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Group analysis identifies differentially elevated biomarkers with distinct outcomes for advanced acute kidney injury in cardiac surgery

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Aim: To investigate early postoperative biomarkers for risk discrimination of advanced acute kidney injury (AKI). **Materials & methods:** Postoperative plasma biomarkers including NGAL, h-FABP, CK-MB, hsTNT, NT-proBNP, IL-6, IL-10 and VEGF were analyzed using group-based method among 426 patients with AKI after cardiac surgery. **Results:** Six patient groups with distinct biomarker patterns were identified. Individual biomarker displayed significant difference across the groups. The groups showed better discrimination for advanced AKI than any single biomarker either with or without adjusting for clinical variables. Average concentration of a single biomarker within each group, mortality and risk of a secondary outcome all demonstrated an approximately U-shaped relationship with proportion of advanced AKI within each group. **Conclusion:** The group-based analysis revealed that the order of the patient groups with an increasing likelihood of advanced AKI had a nonlinear relationship with average concentration of an individual biomarker, mortality and risk of other outcomes.

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Keywords: advanced AKI • biomarkers of AKI • group-based analysis

Serum creatinine measurement is the most common method for evaluating renal function and injury. However, structural renal tubular injury is not always evident by creatinine-based estimates of glomerular filtration rate [1]. Although the incidence of acute kidney injury (AKI) is reaching epidemic dimensions, few tools currently exist for assessing the risk for the progression of this serious disease. Identification of biomarkers for the prognosis of the outcomes among patients with established AKI has become increasingly critical in patient care as several biomarkers of AKI have been investigated for the severity of AKI [2–5]. Patients with advanced AKI conferred worse outcome such as mortality, organ failure and chronic kidney disease [6,7]. The advanced AKI reflects more sustained period of damage at AKI occurrence and more extensive dysfunction in the distal renal tubule that may need more renal support before recovery than those milder AKI [8]. Comparing to the literatures in AKI, there are much fewer literatures on advanced AKI. The lack of earlier biomarkers for advanced AKI is an obstacle to the development of better prognosis and prevention strategies.

Cardiac surgery utilizes cardiopulmonary bypass (CPB) which can cause injury of vital organs due to ischemia reperfusion injury, hemolysis and initiation of inflammatory processes. AKI is a frequent consequence of the systemic inflammatory response after bypass [9]. AKI after cardiac surgery is strongly associated with both short- and long-term morbidity and mortality [10]. Identification of early biomarkers associated with advanced AKI after cardiac surgery would therefore be critical in guiding postsurgical care.

Future Medicine

Prior studies have identified biomarkers of AKI that could serve as a prognostic tool in chronic heart failure (CHF) patients [11,12]. In addition, the inflammatory biomarkers IL-6 and IL-10 have been found to be associated with mortality after cardiac surgery [13]. Furthermore, a wide range of urinary and plasma biomarkers measured at the time of AKI diagnosis have been found to be associated with progression of AKI, with NGAL performing the best [2,14]. These biomarkers reflect activity in a number of biological systems, for example, renal, inflammatory, cardiovascular and metabolic. However, individual biomarkers have not yet been identified as robust predictors of AKI or its progression, so interest in using combinations of biomarkers rather than individual ones at a time has been increasing [15].

In this paper, we apply a group-based method to identify patterns of early postoperative biomarkers that are associated with advanced AKI, which includes higher stage of AKI beyond stage 1 after cardiac surgery [16]. This method has been recently applied to cluster HIV-infected women with chronic kidney disease into distinct groups with differential patterns of urinary kidney injury biomarkers [17]. When using multiple biomarkers together in a regression model, neither the relationship between a single biomarker and the response nor the relationship between the multiple biomarkers is clearly interpretable. However, using the group-based method, we can identify distinct patterns of differentially elevated biomarkers that are associated with distinct patterns of outcomes.

We used data from the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) Consortium, which prospectively enrolled 1219 adults from six institutions between 2007 and 2009. Our primary objective was to determine whether pattern of biomarkers can facilitate the risk discrimination for advanced AKI and other adverse outcomes.

Methods

Study sample

Between July 2007 and December 2009, 1219 adults who underwent cardiac surgery (coronary artery bypass graft and/or surgery for valve disease) and deemed at high risk for AKI were enrolled at six academic centers in North America for the TRIBE-AKI study [18,19]. Specifically, high risk for AKI was defined by the presence of one or more of the following: emergency surgery, preoperative serum creatinine >2 mg/dl (>177 μmol/l), ejection fraction <35% or grade 3 or 4 diastolic dysfunction [20], age >70 years, diabetes mellitus, concomitant CABG and valve surgery or repeat revascularization surgery.

As per study protocol, blood samples were collected preoperatively and postoperatively for up to five consecutive days. The first postoperative blood sample was collected within 6 h of admission to the intensive care unit. Specimen collection was stopped on the third postoperative day in patients who did not show evidence of AKI after surgery.

We excluded patients with histologic evidence of AKI before surgery, prior kidney transplantation, preoperative serum creatinine concentration >4.5 mg/dl (>398 μmol/l) or end-stage renal disease. A total of 426 patients who developed AKI after surgery were included in our analysis of the following plasma biomarkers which were quantified on the first postoperative day: NGAL, h-FABP, CK-MB, IL-6, IL-10, NT-proBNP, hsTNT and VEGF.

Biomarker measurements

Blood samples were collected in EDTA tubes, centrifuged to separate plasma and stored at -80°C. Biomarker concentrations were measured following one freeze-thaw cycle. Concentration of all the eight biomarkers was determined by immunoassay. Concentrations of H-FABP (detection range: 0.7–140 ng/ml) [21], VEGF (detection range: 4.5–144 pg/ml), IL-6 (detection range: 0.6–790 pg/ml) and IL-10 (0.9–840 pg/ml) were measured by using the Randox Evidence Investigator (Randox Laboratories Ltd, WV, USA) [13]. NT-proBNP (detection range: 1.8–2684 pmol/l), CK-MB (detection range: 1.2–303 μg/l) and hsTNT (detection range: 8–10000 ng/l) concentrations were measured using the Beckman Coulter Access II (Beckman Coulter, CA, USA) [22]. NGAL was measured using the Triage NGAL immunoassay in conjunction with the Triage Meter (Biosite, Inc., CA, USA) (detection range: 60–1300 ng/ml). The coefficients of variation for these assays were <20%. All laboratory personnel were blinded to clinical outcomes and samples were analyzed according to the manufacturer's specifications.

