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ORIGINAL RESEARCH

Novel Blood Cytokine-Based Model for Predicting Severe Acute Kidney Injury and Poor Outcomes After Cardiac Surgery

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BACKGROUND: Alterations in serum creatinine levels delay the identification of severe cardiac surgery-associated acute kidney injury. To provide timely diagnosis, novel predictive tools should be investigated.

METHODS AND RESULTS: This prospective observational study consists of a screening cohort (n=204) and a validation cohort (n=198) from 2 centers from our hospital. Thirty-two inflammatory cytokines were measured via a multiplex cytokine assay. Least absolute shrinkage and selection operator regression was conducted to select the cytokine signatures of severe cardiac surgery-associated acute kidney injury. Afterwards, the significant candidates including interferon-γ, interleukin-16, and MIP-1α (macrophage inflammatory protein-1 alpha) were integrated into the logistic regression model to construct a predictive model. The predictive accuracy of the model was evaluated in these 2 cohorts. The cytokine-based model yielded decent performance in both the screening (C-statistic: 0.87, Brier 0.10) and validation cohorts (C-statistic: 0.86, Brier 0.11). Decision curve analysis revealed that the cytokine-based model had a superior net benefit over both the clinical factor-based model and the established plasma biomarker-based model for predicting severe acute kidney injury. In addition, elevated concentrations of each cytokine were associated with longer mechanical ventilation times, intensive care unit stays, and hospital stays. They strongly predicted the risk of composite events (defined as treatment with renal replacement therapy and/or in-hospital death) (OR of the fourth versus the first quartile [95% CI]: interferon-γ, 27.78 [3.61–213.84], interleukin-16, 38.07 [4.98–291.07], and MIP-1α, 9.13 [2.84–29.33]).

CONCLUSIONS: Our study developed and validated a promising blood cytokine-based model for predicting severe acute kidney injury after cardiac surgery and identified prognostic biomarkers for assisting in outcome risk stratification.

Key Words: acute kidney injury ■ biomarker ■ blood cytokines ■ CSA-AKI ■ predictive model

Acute kidney injury (AKI) is one of the major adverse complications frequently occurring after cardiac surgery.[1] Globally, ≈5% to 42% of patients undergoing cardiac surgery experience AKI.[2] Patients with severe AKI are confronted with a 3- to 8-fold higher perioperative mortality, prolonged length of intensive care unit (ICU) and hospital stay, and an increased cost of health care.[3] Moreover, as reported, cardiac surgery-associated acute kidney injury (CSA-AKI) was the second leading condition for AKI in the intensive care setting and contributed to increased mortality.[4] Currently, because of a general lack of effective treatments for severe CSA-AKI, the earlier identification of the condition will offer clinicians essential guidance on the prevention and management of the syndrome. Given that traditional clinical diagnosis based on serum creatinine alterations and clinical risk factors are time-consuming and lack discriminability,[5] increasing

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CLINICAL PERSPECTIVE

What Is New?

- Patients who developed severe acute kidney injury after cardiac surgery had distinct blood immune-inflammatory cytokines signatures within 12 to 24 hours after surgery.
- A novel cytokine-based predictive model incorporating interferon-γ, interleukin-16, and MIP-1α (macrophage inflammatory protein-1 alpha) successfully predicted severe cardiac surgery-associated -acute kidney injury).
- Interferon-γ, interleukin-16, and MIP-1α were all capable of predicting poor outcomes after cardiac surgery.

What Are the Clinical Implications?

- Increased plasma concentrations of interferon-γ, interleukin-16, and MIP-1α each predict higher risk for severe cardiac surgery-associated-acute kidney injury.
- Alterations in plasma immune-inflammatory cytokine concentrations demonstrate promise for early identification of patients likely to develop severe cardiac surgery-associated-acute kidney injury.

Nonstandard Abbreviations and Acronyms

AKI	acute kidney injury
CSA-AKI	cardiac surgery-associated acute kidney injury
ICU	intensive care unit

research has focused on exploring novel biomarkers for identifying risk factors for AKI and improving the diagnostic accuracy and efficiency of the disease. Apart from the well-established urine cell cycle arrest indicators such as neutrophil gelatinase-associated lipocalin (NGAL),[6] kidney injury (KIM1),[7] liver-type fatty-acid-binding protein (L-FABP),[8] insulin-like growth factor-binding protein-7 (IGFBP-7), and tissue inhibitor of metalloproteinase-2 (TIMP2),[9,10] blood biomarkers also deserve investigation for their continuous participation in the inflammatory response during AKI development and in disease prognosis.

According to animal-based studies and clinical observations, intrarenal and systemic inflammation triggered by cellular damage seriously affects tissue injury and repair.[11] During the initial phase, the activation of resident and recruited immune cells is dominated by proinflammatory soluble mediators such as free radical species, cytokines, and chemokines, which tend to amplify and extend cell damage or cell death.[12] Furthermore, the inflammatory response in the bloodstream can exacerbate tissue injury in both the kidney and remote organs.[13] Overall, cellular and molecular immune signatures play a crucial role throughout the initiation and progression of CSA-AKI, and they might be promising indicators for predicting AKI severity and clinical prognosis after cardiac surgery. Previous publications demonstrated that blood biomarkers such as interleukin (IL)-8, IL-6, IL-10, IL-18, monocyte chemotactic protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α) were associated with CSA-AKI.[14–18] However, in practice, their sensitivity and/or specificity remain unsatisfactory, which might be because of the instability of subjectively selected single markers and the lack of independent validation. Therefore, it is necessary to consider a more comprehensive landscape of immune-inflammation cytokines and integrate multiple biomarkers into a single model to improve the accuracy and robustness of disease prediction.

In this study, we conducted a 32 immune-inflammation cytokine assay in patient plasma and used a machine-learning strategy of feature selection to develop a multibiomarker-based model for predicting severe CSA-AKI. Moreover, we also evaluated the capability of each selected biomarker in the risk stratification of severe AKI and associated outcomes.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files.

Subject Enrollment and Data Collection

Two separate prospective cohorts (the screening cohort and validation cohort) from 2 cardiac surgery centers were included in this study. Eligible participants underwent cardiac surgery in the 2 centers from our hospital. Patients were excluded if they met any of the following criteria: (1) aged <10 years or >80 years; (2) history of renal transplantation or dialysis; (3) exposure to nephrotoxic drugs within 2 weeks before surgery; (4) comorbidity of advanced chronic kidney disease; and (5) recent urinary tract infection or obstruction. Finally, 204 and 198 patients were successfully enrolled in the screening and validation cohorts, respectively. Perioperative clinical data were collected by reviewing the hospital database. The study protocols were approved by the Ethics Committees of Shanghai Ruijin Hospital, and all patients provided written informed consent before study inclusion.

Sample Collection and Cytokine Measurement

Fasting venous blood samples were uniformly collected from all participants into EDTA anticoagulation tubes on the morning (8:00 AM) of the first postoperative day (between 12 and 24 hours after cardiac surgery). At a freezing temperature, the samples were centrifuged at 1500*g* for 15 minutes. Supernatant plasma was stored at −80°C before analysis. Pre- and postoperative serum creatinine concentrations were measured by the identical testing platform in the clinical laboratory of the hospital. A total of 32 plasma cytokines (including cutaneous T-cell-attracting chemokine (CTACK), acidic fibroblast growth factor (FGFa), granulocyte-colony-stimulating factor (G-CSF), hepatocyte growth factor (HGF), interferon-alpha2 (IFN-α2), interferon-gamma (IFN-γ), IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-12p40, IL-16, IL-17α, IL-18, interferon gamma-induced protein-10 (IP-10), MCP-1, monocyte chemotactic protein-3 (MCP-3), macrophage-colony stimulating factor (M-CSF), migration inhibitory factor (MIF), monokine induced by interferon-gamma (MIG), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), stem cell factor (SCF), stem cell growth factor-beta (SCGF-β), stromal-derived factor-1 alpha (SDF-1α), and TNF-α), MIP-1β (macrophage inflammatory protein-1 beta), SCF, SCGF-β, SDF-1α, and tumor necrosis factor-α) were measured using the Bio-Plex Pro Human Cytokines Assay (Bio-Rad, US) according to the manufacturer's instructions.

Diagnostic Criteria and Outcome Definition

The diagnosis of AKI was made according to the Acute Kidney Injury Network (AKIN) criteria, which referred to the 48-hour change in creatinine levels after the operation. An over 50% increase or a 0.3 mg/dL increase in serum creatinine from baseline after cardiac surgery was defined as stage 1, with doubled serum creatinine from baseline defined as stage 2 and tripled serum creatinine from baseline defined as stage 3.[19] In this study, mild AKI was determined as AKIN stage 1, while severe AKI represented AKIN stages 2 or 3. The composite events were defined as treatment with renal replacement therapy and/or in-hospital death. Prolonged length of mechanical ventilation, ICU stay, and hospitalized duration were also considered associated outcomes.

