokine

IFN- γ remained persistently elevated 24 hours after surgery, which could indicate initiation of anti-inflammatory or reparative responses after injury. We also noted a correlation between all inflammatory biomarkers immediately after surgery, indicating activation of both Th1 and Th2 pathways during cardiac surgery.

Contact with CPB is a known trigger of the proinflammatory response after cardiac surgery. In a study of 16 patients undergoing cardiac surgery, the authors showed a positive correlation between CPB duration and IL-6 levels, although the association of CPB duration with Th1 and Th2 pathway mediators has not been studied.³² We demonstrate that mediators in both the Th1 and Th2 pathway increased with higher duration of CPB. A randomized controlled trial demonstrated lower AKI and respiratory complications in persons who underwent off-pump surgery compared with on-pump surgery.³⁻⁵ However, there was a higher need for revascularization and lower survival, possibly as a result of technical challenges associated with off-pump surgery.^{6,7} Thus, although performing off-pump surgery currently is not recommended, therapeutic inhibition of the inflammatory pathways activated in cardiac surgery could potentially reduce complications in patients undergoing cardiac surgery. Such targeted inhibition of inflammation was shown to be beneficial in patients with coronary artery disease.³³

Finally, we demonstrated the association of Th1 and Th2 cytokines with complications after cardiac surgery. We examined AKI, which not only imposes the greatest cost after cardiac surgery but also increases the risk of mortality.³⁴ Identifying persons at greater risk for these complications is an important priority, and inflammatory biomarkers could play an important role in this regard.³⁵ Prior studies have examined the association of inflammatory mediators in several pathways as predictors with these complications. For example, in pediatric studies, IL-6, IL-8, and IL-10 levels were associated with higher risk of AKI and prolonged ventilation.³⁶⁻³⁸ In the TRIBE-AKI cohort, we reported that IL-6 and IL-10 were associated with higher risk of AKI.¹³ In this study, we examined cytokines in the Th1 and Th2 pathways, which were not examined in the prior studies. We found that IL-4 and IL-13 were independently associated with AKI and mortality after cardiac surgery, whereas IFN- γ was associated with AKI but not mortality.

Our findings may provide insight into the underlying inflammatory mechanisms activated in cardiac surgery. First, the high postoperative correlations among cytokines of both Th1 (IFN- γ) and Th2 (IL-4 and IL-13) pathways, as well as association of all of these with postoperative complications, indicate a

global activation of T cell-mediated immunity in cardiac surgery rather than predominant Th1- or Th2-cell activation. Second, although the association of Th2 biomarkers with worse outcomes may seem contrary to animal models that show that these cytokines are associated with repair, we believe that the levels of IL-4 and IL-13 measured early after surgery may reflect the degree of underlying injury rather than activation of repair mechanisms. This belief is supported by the high correlation between all cytokines in our study regardless of pathway. Repair is generally activated a few days after injury, and measurement of IL-4 and IL-13 a few days after injury may better reflect repair. Alternatively, aberrant activation of IL-4 and IL-13 also could lead to fibrosis and worse outcomes. Measurement of these cytokines 3 to 7 days after AKI may provide further insight into their role in repair and may be associated with improved outcomes.

Our study has several strengths. First, given the large sample size and event rate, we controlled our analysis for important covariates to demonstrate the independent association of biomarkers with complications. Second, we tested the association of biomarkers with complications defined by the Society of Thoracic Surgeons, which are associated with high morbidity and mortality. Finally, we ascertained vital status on 98% of our cohort. Our study also has some limitations. First, demonstration of association is not evidence of causality; increase in the biomarkers may merely reflect a normal response to injury, and inhibition of these biomarkers may not reduce complications. Second, we could not control our analysis for other confounders such as smoking status or steroid use during surgery. Third, we did not adjust for other markers of inflammation such as C-reactive protein because we did not have this available. Fourth, these findings will need to be replicated in an external validation cohort. Finally, the TRIBE-AKI cohort included participants who underwent cardiac surgery with high risk of AKI, and our findings are generalizable only to this subset of patients.

In conclusion, our study demonstrates that cytokines IFN- γ , IL-4, and IL-13 increased after cardiac surgery and in proportion to the duration of CPB. These cytokines were highly correlated and were all associated with postoperative complications of AKI and 1-year mortality. Our findings suggest that plasma levels of cytokines could help identify patients at higher risk of complications and targeted inhibition of these mediators of inflammation could reduce complications. Moreover, the results also could be used in clinical practice to risk stratify patients and provide better prognostic estimates in cardiac surgery.

DISCLOSURE

JLK has received research fees from Bioporto and Astute Medical and consulting fees from Baxter, Astute Medical, and Sphingotec. MGS received grant support from Cricket Health, Inc. and consultancy fees from the University of Washington and has equity in TAI diagnostics and Cricket Health, Inc. SGC and CRP are on the Advisory Board of RenalytixAI and both own equity in this company. SGC received consulting fees from Goldfinch Bio, CHF Solutions, Quark Biopharma, Janssen Pharmaceuticals, and Takeda Pharmaceuticals. CRP is on the Data Safety and Monitoring Board of Genfit. All the other authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

[Supplementary File \(Word\)](#)

Table S1. Detection range and coefficient of variation of biomarkers.

Table S2. List of covariates used in multivariable models.

Table S3. Association of preoperative biomarkers with acute kidney injury after cardiac surgery.

Table S4. Association of preoperative biomarkers with 1-year mortality after cardiac surgery.

Figure S1. STARD flow diagram of study participants.

Figure S2. Association of cardiopulmonary bypass duration with postoperative plasma biomarker levels.

STROBE Statement. Checklist of items that should be included in reports of observational studies.

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