Study outcomes

AKI was defined as a >50% or >0.3 mg/dl increase in serum creatinine from preoperative baseline, corresponding to AKI Network Stage 1 or higher. The primary outcome of this paper is advanced AKI which indicated AKI Network Stage reached stage 2 or higher, and was defined as >100% increase in serum creatinine from preoperative baseline or the requirement of dialysis [23]. Mortality data after discharge were obtained via several overlapping

approaches. For patients living in the USA, we called patients' homes, searched the National Death Index and reviewed hospital records. For patients living in Canada, we used phone calls as well as data held at the Institute for Clinical Evaluative Sciences to identify the patients who died using unique, encoded identifiers that were linked to the patients at the Institute for Clinical Evaluative Sciences. Death status and date of death were recorded until the last date of follow-up, namely 21 February 2012.

Statistical analysis

Demographic and clinical baseline characteristics were summarized numerically. Continuous biomarker variables were assessed with analysis of variance (ANOVA) after log-transformation for normalization, and categorical variables were assessed with χ^2 tests when all cell counts were greater than five or Fisher's exact tests otherwise. One-way ANOVA F-test was performed to compare each of the biomarkers between patients with stage I AKI only and advanced AKI. An ANOVA test with patient-specific random effects was performed to compare pre and postoperative concentration for each biomarker. We used logistic regression to assess the association of biomarker sextiles with advanced AKI and used the following clinical variables for adjustment: patient age, gender, race, indicator of CHF, indicator of hypertension, indicator of diabetes, indicator of myocardial infarction, CPB time, indicator of nonelective cardiac surgery, indicator of greater than 50% left ventricular ejection fraction and preoperative serum creatinine concentration. The estimates of glomerular filtration rate which was derived from creatinine were also considered as an adjusting variable but we did not use it because it was not statistically significant after adjusting the other clinical variables while serum creatinine was significant. Reviewer also suggested to add extracardiac arteriopathy, but our dataset did not contain it and we therefore was unable to adjust for it. We used sextiles to facilitate comparison with the six groups of distinct biomarker patterns that we identified with the group-based analysis of multivariate biomarkers. Area under receiver operating characteristic (ROC) curve was calculated for each logistic regression model where the ROC curve is a plot sensitivity against 1-specificity for a range of cut-off values in a biomarker [24]. The regression analysis could not illuminate whether the biomarkers were elevated in sync or there were different patterns of change, which we examined instead via group-based analysis as follows.

To discover phenotypes of the eight plasma biomarkers for advanced AKI, group-based analyses [16,17] were performed using SAS software Version 9.4 (SAS, NC, USA) [25] after logarithmic transformations of the biomarkers to normalize their right-skewed distributions. The group-based method does not require a patient to have measurements on all eight biomarkers but uses his/her available biomarker measurements. The goal of fitting a group-based model was to obtain discrete groups from the totality of eight biomarkers, with each group representing a distinct pattern of the biomarker concentrations. A group potentially has its own prognosis for advanced AKI and phenotype of patient outcomes. The supervised group analysis [26,27] was adopted in this paper by including advanced AKI as a binary response together with the continuous responses of the eight biomarker concentrations, since the group-based method can accommodate mixed continuous and binary responses. The discrete groups were obtained through maximizing the observed data likelihood of the eight biomarkers and the binary outcome in a given model for the groups. In layman language, the likelihood of a given model is the probability of actually observing the responses in the data under the model. The group-based biomarker analysis in this paper is technically similar to the group-based trajectory analysis that was developed previously [16]. The group-based trajectory analysis assumes each group has its own response pattern of repeated measures over time while the group-based biomarker analysis assumes each group having its own pattern of the multiple biomarker responses. The main difference between the two approaches is whether responses are ordered or not. The group-based trajectory analysis repeatedly treats measured responses as ordered in time and uses time as a polynomial covariate (e.g., linear, quadratic or cubic, etc.) while our group-based biomarker analysis treats the multiple biomarkers and the binary response of advanced AKI as unordered responses. There are three steps in obtaining the final groups. In the first step, we perform the group-based analysis with the number of groups respectively fixed at 1, 2, . . . , 7, 8. So there are eight models in total. In the second step, we calculated Bayesian Information Criteria (BIC) [28] for each of the eight models. The model with smallest BIC was selected as the final model which has G number of groups, for example, $G = 6$. In the third step, using the estimates from the final model, a patient i was classified into one of the G groups for which

Table 1. Patient characteristics and biomarker concentrations before and first measurement after surgery.