Statistical Analysis

For continuous variables, normally distributed variables are expressed as the mean and standard deviation, while non-normally distributed variables are shown as the median and interquartile range. Categorical variables are summarized as frequencies with proportions. The unpaired Student *t*-test or Mann–Whitney *U* test was used for comparing the difference between 2 groups. Three-group comparisons were accomplished by analysis of variance or the Kruskal–Wallis test. The Chi-square test or Fisher exact test was used to evaluate the association between 2 categorical variables. For the concentrations of the 32 cytokines, we normalized the order of magnitude through decimal scaling. Simple logistic regression was performed to examine the association between each cytokine and severe AKI. Least absolute shrinkage and selection operator regression (using the "glmnet" R package) was performed using cytokines with a significant level.[20] Using the least absolute shrinkage and selection operator-selected cytokines or clinical variables, candidate models for predicting severe AKI were developed in the screening cohort by binary logistic regression and then applied in the validation cohort. The goodness-of-fit of the models was evaluated by the Akaike information criterion and the Bayesian information criterion. Lower Akaike information criterion and Bayesian information criterion statistics indicate a better model. Receiver operating characteristic curve analysis was conducted to assess the model discrimination. The area under the curve, which is equal to the C-statistic, was calculated. The Brier score was used to assess the calibration of the model. Decision curve analysis was conducted to evaluate the net benefit of the cytokine-based model over the clinical variable model and established-biomarker model in both the screening and validation cohorts.[21] A nomogram for the optimum model was calculated using the "rms" R package. Receiver operating characteristic curves were also formulated to assess the discriminability of each selected cytokine for predicting severe AKI and the composite events. Based on the highest Youden Index, the optimal cutoff values were determined in the screening cohort. Afterwards, according to these cut-off values, the specificity and sensitivity of each cytokine for predicting severe AKI and the composite events were also calculated in the validation cohort. Spearman test was performed to examine the correlation between the selected cytokines and AKI severity (defined as creatinine alteration). Multivariable logistic regression was performed to examine whether the association between each cytokine and severe AKI or the composite events was independent of other clinical cofounding factors. The odds ratios (ORs) with 95% CIs of each cytokine are reported.

The data were analyzed using SPSS version 22.0 (SPSS, Inc., Chicago, IL) and statistical packages in R (The R Foundation; http://www.r-project.org; version 3.6.1), and diagrams were plotted by GraphPad

Prism 8.0 (GraphPad Software, USA). Significance levels were set at $P<0.05$ for 2-tailed tests in all analyses.

RESULTS

Clinical Characteristics of the Study Cohorts

Overall, among 204 participants in the screening cohort, 58 patients (28.4%) developed mild AKI (AKIN stage 1), and 42 patients (20.6%) were diagnosed with severe AKI (AKIN stage 2–3). In addition, 45 (22.7%) out of 198 patients in the validation cohort developed severe AKI, while 56 patients (28.3%) developed mild AKI. The clinical characteristics of the 2 cohorts are comparable (see Table S1). For each cohort, the patients' clinical and operative characteristics are summarized in Table 1. In the screening cohort, no significant differences were observed among the control, mild AKI, and severe AKI groups in age, sex, cardiac function, hyperlipidemia, and previous myocardial infarction, while in the validation cohort, sex, hyperlipidemia, and myocardial infarction varied among the 3 groups. In terms of perioperative surgical characteristics, concomitant surgery, and prolonged cardiopulmonary bypass time were significantly different between the severe AKI group and the other groups in both cohorts. Correspondingly, alteration in serum creatinine, use of renal replacement therapy, and death frequency were higher in the severe AKI group, along with prolonged ventilation time, ICU stay, and hospital duration.

Potential Blood Cytokine Markers for Severe AKI

To detect the distinct blood immune cytokine signature between the severe and non-severe AKI groups, we measured 32 cytokines in the screening cohort. The hierarchical clustering of severe AKI, mild AKI, and non-AKI based on these 32 cytokines is shown in Figure 1A. According to the clustering graph, distinct cytokine-enriching signatures were found among the severe AKI group, mild-AKI group, and non-AKI group. Twenty-two cytokines were particularly enriched in the severe AKI group. In line with the hierarchical clustering results, simple logistic regression analysis revealed that these 22 cytokines were significantly associated with severe AKI ($P<0.05$) (Figure 1B). Because of the relatively high dimensionality of the data, least absolute shrinkage and selection operator regression, a machine learning variable selection method, was conducted based on the 22 differentially enriched cytokines to identify the major representative cytokine signatures (Figure S1). Based on lambda that gives 1 standard error from the minimum cross-validated error, a parsimonious model was acquired, from which we identified 3 significant variables including IFN-γ, IL-16, and MIP-1α as representative blood biomarkers for predicting severe AKI. In line with known biological functions, these 3 cytokines could mediate immune cell activation and other cytokine production and are considered critical inflammatory factors in the initiation and progression of various types of renal injury.[22–27] Therefore, the 3 biomarkers might be promising for predicting severe AKI after cardiac surgery.

Construction and Validation of the Cytokine-Based Predictive Model for Severe AKI

To specify the distinct concentrations of the selected biomarkers between patients with severe and non-severe AKI, we compared the levels of IFN-γ, IL-16, and MIP-1α between the 2 groups. In both cohorts, patients with severe AKI had approximately twice as much the concentrations of these 3 cytokines than patients with non-severe AKI (Figure 2). Then, the 3 factors were included in a logistic regression model to build a prediction model for identifying patients at higher risk of severe AKI after cardiac surgery. Since previous studies indicated that clinical factors such as history of hypertension,[28,29] concomitant surgery and prolonged cardiopulmonary bypass time were predictors for a higher risk of AKI after cardiac surgery,[30–32] we also compared the predictive ability of the cytokine-based model with these traditional clinical factor-based models. Notably, previous publications demonstrated that postoperative TNF-α and MCP-1 are useful blood biomarkers for predicting CSA-AKI.[18,33,34] Thus, we also compared the effectiveness of the novel cytokine-based model to the established biomarker-based model incorporating TNF-α and MCP-1.

Finally, 3 logistic regression models [including the cytokine-based model (Model 1), the clinical factor-based model (Model 2) and the established plasma biomarker-based model (Model 3)] were developed in the screening cohort and tested in the validation cohort (see Table 2). In both cohorts, compared with Model 2 and Model 3, Model 1 yielded lower Akaike information criterion and Bayesian information criterion values and had better calibration (screening cohort: Brier Score: Model 1: 0.10, Model 2: 0.15, Model 3: 0.15; validation cohort: Brier Score: Model 1: 0.11, Model 2: 0.17, Model 3: 0.17) and discrimination performance (screening cohort: C-statistic; Model 1, 0.87 [0.80–0.94]; Model 2, 0.68 [0.60–0.76]; Model 3, 0.80 [0.73–0.87]; validation cohort: C-statistic; Model 1, 0.86 [0.79–0.92]; Model 2, 0.63 [0.54–0.73]; Model 3, 0.76 [0.68–0.83]).

Table 1. Characteristics of the Patients at Baseline in Screening and Validation Cohorts

	Screening Cohort				Validation Cohort			
	Non-AKI (n=104)	Mild-AKI (AKIN 1, n=58)	Severe-AKI (AKIN 2/3, n=42)	*P* Value	Non-AKI (n=97)	Mild-AKI (AKIN1, n=56)	Severe-AKI (AKIN2/3 n=45)	*P* Value
Age, y, mean (SD)	53.9 (12.8)	55.1 (12.8)	57.2 (11.7)	0.37	56.2 (13.3)	58.5 (12.0)	52.6 (13.5)	0.076
Male, n (%)	77 (74.0%)	45 (77.6%)	26 (61.9%)	0.197	75 (77.3%)	49 (87.5%)	26 (57.8%)	0.002
LVEF, % median (IQR)	60.0 (58.0–63.0)	61.5 (58.2–65.0)	60.5 (58.5–63.0)	0.336	60.0 (56.0–63.0)	60.0 (58.0–63.0)	61.0 (58.0–65.0)	0.117
NYHA class III or IV, n (%)	19 (18.3%)	18 (31.0%)	11 (26.2%)	0.165	29 (29.9%)	21 (37.5%)	10 (22.2%)	0.25
Diabetes mellitus, n (%)	18 (17.3%)	11 (19.0%)	2 (4.8%)	0.083	18 (18.6%)	9 (16.1%)	4 (8.9%)	0.339
Hypertension, n (%)	54 (51.9%)	34 (58.6%)	30 (71.4%)	0.096	49 (51.0%)	38 (67.9%)	30 (66.7%)	0.066
Hyperlipidemia, n (%)	54 (51.9%)	32 (55.2%)	26 (61.9%)	0.547	57 (58.8%)	25 (44.6%)	17 (37.8%)	0.043
AMI history, n (%)	8 (7.7%)	5 (8.6%)	1 (2.4%)	0.506	13 (13.4%)	6 (10.7%)	0 (0.0%)	0.018
Surgery type								
CABG or Valve surgery,	45 (43.3%)	21 (36.2%)	8 (19.0%)	0.022	52 (53.6%)	17 (30.4%)	12 (26.7%)	<0.001
CABG and Valve surgery, n (%)	59 (56.7%)	37 (63.8%)	34 (81.0%)		45 (46.4%)	39 (69.6%)	33 (73.3%)	
CPB time, h median (IQR)	102.0 (81.0–130.0)	133.0 (111.0–164.0)	147.5 (123.8–195.8)	<0.001	105.0 (83.0–131.0)	144.0 (120.0–170.0)	163.0 (115.5–202.8)	<0.001
CPB time >120 min, n (%)	20 (19.2%)	23 (39.7%)	18 (42.9%)	0.003	20 (20.6%)	28 (50.0%)	19 (42.2%)	0.002
Delta creatinine, μmol/L median (IQR)	15.3 (2.5–29.5)	65.4 (54.3–75.0)	174.9 (110.5–224.4)	<0.001	12.0 (4.5–24.4)	63.5 (55.9–73.1)	142.9 (103.9–238.3)	<0.001
Outcomes								
RRT or (and) in-hospital death, n (%)	2 (1.9%)	2 (3.4%)	13 (31.0%)	<0.001	2 (2.1%)	0 (0.0%)	17 (37.8%)	<0.001
Hospitalized time, d median (IQR)	13.0 (10.0–18.0)	14.0 (11.0–20.8)	20.5 (13.2–27.8)	<0.001	13.0 (10.0–15.0)	14.0 (12.0–20.2)	21.0 (12.0–26.0)	<0.001
ICU stay, d median (IQR)	2.0 (1.0–5.0)	4.0 (2.0–5.8)	6.5 (5.0–14.5)	<0.001	3.0 (2.0–4.0)	2.0 (2.0–5.0)	6.0 (2.0–10.0)	<0.001
Ventilation time, h median (IQR)	15.0 (12.0–20.5)	20.5 (15.2–43.5)	48.5 (24.5–134.0)	<0.001	16.0 (11.0–20.0)	17.5 (12.8–27.2)	38.0 (19.0–128.0)	<0.001

AMI indicates acute myocardial infarction; CABG, coronary artery bypass graft; CPB, cardiopulmonary bypass; ICU, intensive care unit; IQR, interquartile range; LVEF, left ventricle ejection fraction; NYHA, New York Heart Association functional classification; and RRT, renal replacement therapy.

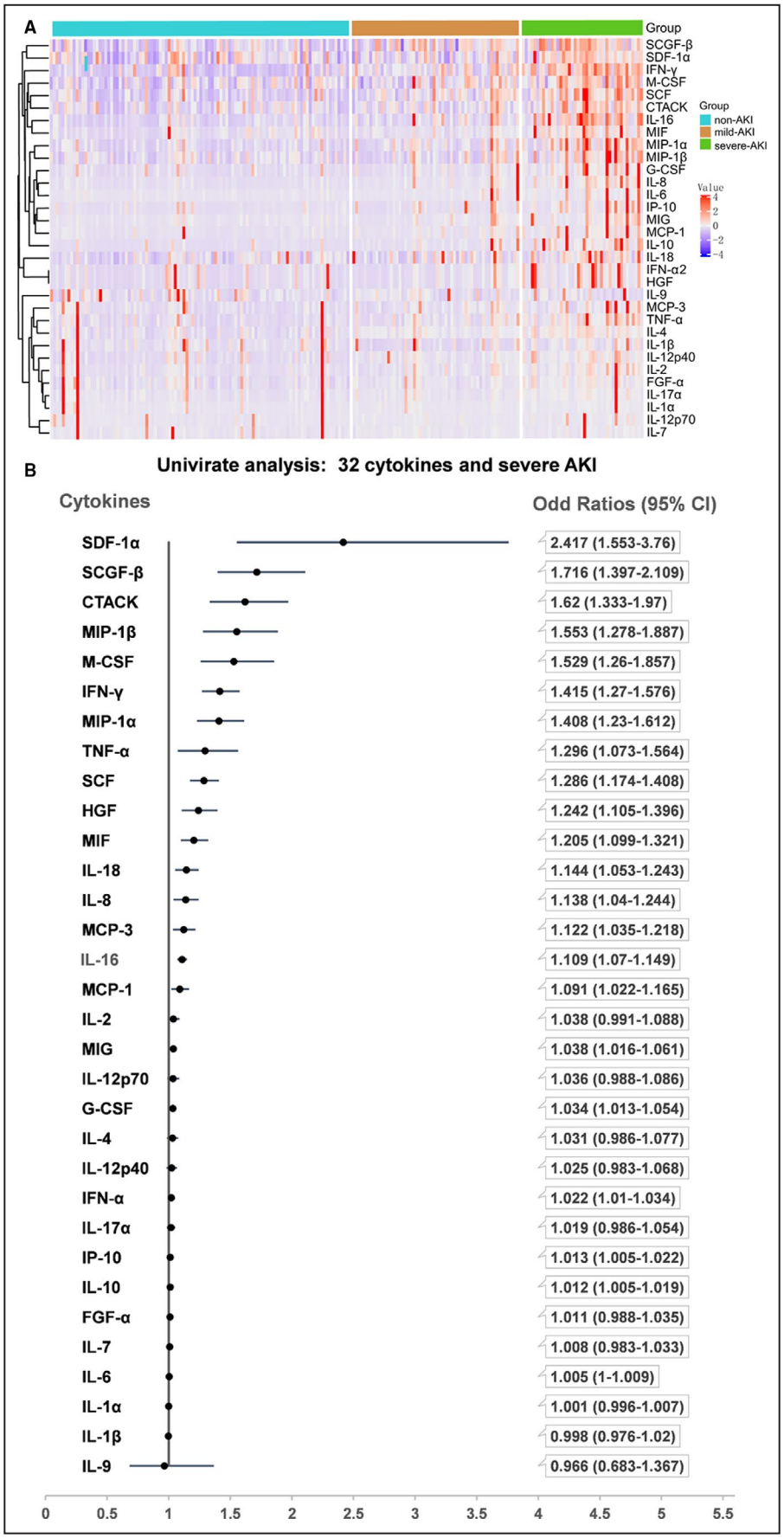

A
Group
SCGF-β
SDF-1α
IFN-γ
M-CSF
SCF
CTACK
IL-16
MIF
MIP-1α
MIP-1β
G-CSF
IL-8
IL-6
IP-10
MIG
MCP-1
IL-10
IL-18
IFN-α2
HGF
IL-9
MCP-3
TNF-α
IL-4
IL-1β
IL-12p40
IL-2
FGF-α
IL-17α
IL-1α
IL-12p70
IL-7
Group
non-AKI
mild-AKI
severe-AKI
Value
4
2
0
−2
−4
B
Univirate analysis: 32 cytokines and severe AKI
Cytokines
Odd Ratios (95% CI)
SDF-1α 2.417 (1.553-3.76)
SCGF-β 1.716 (1.397-2.109)
CTACK 1.62 (1.333-1.97)
MIP-1β 1.553 (1.278-1.887)
M-CSF 1.529 (1.26-1.857)
IFN-γ 1.415 (1.27-1.576)
MIP-1α 1.408 (1.23-1.612)
TNF-α 1.296 (1.073-1.564)
SCF 1.286 (1.174-1.408)
HGF 1.242 (1.105-1.396)
MIF 1.205 (1.099-1.321)
IL-18 1.144 (1.053-1.243)
IL-8 1.138 (1.04-1.244)
MCP-3 1.122 (1.035-1.218)
IL-16 1.109 (1.07-1.149)
MCP-1 1.091 (1.022-1.165)
IL-2 1.038 (0.991-1.088)
MIG 1.038 (1.016-1.061)
IL-12p70 1.036 (0.988-1.086)
G-CSF 1.034 (1.013-1.054)
IL-4 1.031 (0.986-1.077)
IL-12p40 1.025 (0.983-1.068)
IFN-α 1.022 (1.01-1.034)
IL-17α 1.019 (0.986-1.054)
IP-10 1.013 (1.005-1.022)
IL-10 1.012 (1.005-1.019)
FGF-α 1.011 (0.988-1.035)
IL-7 1.008 (0.983-1.033)
IL-6 1.005 (1-1.009)
IL-1α 1.001 (0.996-1.007)
IL-1β 0.998 (0.976-1.02)
IL-9 0.966 (0.683-1.367)
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5

Figure 1. Association of 32 blood cytokines and acute kidney injury (AKI) after cardiac surgery.
A, Hierarchical clustering of samples based on the 32 cytokines among the non-AKI, mild-AKI and severe AKI groups. **B**, Forest plot of univariate logistic analysis of the 32 cytokines and severe AKI after cardiac surgery. AKI was defined by the AKIN criteria: mild AKI=AKIN stage 1, severe AKI=AKIN stage 2/3. AKI indicates acute kidney injury; IFN, interferon; IL, interleukin; MIP, macrophage inflammatory protein; TNF, tumor necrosis factor; CTACK, cutaneous T-cell-attracting chemokine; FGFa, acidic fibroblast growth factor, G-CSF, granulocyte-colony-stimulating factor; HGF, hepatocyte growth factor; IP-10, interferon gamma-induced protein-10, MCP, monocyte chemotactic protein; M-CSF, macrophage-colony stimulating factor; MIF, migration inhibitory factor; MIG, monokine induced by interferon-gamma ; SCF, stem cell factor; SCGF-β, stem cell growth factor-beta; SDF-1α, stromal-derived factor-1 alpha.

Additionally, according to the decision curve analysis, Model 1 demonstrated an increased net benefit over Model 2 and Model 3 for predicting severe AKI in both cohorts (see Figure 3A through 3D). Afterwards, for clinical usage, a prognostic nomogram based on Model 1 for severe AKI with scales for the particular 3 cytokines was established (Figure 3E).[35] The model had a sensitivity of 0.79 and a specificity of 0.83 in the screening cohort and a sensitivity of 0.73 and a specificity of 0.88 in the validation cohort.

Application of the Cytokine-Based Predictive Model in Age/Sex Stratified Groups

Considering the male-dominating sex distribution as well as the wide range of patients' ages, we also tested the performance of our novel predictive model in different age and sex groups. The cytokine-based model was shown to be useful in both the male and female groups, as it showed good performance in both groups, with a relatively better discriminability among female patients (C-statistic: 0.87, Brier 0.13 in women versus C-statistic 0.86, Brier 0.10 in men). Furthermore, we also divided the study population into a young and middle-aged group (aged <60 years) and an older group (aged ≥60 years) to test the predictive value of our model. The cytokine-based model showed decent performance in different age groups, with better discriminability and calibration in older patients (young and middle-aged: C-statistic 0.84, Brier 0.12, Older: C-statistic: 0.91, Brier 0.08) (Table S2).

Predictive Performance of Cytokines for Severe AKI and Outcomes

To assess the prognostic value of each biomarker from our predictive model, we conducted receiver operating characteristics analysis and calculated the sensitivity, specificity, and cutoff value of the best performance in predicting severe CSA-AKI. As shown in Figure 4A and 4B, the area under the receiver operating characteristic curves (AUC), represented by the C-statistic, indicated that each cytokine had good discriminability for severe CSA-AKI, with IFN-γ (screening cohort: C-statistic: 0.86, 95% CI, 0.79–0.92; validation cohort: C-statistic: 0.82; 95% CI, 0.76–0.89) and IL-16 (screening cohort: C-statistic: 0.82; 95% CI, 0.74–0.90; validation cohort: C-statistic: 0.83; 95% CI, 0.75–0.91) displaying better performance than MIP-1α (screening cohort: C-statistic: 0.77; 95% CI, 0.69–0.86; validation cohort: C-statistic: 0.79; 95% CI, 0.72–0.87). Such satisfactory predictive value of each cytokine was also observed in the age- and sex-stratified groups, in which IFN-γ and IL-16 also outperformed MIP-1α in predicting severe CSA-AKI (Figure 5, left). In addition, with almost the same threshold value from the screening cohort, IL-16 and MIP-1α also had higher specificity (screening cohort: IL-16: 0.89, MIP-1α: 0.97; validation cohort: IL-16: 0.88, MIP-1α: 0.83), while IFN-γ showed the highest sensitivity (0.88 in the screening cohort and 0.76 in the validation cohort) for severe CSA-AKI prediction.