Parameter†	AKI stage 1, n = 367	Advanced AKI, n = 59	p-value			
Age (std. dev.)	72.2 (9.6)	70.5 (10.9)	0.22			
Male	71.1% (261)	39 (66.1%)	0.43			
Nonwhite	6.5% (24)	5 (8.5%)	0.58			
CHF	31.3% (115)	24 (40.7%)	0.16			
Hypertension	82.0% (301)	52 (88.1%)	0.25			
Diabetes	45.7% (168)	29 (49.2%)	0.63			
MI	25.9% (95)	18 (30.5%)	0.46			
Postsurgery biomarker values				**All patients with AKI, n = 426**		
				Presurgery	**Postsurgery**	**p-value**
Serum creatinine	1.26 (0.37, n = 364)	1.48 (0.52, n = 58)	<0.0001	1.16 (0.37, n = 426)	1.29 (0.40, n = 422)	<0.0001
NGAL, ng/ml	239.8 (131.0, n = 354)	281.4 (144.2, n = 55)	0.03	92.2 (76.7, n = 408)	245.4 (133.5, n = 409)	<0.0001
h-FABP, ng/ml	50.3 (36.2, n = 296)	90.2 (51.2, n = 44)	<0.0001	5.70 (3.58, n = 333)	55.5 (40.7, n = 340)	<0.0001
CK-MB, μg/ml	35.0 (40.5, n = 299)	60.8 (67.3, n = 44)	0.0004	3.21 (11.5, n = 342)	38.3 (45.5, n = 343)	<0.0001
IL10, pg/ml	99.3 (119.2, n = 296)	109.8 (143.2, n = 44)	0.60	2.15 (10.3, n = 317)	100.7 (122.3, n = 340)	<0.0001
NT-proBNP, pmol/l	166.7 (249.5, n = 296)	220.0 (268.4, n = 43)	0.20	210.3 (326.0, n = 332)	173.5 (252.2, n = 339)	<0.0001
hsTNT, ng/l	796.2 (1070.6, n = 298)	1457.4 (2065.5, n = 44)	0.001	105.7 (579.6, n = 332)	881.3 (1259.2, n = 342)	<0.0001
IL6, pg/ml	277.4 (222.8, n = 296)	436.6 (289.8, n = 44)	<0.0001	12.0 (43.8, n = 324)	298.0 (238.1, n = 340)	<0.0001
VEGF, pg/ml	9.3 (12.7, n = 292)	6.89 (6.73, n = 43)	0.23	16.4 (22.1, n = 323)	8.98 (12.2, n = 335)	<0.0001
EGFR, ml/min/1.73 m^2	57.5 (17.5, n = 364)	50.3 (20.7, n = 58)	0.005	63.7 (19.8, n = 426)	56.5 (18.1, n = 422)	<0.0001
Outcomes						
Long-term mortality	59 (16.4%)	26 (44.1%)	<0.0001			

†Continuous variables are presented as mean (standard deviation, sample size) except for age.
Discrete variables are presented as percentage (number).
AKI: Acute kidney injury; CHF: Chronic heart failure; MI: Myocardial infarction, std. dev.: Standard deviation.

s/he had the largest posterior probability as:

$$\text{posterior P(i in Group g)} = \frac{\text{f(Bl, …, B8 | i in Group g)} \times \text{f(advanced AKI | i in Group g)} \times \text{[prior P of Group g]}}{\sum_{c=1}^{G} \text{f(Bl, …, B8 | i in Group c)} \times \text{f(advanced AKI | i in Group c)} \times \text{[prior P of Group c]}},$$

where i denotes ith patient and P denotes probability, B1, . . . , B8 denote the eight biomarkers and f denotes the likelihood of the observed responses for patient i in the given group. The prior probability refers to the probability model used for estimating prevalence of a group. All the terms in the formula can be calculated with the final model estimates. The denominator in the formula is the likelihood for the observed responses that was maximized for all the patients across all the groups. The group-based method has the advantage to possibly increase the discrimination ability of the biomarkers, since patients can be grouped based on certain distinct patterns of the biomarker concentrations (e.g., a pattern can have a high concentration in biomarker 1, intermediate concentration in biomarker 2 and low concentration in biomarker 3) instead of grouping based on similar biomarker values.

We then compared demographic and clinical characteristics of our cohort, stratified by the groups, using χ^2/Fishers' exact test and ANOVA F-test for categorical and continuous variables, respectively. To assess the group reliability and stability, Jaccard index was calculated [29].

Results

Descriptive statistics in the study sample

Patient characteristics are shown in Table 1. Among 426 patients with AKI, 59 patients (13.8%) reached AKI Network Stage 2 or higher, and among them, 26 (44.1%) died. Among the 367 patients with stage 1 AKI, 59 (16.4%) died. Among 426 patients with AKI, 69 patients had only one of the biomarkers. One, two, two and eight patients had 3, 4, 6 and 7 of the biomarkers. No patients had only two or five biomarkers. Three hundred

Table 2. Discriminative ability for advanced acute kidney injury of single biomarker and group of panel biomarkers.

Biomarker	No adjusting for clinical variables			Adjusting for clinical variables			
	Odds ratio (95% CI) of 6th vs1st sextile	p-value	AUC[†]	Odds ratio (95% CI) of 6th vs 1st sextile	p-value	AUC[†]	p-value of the ROC increment[‡]
IL-10	1.09 (0.38, 3.15)	0.79	0.55	1.30 (0.37, 4.62)	0.59	0.77	0.12
NGAL	2.63 (0.85, 8.09)	0.090	0.59	1.09 (0.28, 4.17)	0.54	0.76	0.38
h-FABP	9.83 (3.11, 31.1)	0.0002	0.71	4.97 (1.19, 20.8)	0.031	0.78	0.20
CK-MB	7.90 (2.16, 28.9)	0.0023	0.66	2.79 (0.48, 16.4)	0.41	0.77	0.29
hsTNT	2.59 (0.90, 7.42)	0.085	0.63	0.33 (0.067, 1.60)	0.62	0.75	0.93
IL-6	6.14 (1.91, 19.8)	0.0035	0.64	6.17 (1.42, 26.8)	0.034	0.78	0.18
NT-proBNP	6.31 (1.31, 30.4)	0.027	0.60	6.24 (0.66, 59.3)	0.50	0.76	0.39
VEGF[§]	0.73 (0.29, 1.84)	0.51	0.54	0.97 (0.33, 2.86)	0.67	0.76	0.07
Groups of panel biomarkers	¶		0.77	¶	<0.0001	0.84	0.0023

[†]This column lists the area under the ROC (AUC) for the biomarker and clinical variables. The clinical variables including patient age, gender, race, indicator of chronic heart failure, indicator of hypertension, indicator of diabetes, indicator of myocardial infarction, CPB time, surgery type, ejection fraction, serum creatinine concentration (without any biomarker) had an ROC of 0.76.
[‡]This column gives the p-value for comparing the AUC with only the clinical variables and the AUC with the clinical variables plus the individual biomarker or group.
[§]The first, second and third sextiles of VEGF were inseparable and combined into the third sextile; the fourth and the fifth sextiles of VEGF were also inseparable and combined into the fifth sextile. Therefore the odds ratio and the p-value are both based on sixth versus third sextile.
¶The odds ratio was not calculated as the first group had no advanced AKI.
AKI: Acute kidney injury; AUC: Area under the curve; CPB: Cardiopulmonary bypass; ROC: Receiver operating characteristic.