As severe CSA-AKI predominantly results in poor outcomes such as renal replacement therapy and in-hospital death, we also evaluated the prognostic capability of the 3 cytokines for these composite events. According to Figure 4C and 4D, IL-16 had a stronger ability to predict the composite events in both cohorts (screening cohort: C-statistic: 0.87, 95% CI, 0.77–0.96; validation cohort C-statistic: 0.91, 95% CI, 0.82–1.00), with a cut-off value of ≈11.89 (10^{-2} ng/mL). IL-16 had the strongest sensitivity (0.88 in the screening cohort and 0.95 in the validation cohort) for predicting the composite events. Secondary to IL-16, IFN-γ showed a C-statistic of 0.81 in the screening cohort and 0.82 in the validation cohort, while MIP-1α displayed C-statistics of 0.75 and 0.81 in the screening and validation cohorts, respectively. Moreover, the prognostic roles of these cytokines were not disturbed by age or sex stratification. In the subgroups, the 3 biomarkers yielded decent prognostic values for the composite outcomes, with IL-16 also displaying better discriminability (Figure 5, right).

Associations of Cytokine Levels and Perioperative Prognostic Features

To explore the concentration-dependent effect of the 3 cytokines on the risk stratification of severe AKI and the composite events, we further analyzed the association between the cytokine levels and disease severity after pooling the 2 cohorts together. Disease severity was evaluated by various

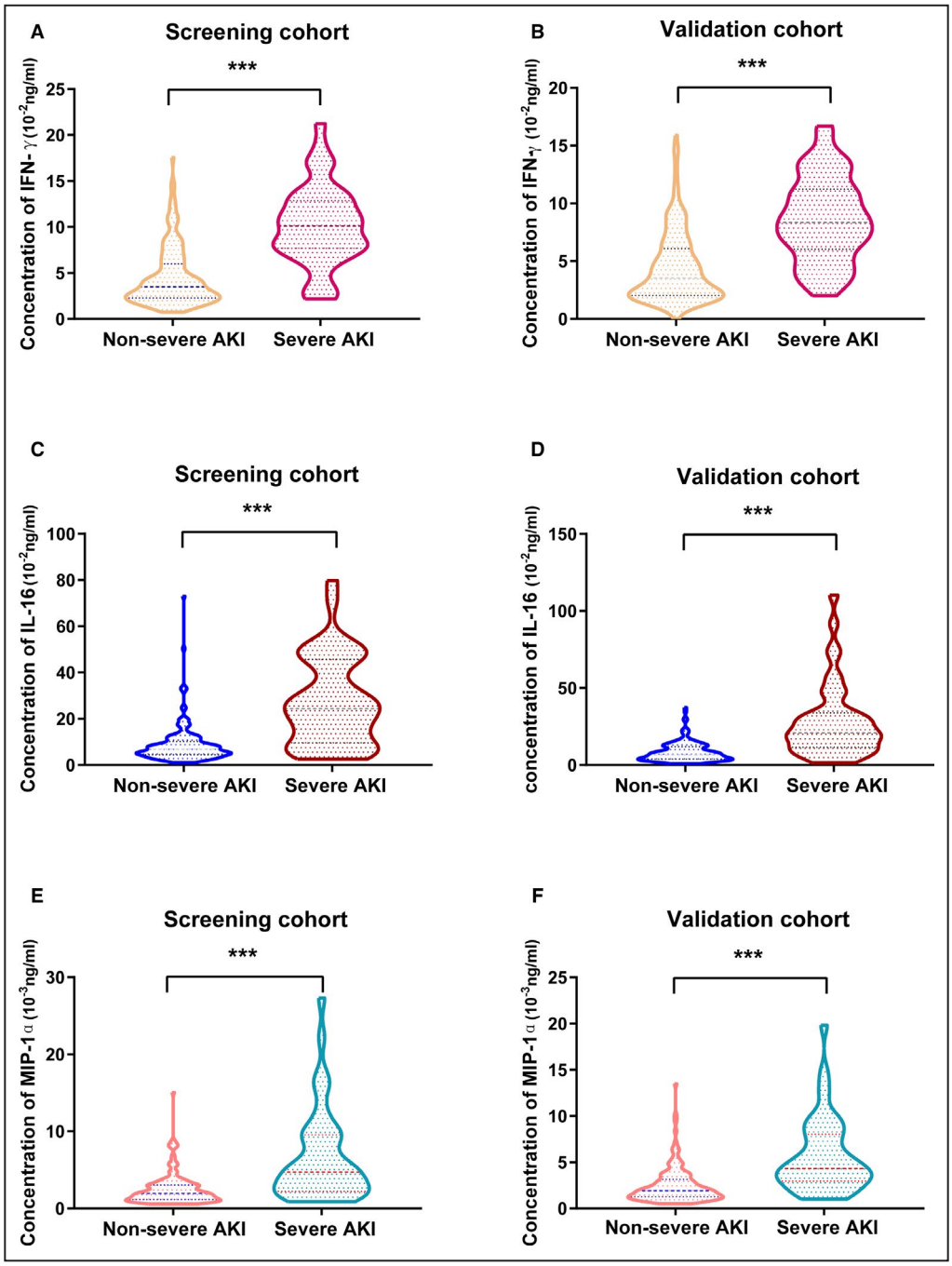


Figure 2. Variation in the concentrations of the selected cytokines between the severe and non-severe AKI (acute kidney injury) groups in the screening and validation cohorts.
A, comparison of interferon-γ between the severe and non-severe AKI groups in the screening cohort. **B**, A comparison of interferon-γ between the severe and non-severe AKI groups in the validation cohort. **C**, Comparison of interleukin-16 between the severe and non-severe AKI groups in the screening cohort. **D**, Comparison of interleukin-16 between the severe and non-severe AKI groups in the validation cohort. **E**, Comparison of MIP-1α (macrophage inflammatory protein-1 alpha) between the severe and non-severe AKI groups in the screening cohort. **F**, Comparison of MIP-1α between the severe and non-severe AKI groups in the validation cohort. In the violin plots, the horizontal dotted lines indicate the first quartile and the third quartile of the cytokine levels, and the horizontal dashed line within the violin represents the median cytokine levels. Statistical significance was determined by a non-parametric test (***P<0.0001). AKI indicates acute kidney injury; IFN-γ , interferon-gamma; IL-16, interleukin-16; and MIP-1α, macrophage inflammatory protein-1 alpha.

Table 2. Performance of the Predictive Models for Predicting acute kidney injury (AKI) After Cardiac Surgery in the Screening and Validation Cohorts

	Screening Cohort				Validation Cohort	
	AIC	BIC	Brier	C-statistics	Brier	C-Statistics
Model 1	142.78	156.05	0.10	0.87 (0.80–0.94)	0.11	0.86 (0.79–0.92)
Model 2	201.50	214.80	0.15	0.68 (0.60–0.76)	0.17	0.63 (0.54–0.73)
Model 3	192.16	202.11	0.15	0.80 (0.73–0.87)	0.17	0.76 (0.68–0.83)

Model 1: MIP-1α (macrophage inflammatory protein-1 alpha)+interleukin-16+interferon-γ; Model 2: Hypertension+surgery type (=1)+cardiopulmonary bypass time (>120 minutes); Model 3: MCP-1 (monocyte chemotactic protein-1) +TNF-α (tumor necrosis factor-alpha). AIC indicates Akaike Information Criterion; AKI, acute kidney injury; and BIC: Bayesian Information Criterion were used to evaluate goodness-of-fit for the models.

parameters, including AKI severity (expressed as the elevation of creatinine after surgery), composite events, and perioperative indicators that reflect patients' clinical course. Spearman correlation tests revealed that IFN-γ, IL-16, and MIP-1α strongly correlated with creatinine alteration (IFN-γ, r=0.63; IL-16, r=0.52; MIP-1α, r=0.47), implying a significant association between the biomarkers and AKI severity (Figure S2). To further evaluate the performance of these biomarkers in classifying the risk of severe AKI or composite events, we subsequently divided the patients into 4 groups according to the quartiles of cytokine concentrations. Notably, as the cytokine levels increased, patients were more likely to develop severe AKI and experience composite events, along with a prolonged length of mechanical ventilation, ICU stay and hospital stay (see Table S3). Among the 3 cytokines, IFN-γ had an excellent performance for gradient risk stratification. Specifically, patients with IFN-γ levels in the third and fourth quartiles (Q3 and Q4) had a concentration-dependent significantly higher frequency of poor outcomes and delayed clinical recovery than patients with IFN-γ levels in the first quartile (Q1). Patients with IL-16 or MIP-1α concentrations in Q4 showed a significantly higher incidence of severe AKI and composite events, while compared with Q1 group patients, the frequency of these events in the Q2 and Q3 groups lacked statistical significance.