and thirty patients had all the eight biomarkers. There were 14 AKI patients without any quantified biomarker. Among the 14 patients, four patients had advanced AKI who later died and additional four patients died without advanced AKI. Patients with advanced AKI also had significantly higher concentrations of the first postoperative biomarkers for NGAL, h-FABP, CK-MB, hsTNT and IL-6 than patients in stage 1 AKI (Table 1). The Pearson correlation coefficients varied from 0.01 to 0.78 among the eight biomarkers measured at the first postoperative occasion. The actual values for all the pairwise correlation coefficients among the eight biomarkers are given in the Supplementary Table.

Concentrations of NGAL, h-FABP, CK-MB, hsTNT, IL-6 and IL-10 showed significant elevations at the first postoperative time point relative to the preoperative time point, while NT-proBNP and VEGF had significantly reduced postoperative concentrations (Table 1).

Association of individual plasma biomarker with advanced AKI

We next examined associations of biomarker concentrations at the first postoperative time point with odds of advanced AKI (Table 2). In unadjusted analysis, we found that the highest sextile of each biomarker was significantly associated with advanced AKI for h-FABP, CK-MB, NT-proBNP and IL-6. For example, the highest sextile of h-FABP was associated with a 9.8-fold odds of AKI relative to the lowest sextile ($p = 0.0002$). The highest sextiles of NGAL and hsTNT were also associated with greater odds of advanced AKI, although associations did not reach statistical significance. By contrast, IL-10 and VEGF showed little association with AKI. After adjusting for clinical variables including patient age, gender, race, indicator of CHF, indicator of hypertension, indicator of diabetes, indicator of myocardial infarction, CPB time, surgery type, ejection fraction and serum creatinine concentration, only h-FABP and IL-6 remained significantly associated with advanced AKI with an odds ratio (OR; 95% CI) of 5.0 (1.2, 20.8) and 6.2 (1.4, 26.8), respectively (Table 2). None of the preoperative biomarkers were significantly associated with advanced AKI even in unadjusted model (data not shown).

Group discrimination of advanced AKI via panel biomarkers

We determined the optimal number of groups using the BIC by varying the group sizes between 1 and 8 in eight separate models, finding that a 6-group model yielded the best fit. The Jaccard index was 0.82, indicating a high stability and reliability of these groups. We labeled the groups in ascending order by group proportion of advanced AKI, with group 1 having the lowest proportion (0%) and group 6 having the highest proportion (43.5%). By comparison, the most predictive biomarker, h-FABP, had advanced AKI ranging from 7.1 to 41.0% across the sextiles. When analyzed as an ordinal variable, we found that group order had higher discrimination for advanced

Table 3. Quantiles of group average biomarker value[†] and proportion of advanced acute kidney injury.

Biomarker	Group label (n of patients)						p-value[‡]
	1 (n = 18)	2 (n = 146)	3 (n = 159)	4 (n = 17)	5 (n = 17)	6 (n = 69)	
NGAL, ng/ml	44	83>	43<	38	85>	87>	<0.0001
h-FABP, ng/ml	62	77>	43<	19<	93>	91>	<0.0001
CK-MB, μg/ml	81	65	33<	4<	99>	89>	<0.0001
hsTNT, ng/l	94>	67	39<	5<	98>	89>	<0.0001
NT-proBNP, pmol/l	68	81>	63	52	54	88>	<0.0001
IL-6, pg/ml	76	81>	54<	44	80>[§]	83>	<0.0001
IL-10, pg/ml	28<	79>	62	45	79	81>	<0.0001
VEGF, pg/ml	63	55	57	68	55	52	0.298
% (n) advanced AKI	0 (0)	6.2 (9)	6.9 (11)	11.8 (2)	41.2 (7)	43.5 (30)	<0.0001

[†]The cell numbers are the quantiles in percentage for group average value at the first postoperative measurement. The quantiles were calculated according to the preoperative biomarker values for VEGF and the first postoperative values for all other biomarkers among AKI patients.
[‡]The p-values are for the ANOVA F-test of each biomarker concentration across different groups.
[§]Denotes that p-value is significant at $\alpha = 0.05$ for testing the difference in a biomarker concentration between those with and without advanced AKI within a same group between those with and without advanced AKI. > and < indicate statistically higher or lower values comparing to first postoperative values among non-AKI patients.
AKI: Acute kidney injury; ANOVA: Analysis of variance.

AKI than h-FABP or any other individual biomarker. The area under the ROC curve of the groups before and after adjusting for the clinical variables was 0.77 and 0.84, respectively, higher than that of any individual biomarker (Table 2). The increment in ROC of the model with clinical variables plus the group of panel biomarkers over the model with only the clinical variables was highly significant at 8.2% with p-value 0.0023, and the increment was not significant for any individual biomarker (Table 2).

Overall, all eight biomarkers except VEGF showed statistically significant differences across the groups using the ANOVA F-test (Table 3 & Figure 1). Within each group, there was no significant difference between patients with versus without advanced AKI except for IL-6 in group 5 (Table 3, Group 5). A same biomarker can be empirically compared across different groups using either the group average values or their corresponding quantiles. The group average biomarker values were displayed in Figure 1. The quantiles of the group average values were given in Table 3. The quantiles additionally allow us to compare relative concentrations of different biomarkers within a same group, for example, in group 4 only the quantile for NT-ProBNP was above the median and the quantiles for all other biomarkers (except VEGF) were below median.

Relationships of group with average concentration of an individual biomarker & mortality

Average concentration of an individual biomarker did not follow monotone relationships with the group order of increasing proportion of advanced AKI, and actually the relationship appeared approximately 'U-shaped' as groups 3 and 4 tend to have lowest average values of an individual biomarker but higher risk of advanced AKI than groups 1 and 2 (Table 3 & Figure 1). Mortality and other secondary outcomes also displayed approximately U-shaped relationship with the group order. A higher biomarker value (except for VEGF) could have either rather low or very high risk of advanced AKI. Figure 2 showed the differential relationships between the group order of advanced AKI and mortality. Group 1 – the group with no advanced AKI – had the second highest mortality rate, group 4 – the group with moderate risk of advanced AKI – had the lowest mortality rate. Group 6 had both the highest risk of advanced AKI and mortality.

Specifically, group 1 had a significantly lower concentration of first postoperative IL-10 than patients in any other group (Table 3). Except for IL-10 and NGAL, group 1 and group 2 both had elevated concentration for other biomarkers. The two largest groups, 2 and 3, had a similarly low proportion of advanced AKI at 6.2 and 6.9%, respectively but moderate mortality rates of 13 and 16%, respectively. However, these two groups displayed very different biomarker patterns. Patients in group 3 had significantly lower values of NGAL, h-FABP, CK-MB, hsTNT and IL-6 than patients in group 2. Further examination revealed that there were a higher proportion of patients with advanced AKI in group 2 than group 3 among those who died, and they tended to have higher biomarker values. Despite a moderate proportion of 12% for advanced AKI, patients in group 4 had the lowest mortality rate of 5.9% and lowest concentrations in all biomarkers among all the groups. Some of the biomarkers

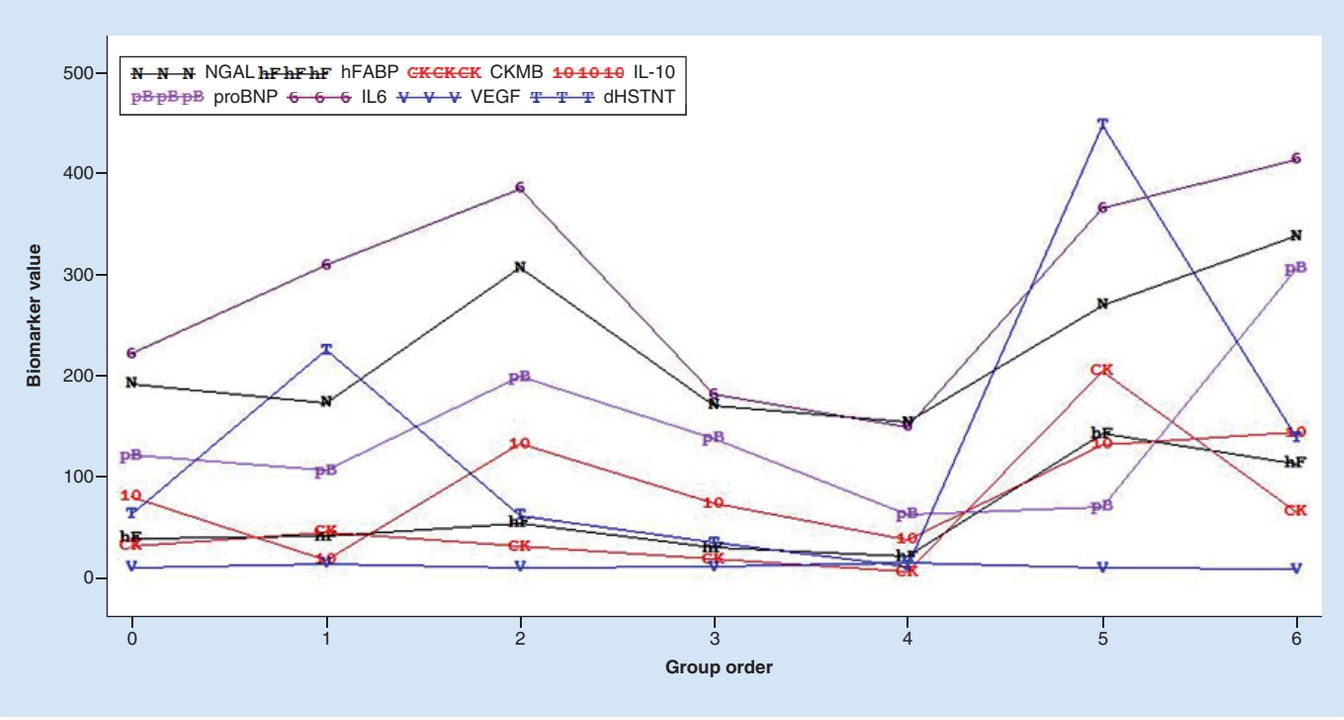

Figure 1. Average biomarker concentrations by group. All biomarkers are plotted in their actual values with corresponding units except that hsTNT was plotted as dTNTHS at one tenth of its actual value to make it have a similar scale with other biomarkers in the plot. Biomarker values of group 0 refer to the preoperative values.