Furthermore, we found a significant association between the 3 cytokines (stratified as quartiles) and the risks of both severe AKI and composite events. In crude models and after adjusting for cofounders (age, sex, prolonged cardiopulmonary bypass time, cardiac function, diabetes mellitus, hypertension), high quantiles of IFN-γ, IL-16, and MIP-1α indicated an increased risk for both severe AKI and composite events (P for trend<0.0001) (see Table 3). Specifically, the upper quartiles of IFN-γ displayed a significantly higher adjusted OR, from 2.84 at Q2 (P<0.001) to 37.53 at Q4 (P<0.001), compared with that at Q1. For IL-16 and MIP-1α, compared with Q1, the highest quartile showed a significantly higher risk for severe AKI and the composite outcomes, while Q2 and Q3 displayed higher ORs without statistical significance. When examined as continuous variables, with each unit increase, the multivariate-adjusted ORs of IFN-γ, IL-16, and MIP-1α were 1.41 (95% CI, 1.30–1.53; P<0.0001), 1.12 (95% CI, 1.08–1.15; P<0.0001), and 1.50 (95% CI, 1.34–1.68; P<0.0001), respectively, for severe AKI. For the composite events, the multivariate-adjusted ORs of IFN-γ, IL-16 and MIP-1 were 1.26 (95% CI, 1.16–1.38; P<0.0001), 1.10 (95% CI, 1.07–1.13; P<0.0001), and 1.23 (95% CI, 1.12–1.34; P<0.0001), respectively. Overall, the 3 cytokines were also promising biomarkers for assisting in the risk stratification of severe AKI, perioperative recovery time, and composite events.

DISCUSSION

In this study, based on a multiplex cytokine assay as well as the combination of a machine learning signature selection approach and logistic regression, we developed and validated a novel predictive model incorporating IFN-γ, IL-16, and MIP-1α for predicting severe CSA-AKI. The performance of the predictive model was satisfactory, with decent accuracy and discriminability in 2 separate cohorts and different age/sex groups. Additionally, IFN-γ, IL-16, and MIP-1α were all capable of predicting severe AKI and composite events, and higher concentrations of each cytokine were associated with higher risks of both AKI severity and poor outcomes after cardiac surgery. Our study provides a robust predictive tool for severe CSA-AKI and blood inflammatory biomarkers for enhancing the risk stratification of CSA-AKI and relevant clinical outcomes.

CSA-AKI, characterized by abrupt renal dysfunction after cardiac surgery, is a threatening clinical complication associated with poor patient and renal prognosis. Evidence is mounting that patients with severe AKI who have doubled serum creatinine levels have a >2-fold in-hospital mortality, a longer length of ICU and hospital stay, and an excess of healthcare costs.[3,36] Given the delayed diagnosis of the disease according to traditional creatinine alterations, a few[8]

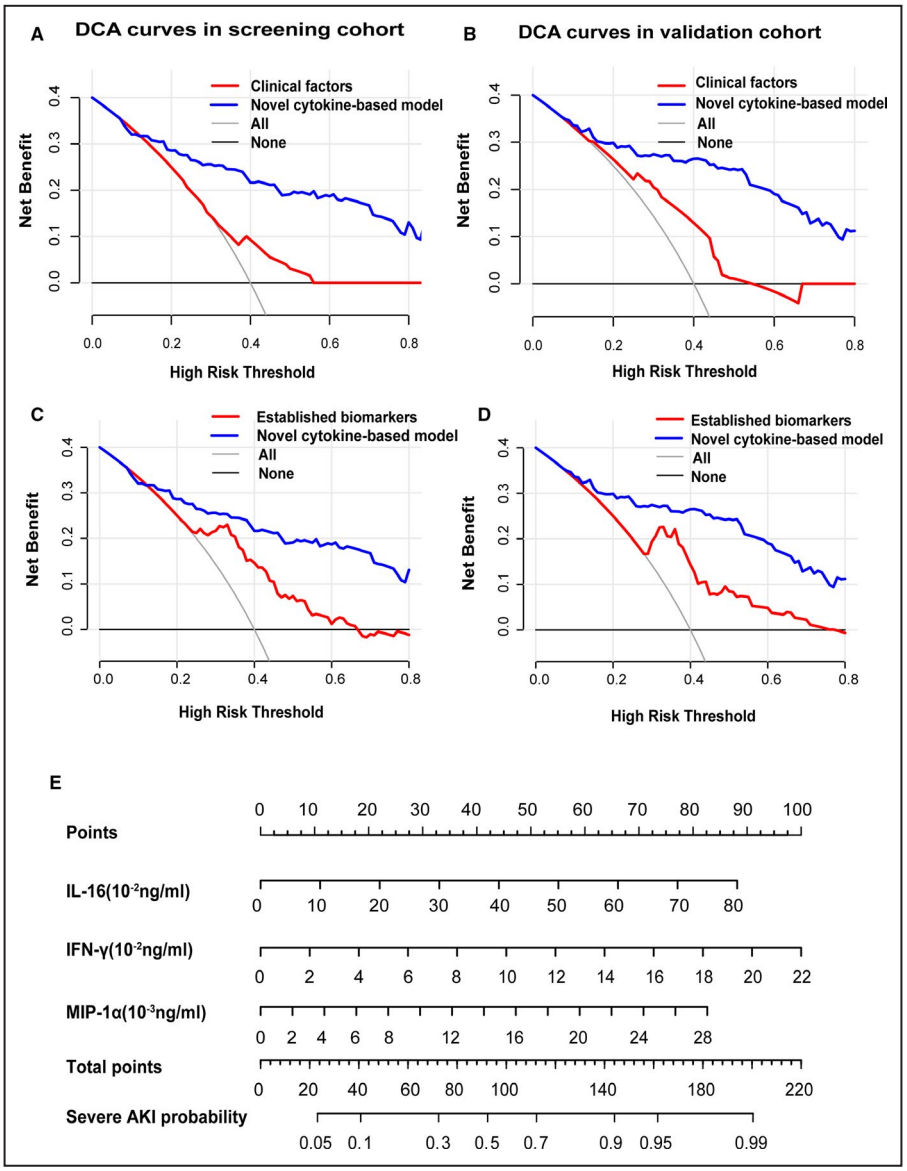
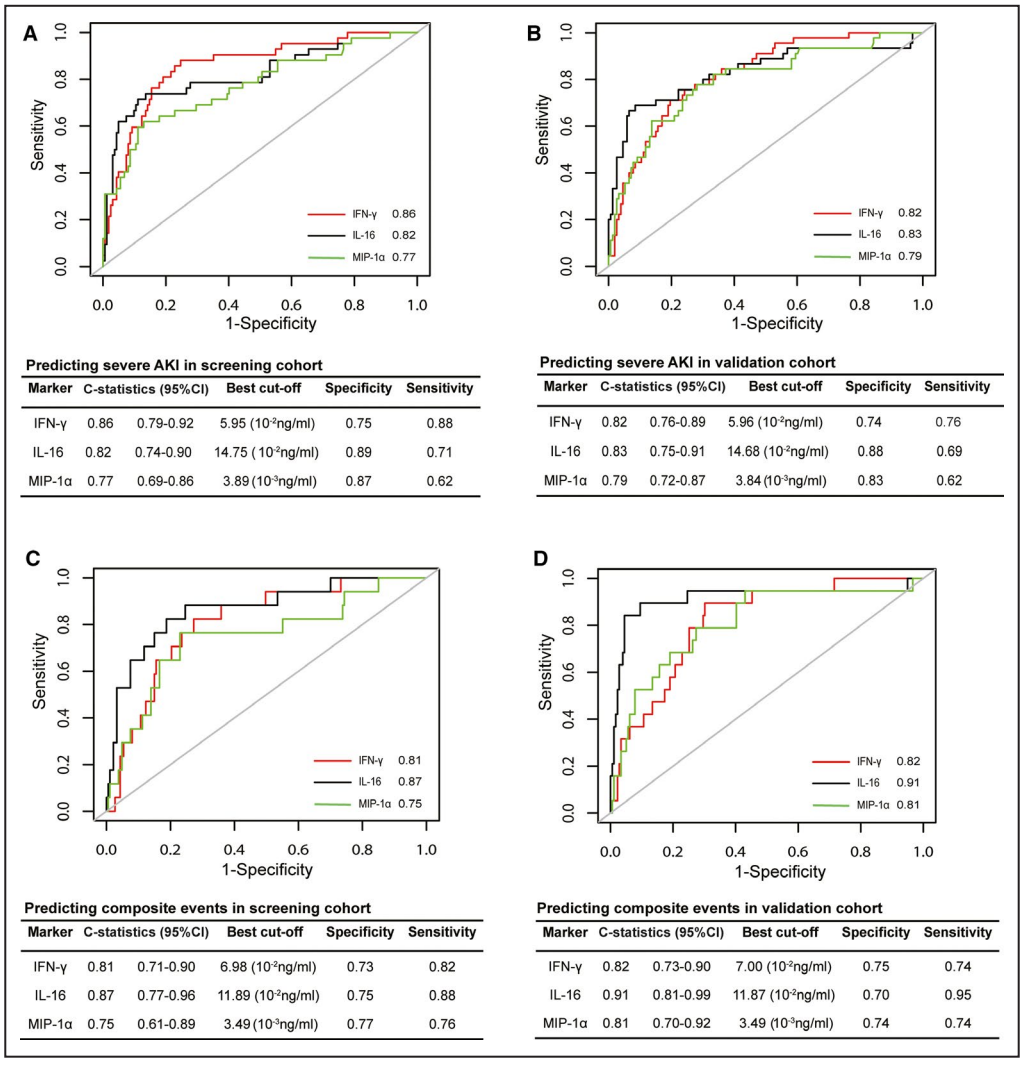


Figure 3. Decision curve analysis (DCA) and prognostic nomogram of the clinical factor-based model.