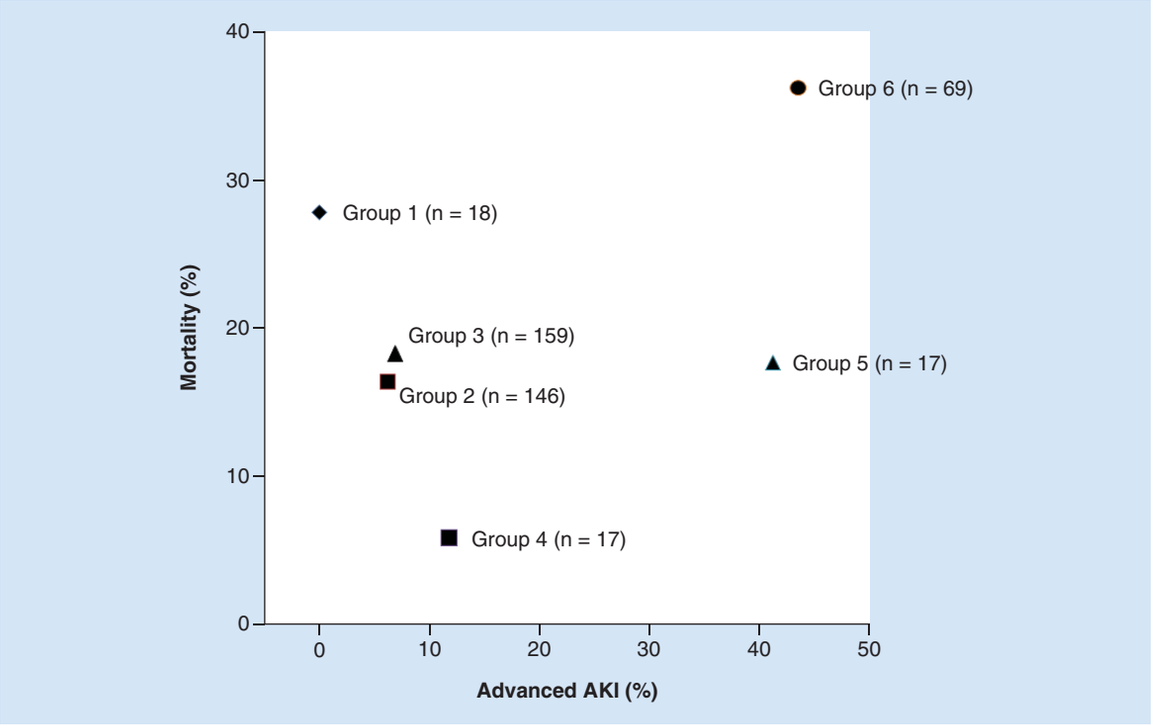

Figure 2. Association of advanced acute kidney injury with long-term mortality by biomarker group.
AKI: Acute kidney injury.

Table 4. Group-wise outcomes and baseline characteristics.

Group label (n patients)	1 (n = 18)	2 (n = 146)	3 (n = 159)	4 (n = 17)	5 (n = 17)	6 (n = 69)	p-value
% (n total death)	27.8 (5)	16.4 (24)	18.3 (29)	5.9 (1)	17.7 (3)	36.2 (25)	0.001
Duration of AKI							<0.0001
% (n) 1–3 days	100 (18)	72.6 (106)	81.1 (129)	82.4 (14)	64.7 (11)	46.41 (32)	
% (n) 4 or more	0 (0)	27.4 (40)	18.9 (30)	17.6 (3)	35.3 (6)	53.6 (37)	
Hospitalization							<0.0001
% (n) 1–6 days	66.7 (12)	34.2 (50)	49.1 (78)	64.7 (11)	17.7 (3)	21.7 (15)	
% (n) 7 or more	33.3 (6)	65.8 (96)	50.9 (81)	35.3 (6)	82.3 (14)	78.3 (54)	
Days of ICU							0.0249
% (n) 1 day	16.7 (3)	30.1 (44)	35.2 (56)	47.1 (8)	23.5 (4)	15.9 (11)	
% (n) 2 or more	83.3 (15)	69.9 (102)	64.8 (103)	52.9 (9)	76.5 (13)	84.1 (58)	
Days of ventilation							0.0013
% (n) 0 day	5.6 (1)	21.9 (32)	18.2 (29)	35.3 (6)	17.6 (5)	20.3 (14)	
% (n) 1 day	77.8 (14)	58.9 (86)	71.1 (113)	58.8 (10)	52.9 (9)	44.9 (31)	
% (n) 2 or more	16.7 (3)	19.2 (28)	10.7 (17)	5.9 (1)	29.4 (5)	34.8 (24)	
Age (SD)	69.5 (11.2)	72.6 (8.5)	72.6 (10.5)	68.3 (9.6)	71.0 (11.8)	71.1 (9.7)	0.377
% (n) Male	72.2 (13)	72.6 (106)	69.8 (111)	70.6 (12)	64.7 (11)	68.1 (47)	0.975
% (n) CHF	55.6 (10)	31.5 (46)	29.6 (47)	5.9 (1)	41.2 (7)	40.6 (25)	0.022
% (n) Hypertension	73.7 (13)	84.3 (123)	82.4 (131)	88.2 (15)	76.5 (13)	84.1 (58)	0.767
% (n) MI	27.8 (5)	26.7 (39)	28.9 (46)	35.3 (6)	23.5 (4)	18.8 (13)	0.649
% (n) Diabetes	47.4 (9)	45.2 (66)	47.8 (76)	58.8 (10)	35.3 (6)	43.8 (30)	0.786
% (n) Elective surgery	38.9 (7)	26.7 (39)	28.3 (45)	23.5 (4)	35.3 (6)	30.4 (21)	0.864
CPB time (SD)	135.1 (53.7)	135.3 (52.1)	99.2 (48.5)	9.7 (26.0)	226.7 (88.4)	180.6 (71.4)	<0.0001
Baseline EGFR (SD)	77.1 (17.9)	51.8 (16.5)	61.4 (17.1)	58.4 (19.5)	61.4 (18.3)	48.4 (15.7)	<0.0001

AKI: Acute kidney injury; CHF: Chronic heart failure; CPB: Cardiopulmonary bypass; MI: Myocardial infarction; SD: Standard deviation.

were not significantly lower due to the small number of patients in group 4. The patients in groups 5 and 6 had highest proportions of advanced AKI at 41 and 43%, respectively; however, the two groups differed significantly in mortality rates which were 18 and 36%, respectively. Both groups had higher concentrations of h-FABP and CK-MB than patients in other groups and non-AKI patients, group 5 had lower concentration of NT-proNBP than group 6.

Association of group and other outcomes

The association of the group order with other outcomes was statistically significant with approximately U-shaped relationship as well. Groups 3 and 4 had shorter duration of hospitalization and AKI than groups 2, as 5 and 6. Groups 3 and 4 had shorter duration of ICU and ventilation than groups 1, 2, 5 and 6. So worse outcomes could be associated with either lower or higher risk of having advanced AKI (Table 4 & Figure 3). The prevalence of baseline CHF also demonstrated a U-shaped relationship with the group order (Table 4).

In sum, average concentration of an individual biomarker within a group demonstrated a monotone association with risk of the occurrence of the outcomes, but a U-shaped relationship with risk of advanced AKI.

Comparing group biomarker concentrations with non-AKI patients

Group 1 had significantly lower concentrations of first postoperative IL-10 than non-AKI patients measured at the same occasion (Table 3). Patients in group 3 had significantly lower concentrations of NGAL, h-FABP, CK-MB, hsTNT and IL-6 than non-AKI patients. Patients in group 4 had lower concentrations in all biomarkers than non-AKI patients, although some of the biomarkers did not reach statistical significance due to the small size of group 4. Patients in groups 2, 5 and 6 generally had significantly higher biomarker concentrations than non-AKI patients.

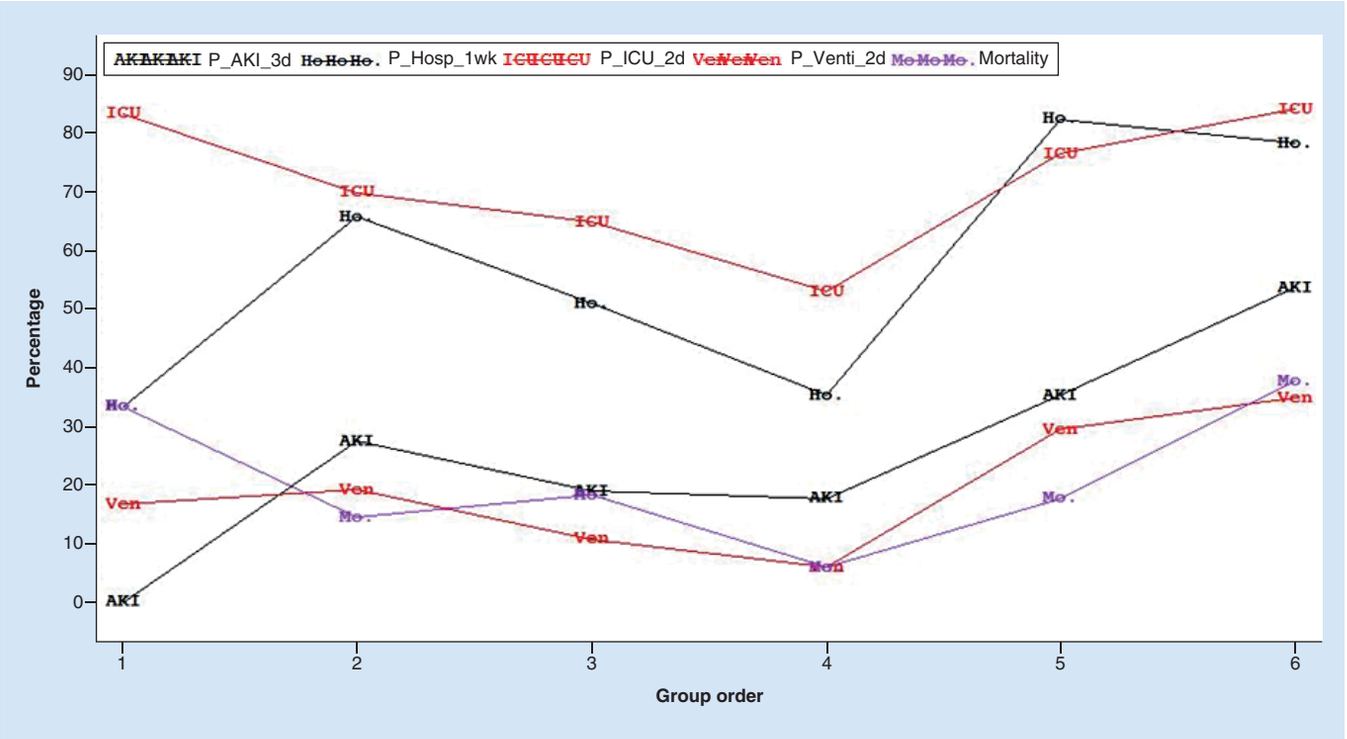

Figure 3. Proportion of the outcomes.
P_AKI_3d: Percentage of patient with the duration of AKI more than 3 day; P_Hosp_1wk: Percentage of patients with the duration of hospitalization 1 week or more; P_ICU_2d: Percentage of patient with the duration of ICU stay 2 day or more; P_Venti_2d: Percentage of patient with the duration of ventilation 2 day or more.

Discussion

Our supervised, group-based method identified distinct patterns of biomarkers at the first postoperative time point and detected approximately U-shaped relationship between risk of advanced AKI within each group and average concentration of a single biomarker, mortality and occurrence of hospitalization outcomes. The groups of the eight panel biomarkers had higher discrimination of advanced AKI than any single biomarker. The group-based method does not restrict to discover linear or monotone relationship between biomarker concentration and risk of advanced AKI. The U-shaped relationship suggested that an elevated biomarker concentration in cardiac surgery patients with AKI does not necessarily indicate advancing AKI and that the elevation of biomarker concentration and occurrence of adverse outcomes could occur in absence of advanced AKI. The difference between our finding of the U-relationship (or nonlinear) and the monotone (using either continuous or dichotomized biomarker value) association in the literature [3,30,31] regarding the association of biomarker and advanced AKI can be easily explained. The linear association of biomarker concentration and AKI described in the literature does not exclude the nonlinear or U-relationship we found in our study. A linear trend between biomarker and outcome often can be statistically significant even when the underlying relationship is nonlinear. The alignment of an individual biomarker concentration with risk of the outcomes but not with advanced AKI suggests that an elevated biomarker concentration predicted worse outcomes regardless of the presence of advanced AKI.