A, The DCA curve for comparing the novel cytokine-based model and clinical factors in the screening cohort. **B**, The DCA curve for comparing the novel cytokine-based model and clinical factors in the validation cohort. **C**, The DCA curve for comparing the novel cytokine-based model and established biomarkers in the screening cohort. **D**, The DCA curve for comparing the novel cytokine model and established biomarkers in the validation cohort. The novel cytokine-based model included interferon-γ, interleukin-16, and macrophage inflammatory protein-1α. Clinical factors included comorbidity of hypertension, concomitant surgery, and cardiopulmonary bypass time. Established biomarkers included monocyte chemotactic protein-1 and tumor necrosis factor-α. The net benefit of the multibiomarker model and clinical predictors was plotted against the threshold probability; the horizontal axis represents the threshold value, which is the reference probability of whether a patient receives treatment, and the vertical axis represents the net benefit rate after the advantages are subtracted from the disadvantages. Under the same threshold probability, the larger net benefit implies that patients can obtain the maximum benefit using the diagnosis from this model. The closer the curve in the DCA graph is to the top, the higher the value of the model diagnosis will be. **E**, The nomogram graphically depicts the cytokine-based predictive model for severe cardiac surgery associated-acute kidney injury. Clinical usage: Points are assigned for interferon-γ, interleukin-16, and MIP-1α by drawing a line upward from the corresponding concentration value to the "Points" line. Based on the cumulative point scores of the 3 cytokines, draw a downward vertical line from the "Total Points" line to the "Severe acute kidney injury probability" line of the nomogram to calculate the probability of severe acute kidney injury. AKI indicates acute kidney injury; DCA; decision curve analysis; IFN-γ, interferon-gamma; IL-16, interleukin-16; and MIP-1α, macrophage inflammatory protein-1 alpha.



Predicting severe AKI in screening cohort

Marker	C-statistics (95%CI)	Best cut-off	Specificity	Sensitivity
IFN-γ	0.86	0.79-0.92	5.95 (10^{-3}ng/ml)	0.75	0.88
IL-16	0.82	0.74-0.90	14.75 (10^{-3}ng/ml)	0.89	0.71
MIP-1α	0.77	0.69-0.86	3.89 (10^{-3}ng/ml)	0.87	0.62

Predicting severe AKI in validation cohort

Marker	C-statistics (95%CI)	Best cut-off	Specificity	Sensitivity
IFN-γ	0.82	0.76-0.89	5.96 (10^{-3}ng/ml)	0.74	0.76
IL-16	0.83	0.75-0.91	14.68 (10^{-3}ng/ml)	0.88	0.69
MIP-1α	0.79	0.72-0.87	3.84 (10^{-3}ng/ml)	0.83	0.62

Predicting composite events in screening cohort

Marker	C-statistics (95%CI)	Best cut-off	Specificity	Sensitivity
IFN-γ	0.81	0.71-0.90	6.98 (10^{-3}ng/ml)	0.73	0.82
IL-16	0.87	0.77-0.96	11.89 (10^{-3}ng/ml)	0.75	0.88
MIP-1α	0.75	0.61-0.89	3.49 (10^{-3}ng/ml)	0.77	0.76

Predicting composite events in validation cohort

Marker	C-statistics (95%CI)	Best cut-off	Specificity	Sensitivity
IFN-γ	0.82	0.73-0.90	7.00 (10^{-3}ng/ml)	0.75	0.74
IL-16	0.91	0.81-0.99	11.87 (10^{-3}ng/ml)	0.70	0.95
MIP-1α	0.81	0.70-0.92	3.49 (10^{-3}ng/ml)	0.74	0.74

Figure 4. Performance of cytokines for predicting severe acute kidney injury and composite events. The C-statistics of cytokines (interferon-γ, interleukin-16, MIP-1α [macrophage inflammatory protein-1 alpha]) for predicting severe acute kidney injury in the screening cohort (**A**) and the validation cohort (**B**). The C-statistics of biomarkers (interferon-γ, interleukin-16, MIP-1α) for predicting composite events in the screening cohort (**C**) and the validation cohort (**D**). AKI indicates acute kidney injury; IFN-γ , interferon-gamma; IL-16, interleukin-16; and MIP-1α, macrophage inflammatory protein-1 alpha.

attempts have been made to develop novel biomarkers for CSA-AKI prediction. Among the well-studied biomarkers, most were based on urine samples, such as the so-called cell cycle arrest urine biomarkers urine neutrophil gelatinase-associated lipocalin (uNGAL), urine kidney injury molecule1 (uKIM1), urine liver-type fatty-acid-binding protein (uL-FABP), urine matrix metalloproteinase-7(uMMP-7), urine insulin-like growth factor-binding protein-7 (uIGFBP-7), urine interleukin-18 (uIL-18) and urine tissue inhibitor of metalloproteinase-2 (uTIMP2).[37–39] However, studies on the predictive value of immune-inflammatory blood cytokines are still insufficient. Accumulating studies have mainly focused on confirming the association between single or several subjectively selected cytokines and CSA-AKI, which introduces the possibility of selection bias.[40,41] As a result, the stability of individual blood biomarkers for predicting CSA-AKI varied among different studies.

Unlike previous studies, our study is dedicated to developing and validating a novel and robust predictive tool to identify severe AKI (AKIN stage 2/3) in patients undergoing cardiac surgery. To the best of our knowledge, we might provide the most comprehensive immune-inflammatory blood cytokine profile among different CSA-AKI groups in a relatively larger population. Beyond the previously reported cytokines, we found that a total of 30 blood cytokines differed among the non-AKI, mild AKI and severe AKI groups. In addition, as many as 22 cytokines showed statistical significance in univariate analysis, indicating that the immune-inflammation network rather than

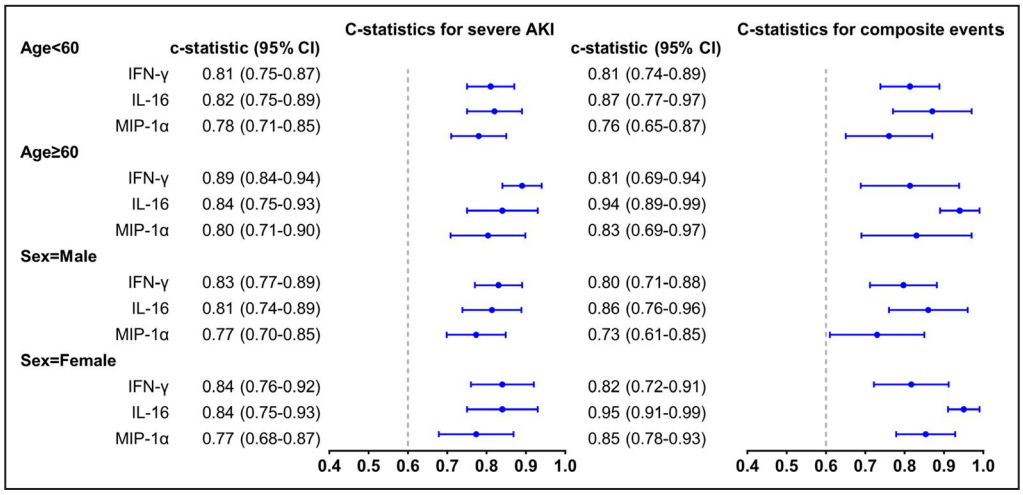


Figure 5. Performance of cytokines for predicting severe acute kidney injury and composite events in age/sex stratified groups.
Left: The C-statistics and 95% CIs of cytokines (interferon-γ, interleukin-16, MIP-1α [macrophage inflammatory protein-1 alpha]) for predicting severe acute kidney injury in the male and female groups as well as the young and middle-aged groups (aged <60 years) and the older group (aged ≥60 years). Right: The C-statistics and 95% CIs of biomarkers (interferon-γ, interleukin-16, MIP-1α) for predicting composite events in different subgroups. AKI indicates acute kidney injury; IFN-γ , interferon-gamma; IL-16, interleukin-16; and MIP-1α, macrophage inflammatory protein-1 alpha.

merely 1 or 2 cytokines might experience profound alterations. To build a robust and representative predictive tool based on such a considerable number of cytokines, we applied least absolute shrinkage and selection operator regression, which is often used for the dimensionality reduction of high-dimensionality data and selects the essential variables with machine learning.[42] The method performs shrinkage and variable selection simultaneously for better prediction while effectively avoiding overfitting.[20] After penalizing the magnitude of regression coefficients and excluding variables with a zero coefficient, IFN-γ, IL-16, and MIP-1α were selected for subsequent model construction. These cytokines were statistically and mathematically appropriate and are also known to play important roles in AKI throughout its pathophysiological process. Specifically, IFN-γ, the key Th-1-derived cytokine, was involved in CD4+ T cell-mediated ischemia-reperfusion injury, was increased in the blood of patients with AKI, and served as an independent risk factor for both AKI and long-term mortality after cardiac surgery.[17,43] MIP-1α, which activates and recruits local leukocytes, has been proven to be an indicator of AKI after acute lung injury.[26] As a T-cell chemoattractant significantly expressed in the proximal and distal straight tubules of the kidney, IL-16 has been shown to contribute to renal ischemia-reperfusion injury in animal models based on the decrease in kidney injury after IL-16 inactivation.[27] For an applicable model in clinical contexts, logistic regression was conducted for model establishment because it is more familiar to clinical physicians. Our predictive model based on these cytokines outperformed both traditional clinical factors and established biomarkers and showed satisfactory performance in both the screening and validation cohorts. Even after subgrouping the whole study population by age and sex, we also observed decent performance of the model, with better predictive value in the female group and older group, which reflects the robustness and stability of the model to some extent. More importantly, each cytokine in the model displayed prognostic value for predicting higher risks of severe CSA-AKI and composite events even in different age and sex groups.