Biomarker concentrations were not all elevated in sync, in other words, different biomarkers were elevated in different groups. Furthermore, elevated concentration in several biomarkers in sync instead of in one or two biomarkers alone was more predictive of advanced AKI and adverse outcomes. A new patient with biomarker measurements can be potentially assigned to a unique group by calculating the posterior probability for each of the groups using the model estimates from the group-based analysis to plug in the formula given in the Method section with the terms f(advanced AKIi in Group g) and f(advanced AKIi in Goup c) removed.

Both group 2 and group 5 had elevated concentration in five biomarkers. Nevertheless, group 2 had elevated NT-ProBNP but not CK-MB and hsTNT while group 5 had elevated CK-MB and hsTNT but not NT-ProBNP.

NT-ProBNP is a marker of cardiac dysfunction and congestion. CK-MB and hsTNT are markers of myocardial ischemia [32]. All the three biomarker concentrations were elevated in group 6. The differential elevation of these cardiac biomarkers may explain the difference in the proportions of advanced AKI between group 2 and groups 5–6. In group 4, all biomarker concentrations were rather low, which may explain its low risk of adverse outcomes.

Regarding mortality, our additional analyses showed a highly significant association between NT-proBNP and mortality after adjusting for the same set of clinical variables. No other biomarker was associated with mortality. Patients in the first sextile of first postoperative NT-proBNP had no mortality and thus the OR was infinity, and the OR of preoperative NT-proBNP was also high for mortality. Those mortalities associated with high NT-proBNP may be related to the biomarker's role in acute cardiac decompensation and heart failure as elevated NT-proBNP has been known to predict cardiac failure [33]. It is also interesting to observe that group 4 had much lower biomarker values of NT-proBNP and the lowest rate of mortality and other secondary outcomes but a moderate risk of advanced AKI. These results may suggest that low concentration of cardiac function biomarkers are associated with significantly better survival [34,35], even in the presence of advanced AKI. The overall low concentration of biomarkers with extremely low concentration of CK-MB and hsTNT, in the absence of elevation in other biomarkers, may explain the low mortality rate in group 4. Groups 5 and 6, which had the highest proportions of advanced AKI, had significantly elevated concentration of both of NGAL and hsTNT. However, patients in group 5 had a significantly lower mortality than those in group 6. The only biomarker which was significantly different between the two groups was NT-proBNP. The nonelevated NT-proBNP concentration in group 5 may explain its lower mortality rate than group 6.

Group 1 had no advanced AKI but had second highest mortality rate. The patients in group 1 had lowest concentration of IL-10 among all the groups and had even lower concentration of IL-10 than the 793 patients without AKI. These findings suggested that concentrations of IL-10, an anti-inflammation biomarker in cardiac injury, might be associated with worse survival even in the absence of advanced AKI. This result is consistent with previous report that IL-10 protects against mortality in cardiac surgery patients [10], and its clinical implication thus warrants further investigation.

Our additional analyses including non-AKI patients also showed that the 763 non-AKI patients who had at least one available plasma biomarker measured at the same time as the AKI patients were exclusively classified into groups 2 and 3 with the exception of only a few patients. This suggests that the phenotypes of biomarkers in each group were very consistent whether the 763 non-AKI patients were included or not.

Our group-based method has several advantages over other nonmodel-based clustering methods. First, our model-based and group-based method can incorporate covariates and outcomes together with the biomarkers themselves. The method does not form groups based on similarity in biomarker values or on distances between observations (such as k-means clustering), but instead assumes that the distribution of each biomarker differs across the groups and thus separates the groups by the difference in the patterns of the multiple biomarkers. In addition, our group-based method can reveal that elevated concentration in theses biomarkers were not all related to each other and in fact showed some independencies. For example, groups 2 and 3 had more elevated NT-proBNP concentrations but lower values of CK-MB than groups 1 and 5. Nonmonotone association among the observed (manifest) variables, in this case the eight plasma biomarkers of AKI, were revealed through the identified groups. Third, our model is quite flexible, as it can incorporate a mix of nominal, ordinal, count and continuous biomarkers. Fourth, the group-based method does not require a patient to have measurements on all biomarkers but uses his/her available measurements. Fifth, multiple measures with varying degree of correlation can be accommodated by the group-based approach, some seemingly low discriminative measures can be dropped for the sake of parsimony, but this is not necessary as these measures may potentially achieve between-group difference.

However, a limitation of our model is that smaller sized groups may not be very representative of an actual patient cohort. Nevertheless, our sensitivity analysis using additional non-AKI patients indicated our results were rather robust in our cohort of cardiac surgery patients despite the small sizes of some groups. Also, as this was a multicenter study, there could also be unmeasured effects of variability between centers dealing with handling and processing of the biosamples. Furthermore, given the relatively low percentage of AKI in our sample, these results should ideally be validated in other independent studies. Last, in the investigation of risk factors that were included as adjusting covariates for advanced AKI, we did not have the variable extracardiac arteriopathy in our dataset, this variable may potentially affect the strength of association of biomarkers with advanced AKI.

Conclusion

The use of group-based method revealed distinct patient groups with differential synchronization of multiple biomarker concentrations. Average concentration of an individual biomarker within a group displayed approximately U-shaped relationship with risk of advanced AKI among cardiac surgery patients with AKI. Elevated concentration of biomarkers was associated with adverse outcomes regardless of the presence of advanced AKI.

Summary points

- Among cardiac surgery patients with AKI, the pre- and the post-surgery biomarker concentrations were significantly different for all the eight plasma biomarkers.
- Among cardiac surgery patients with acute kidney injury (AKI), the first postoperative plasma biomarkers h-FABP and IL-6 were each significantly associated with advanced AKI after adjustment of clinical variables.
- Group-based analysis of multiple biomarkers accommodated patients with varying number of biomarker measurement.
- All except one biomarker showed significant between-group difference.
- Multiple biomarkers displayed differential elevation patterns across groups of patients.
- Biomarker concentration is positively associated with risk of advanced AKI but in a nonlinear fashion.
- Biomarker concentrations demonstrated an approximately U-shaped, nonlinear relationship with risk of advanced AKI.
- Elevated biomarker concentration was associated with worse outcomes of hospitalization and increased mortality regardless of the presence of advanced AKI.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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