Since the 3 cytokines strongly correlated with the change in creatinine levels, which indicates the severity of AKI, we further proved that each cytokine could serve as a predictor for a higher risk of severe AKI and associated outcomes. Among the 3 biomarkers, IFN-γ showed the best performance in the risk stratification of severe AKI and associated outcomes in patients undergoing cardiac surgery. After adjusting for the cofounders, the third and fourth quantiles of IFN-γ denoted an 11.46-fold risk and a 48.62-fold risk of severe AKI, respectively, whereas with the same category, the postoperative levels of IFN-γ denoted an 11.01-fold risk and a 27.78-fold risk of composite events. In line with the risk factor analysis, IFN-γ also showed excellent discriminability for classifying severe AKI in both the screening and validation cohorts. Our study further revealed IFN-γ as a predictor assisting in

Table 3. Multivariate Logistic Regression Analyses of IFN-γ, IL-16, MIP-1α, for Predicting Higher Risk of Severe AKI and Composite Events After Cardiac Surgery

		Severe AKI				Composite Events: RRT or (and) in-Hospital Death			
Cytokines	Range	Crude OR (95% CI)	*P* Value	Adjusted OR* (95% CI)	*P* Value	Crude OR (95% CI)	*P* Value	Adjusted OR* (95% CI)	*P* Value
IFN-γ (10^{-2} ng/mL)	Continuous	1.39 (1.29–1.50)	<0.0001	1.41 (1.30–1.53)	<0.0001	1.26 (1.17–1.37)	<0.0001	1.26 (1.16–1.38)	<0.0001
Q1	0–2.43	1	Ref	1	Ref	1	Ref	1	Ref
Q2	2.43–4.28	2.84 (0.73–11.04)	0.1316	3.53 (0.87–14.23)	0.0767	2.04 (0.18–22.87)	0.5629	2.11 (0.19–23.96)	0.5469
Q3	4.28–7.87	9.21 (2.66–31.92)	0.0005	11.46 (3.16–41.56)	0.0002	11.11 (1.39–88.52)	0.023	11.01 (1.36–88.92)	0.0244
Q4	7.97–21.26	37.53 (11.15–126.31)	<0.0001	48.62 (13.55–174.44)	<0.0001	29.49 (3.90–223.16)	0.001	27.78 (3.61–213.84)	0.0014
P for trend			<0.0001		<0.0001		<0.0001		<0.0001
IL–16 (10^{-2} ng/mL)	Continuous	1.13 (1.09–1.16)	<0.0001	1.12 (1.08–1.15)	<0.0001	1.10 (1.07–1.13)	<0.0001	1.10 (1.07–1.13)	<0.0001
Q1	0.94–4.66	1	Ref	1	Ref	1	Ref	1	Ref
Q2	4.66–7.90	1.57 (0.54–4.58)	0.4123	1.65 (0.55–5.00)	0.3724	2.04 (0.18–22.87)	0.5629	1.92 (0.17–21.73)	0.5973
Q3	7.92–13.78	1.96 (0.69–5.51)	0.204	2.16 (0.72–6.45)	0.1678	2.04 (0.18–22.87)	0.5629	2.00 (0.17–22.90)	0.5773
Q4	14.35–110.36	24.15 (9.66–60.37)	<0.0001	24.86 (9.31–66.35)	<0.0001	44.29 (5.91–332.05)	0.0002	38.07 (4.98–291.07)	0.0005
P for trend			<0.0001		<0.0001		<0.0001		<0.0001
MIP-1α (10^{-3} ng/mL)	Continuous	1.45 (1.31–1.60)	<0.0001	1.50 (1.34–1.68)	<0.0001	1.22 (1.13–1.32)	<0.0001	1.23 (1.12–1.34)	<0.0001
Q1	0.52–1.34	1	Ref	1	Ref	1	Ref	1	Ref
Q2	1.35–2.21	1.15 (0.42–3.11)	0.7835	1.41 (0.50–3.98)	0.5172	0.24 (0.03–2.23)	0.212	0.28 (0.03–2.60)	0.2626
Q3	2.22–3.81	2.21 (0.90–5.44)	0.0829	3.01 (1.17–7.72)	0.0223	1.83 (0.52–6.44)	0.3496	2.05 (0.57–7.43)	0.2732
Q4	3.82–27.34	13.36 (5.88–30.36)	<0.0001	20.06 (8.06–49.94)	<0.0001	7.56 (2.52–22.71)	0.0003	9.13 (2.84–29.33)	0.0002
P for trend			<0.0001		<0.0001		<0.0001		<0.0001

AKI indicates acute kidney injury; IFN-γ, interferon-gamma; IL-16, interleukin-16; and MIP-1α, macrophage inflammatory protein-1 alpha; OR indicates odd ratio; Q1, the first quartile; Q2, the second quartile; Q3, the third quartile; Q4, the fourth quartile; and RRT, renal replacement therapy.

*Adjusted for age, sex, diabetes mellitus, hypertension, NYHA Class III or IV, surgery type=concomitant surgery, prolonged cardiopulmonary bypass time, preoperative creatinine.

the risk stratification of severe AKI and perioperative in-hospital outcomes, suggesting its potential significance in the regulation of kidney injury after cardiac surgery. It is also important to note that the fourth quantile of IL-16, which was the strongest indicator for composite events among the 3, held an over 38-fold risk compared with the first quantile. Similarly, for predicting composite events, IL-16 also had an impressive performance with high sensitivity. In comparison, MIP-1α alone showed a slightly weaker predictive ability in predicting outcomes but was still a decent predictor and risk factor for severe AKI, with the fourth quantile exhibiting a 20.06-fold risk for severe AKI and a 9.13-fold risk for composite events compared with the lowest quantile. Our study depicted MIP-1α as a biomarker for severe CSA-AKI and elucidated its relationship with in-hospital outcomes.

Although we have shown the decent performance of our predictive model, as well as the prognostic utility of each cytokine among age- and sex-stratified groups, it is also necessary to note that differences still exist between our results and those of others. For example, we found significantly higher concentrations of IFN-γ among patients who developed severe CSA-AKI, while Greenberg et al did not observe a significant difference when comparing children who had progressed CSA-AKI and unprogressed CSA-AKI.[44] Moreover, Arthur et al reported a weak predictive value of urine MIP-1α for identifying patients with progressed CSA-AKI,[38] which seems contradictory to our observations. These disparities indicate that even though the predictive tool or biomarker exhibited excellent performance, the population and sample source still mattered. After all, the cardiac surgery and pathophysiological conditions differed between children and adults, and metabolic pathways still affected the various alterations in blood and urine cytokines. Hence, when applying these novel biomarkers or models for predicting CSA-AKI or outcomes, appropriate objectives, the time of sample collection, the sample source, the major study population and even the definition of outcomes should also be considered.

Admittedly, our study had some limitations. First, we used 2 separate centers from the same hospital, and external validation from more hospitals should be further performed. In addition, because of the ununified standard for CSA-AKI, the diagnosis was made according to the AKIN criteria, which, though it has high sensitivity, would also miss patients with later AKI, as it limits the increase in serum creatinine within 48 hours after surgery.

CONCLUSIONS

Our study developed and validated a powerful predictive model incorporating IFN-γ, IL-16, and MIP-1α for predicting severe CSA-AKI. We also revealed that these 3 cytokines were promising blood biomarkers for the discrimination and risk stratification of severe AKI and poor in-hospital outcomes following cardiac surgery. With further validation in relatively larger populations, our predictive model might offer clinicians robust indication for identifying patients with a higher risk of severe AKI, which enables timely diagnostic and therapeutic strategies in the clinical management of patients undergoing cardiac surgery.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Material

Tables S1–S3
Figures S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Comparison of patients' characteristics at bassline between screening and validation cohort.

	Screening (n=204)	Validation (n=198)	P value
Age, years, mean (SD)	54.92±12.54	56.0±13.1	0.382
Male, n (%)	148 (72.55%)	150 (75.8%)	0.463
LVEF, %, median (IQR)	60.00 (58.00-64.00)	60.0 (57.0-63.8)	0.173
NYHA class III or IV, n (%)	48 (23.53%)	60 (30.3%)	0.126
Diabetes, n (%)	31 (15.20%)	31 (15.7%)	0.898
Hypertension, n (%)	118 (57.84%)	117 (59.4%)	0.753
Hyperlipidemia, n (%)	112 (54.90%)	99 (50.0%)	0.325
AMI[#] history, n (%)	14 (6.86%)	19 (9.6%)	0.318
Surgery type (CABG or valve surgery), n (%)	74 (36.27%)	81 (40.9%)	0.34
CPB-time, hours, median (IQR)	120.00 (95.00-149.50)	129.0(91.5-162.5)	0.496
CPB time>120 min, n (%)	61 (29.9%)	67 (33.8%)	0.69
Delta creatinine, μmol/L, median (IQR)	58.0 (24.0-91.2)	62.6 (24.6-96.5)	0.408
AKI stage			0.864
Non-AKI, n (%)	104 (51.0%)	97 (49.0%)	
Mild-AKI, n (%)	58 (28.4%)	56 (28.3%)	
Severe-AKI, n (%)	42 (20.6%)	45 (22.7%)	
Outcome			
RRT or (and) in-hospital death, n (%)	17 (8.33%)	19 (9.6%)	0.658
Hospitalized time, days, median (IQR)	14.00 (11.00-20.00)	14.0 (11.0-21.0)	0.966
ICU stay, days, median (IQR)	4.00 (2.00-6.00)	3.0 (2.0-6.0)	0.404
Ventilation time, hours, median (IQR)	20.00 (13.50-47.50)	18.5 (14.0-37.8)	0.430

SD: standard deviation; IQR: interquartile range; LVEF: left ventricle ejection fraction; AMI: acute myocardial infarction; NYHA: New York Heart Association functional classification; AMI: acute myocardial infarction; CPB: cardiopulmonary bypass; CABG: coronary artery bypass graft; ICU: intensive care unit; RRT: renal replacement therapy

Table S2. Performance of cytokine-based model in age/sex stratified groups.

	Severe AKI/ non-severe AKI	C-statistics (95% CI）	Brier Score	Sensitivity	Specificity
Male group	52/246	0.86 (0.79-0.92)	0.10	0.73	0.90
Female group	35/69	0.87 (0.79-0.95)	0.13	0.77	0.87
Younger group	56/173	0.84 (0.78-0.91)	0.12	0.75	0.86
Older group	31/142	0.91 (0.85-0.97)	0.08	0.81	0.92

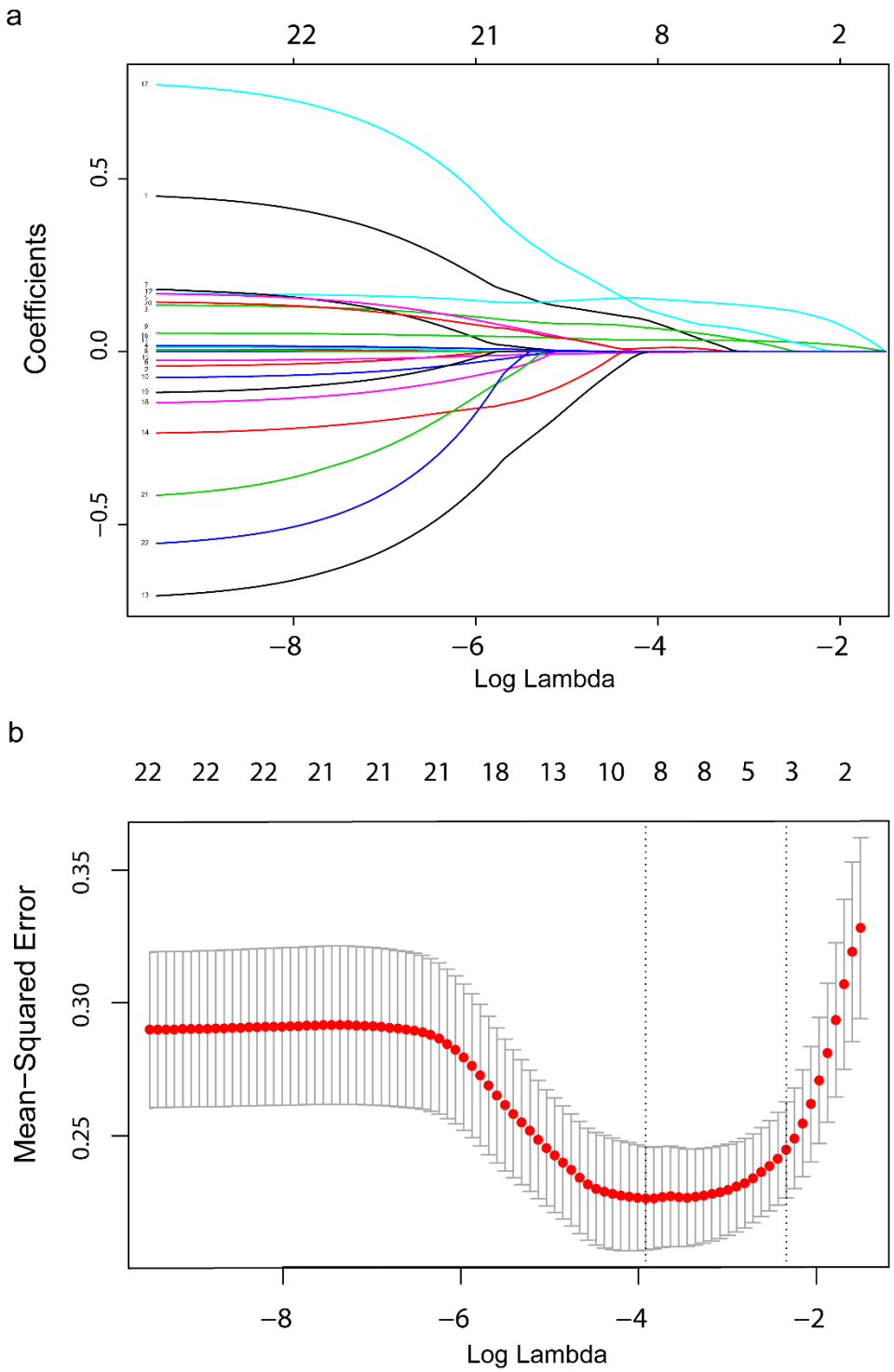
Younger group: age < 60 years old. Older group: age ≥ 60 years old. CI: confidence interval

Table S3. Association of the three cytokines and prognostic features.

Cytokines	Range	Severe AKI		RRT and/or in-hospital deaths		Ventilation time (hours)		ICU stay (days)		Hospitalized time (days)	
		N (%)	P	N (%)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p
IFN-γ (10^{-2}ng/mL)											
Q1	0 - 2.43	3 (3.0%)	ref	1 (1.0%)	ref	13.0 (11.0-17.0)	ref	2.0 (1.0-4.0)	ref	13.0 (10.0-18.0)	ref
Q2	2.43 - 4.28	8 (8.0%)	0.117	2 (2.0%)	0.621	17.0 (12.8-27.2)	0.009	2.0 (1.0-4.0)	0.445	13.0 (10.0-19.0)	0.892
Q3	4.28 - 7.87	22 (22.0%)	<0.0001	10 (10.0%)	0.005	21.0 (14.0-36.0)	<0.0001	4.0 (2.0-6.0)	0.0002	15.0 (11.0-20.2)	0.047
Q4	7.97 - 21.26	54 (53.5%)	<0.0001	23 (22.8%)	<0.0001	43.0 (22.0-125.5)	<0.0001	6.0 (4.0-10.5)	<0.0001	19.0 (12.0-27.0)	0.0003
IL-16 (10^{-2}ng/mL)											
Q1	0.94 - 4.65	6 (5.9%)	ref	1 (1.0%)	ref	16.0 (13.0-20.5)	ref	2.0 (1.0-4.0)	ref	12.0 (9.0-14.0)	ref
Q2	4.68 - 7.90	9 (9.0%)	0.409	2 (2.0%)	0.621	16.0 (11.0-22.0)	0.833	2.0 (2.0-4.0)	0.536	14.0 (11.0-20.0)	0.022
Q3	7.93 - 13.78	11 (11.0%)	0.197	2 (2.0%)	0.621	21.5 (14.2-39.2)	0.046	4.0 (2.0-5.8)	0.008	14.0 (12.0-21.0)	<0.0001
Q4	14.35 - 110.36	61(60.4%)	<0.0001	31 (30.7%)	<0.0001	47.0 (20.5-132.0)	<0.0001	6.0 (4.0-12.5)	<0.0001	18.0 (12.0-27.0)	<0.0001
MIP-1α (10^{-3}ng/mL)											
Q1	0.52 - 1.34	8 (7.9%)	ref	4 (4.0%)	ref	17.0 (11.0-25.0)	ref	3.0 (2.0-4.2)	ref	13.0 (10.0-18.0)	ref
Q2	1.35 - 2.21	9 (9.0%)	0.783	1 (1.0%)	0.369	17.0 (13.0-25.2)	0.999	2.0 (1.0-5.0)	0.913	13.0 (10.0-18.2)	0.994
Q3	2.21 - 3.81	16 (16.0%)	0.077	7 (7.0%)	0.343	18.0 (13.0-33.0)	0.701	3.0 (2.0-5.0)	0.685	15.0 (11.8-21.0)	0.069
Q4	3.82 - 27.34	54 (53.5%)	<0.0001	24 (23.8%)	<0.0001	36.0 (19.0-89.8)	<0.0001	6.0 (4.0-8.0)	<0.0001	17.0 (12.0-25.0)	0.004

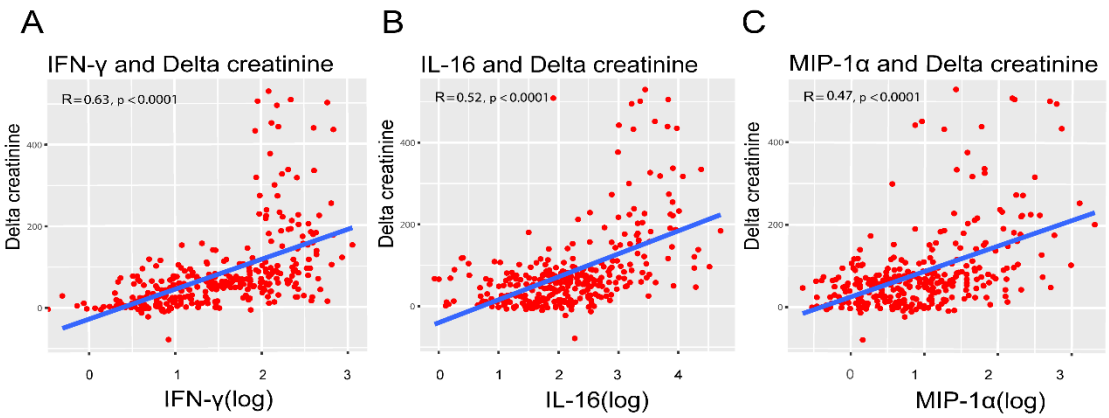
Q1: the first quartile, Q2: the second quartile; Q3: the third quartile; Q4: the fourth quartile; IQR: Interquartile range; RRT: renal replacement therapy

Figure.S1. Feature selection using the least absolute shrinkage and selection operator (LASSO) regression.



A, LASSO coefficient profiles of the 22 significantly differentially enriched cytokines. B, Identification of the optimal penalization coefficient (λ) in the LASSO model with 10-fold cross-validation and the 1 standard error (1-SE criteria). The dotted vertical line was drawn at the optimal λ at minimum criteria and 1 standard error (1-SE criteria). The model at 1-SE criteria was selected as the final model with 3 nonzero coefficients.

Figure S2. Correlations between IFN-γ, IL-16, MIP-1α and alteration of creatinine.



A, correlation between IFN-γ and delta creatinine;B, correlation between IL-16 and delta creatinine; C, correlation between MIP-1α and delta creatinine. Delta creatinine: alteration of serum creatinine after cardiac surgery verse preoperative creatinine. The cytokines concentrations in the plot were ln transformed. Spearman correlation coefficient is shown.