




ORIGINAL RESEARCH

Plasma Metabolites–Based Prediction in Cardiac Surgery–Associated Acute Kidney Injury

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BACKGROUND: Cardiac surgery–associated acute kidney injury (CSA-AKI) is a common postoperative complication following cardiac surgery. Currently, there are no reliable methods for the early prediction of CSA-AKI in hospitalized patients. This study developed and evaluated the diagnostic use of metabolomics-based biomarkers in patients with CSA-AKI.

METHODS AND RESULTS: A total of 214 individuals (122 patients with acute kidney injury [AKI], 92 patients without AKI as controls) were enrolled in this study. Plasma samples were analyzed by liquid chromatography tandem mass spectrometry using untargeted and targeted metabolomic approaches. Time-dependent effects of selected metabolites were investigated in an AKI swine model. Multiple machine learning algorithms were used to identify plasma metabolites positively associated with CSA-AKI. Metabolomic analyses from plasma samples taken within 24 hours following cardiac surgery were useful for distinguishing patients with AKI from controls without AKI. Gluconic acid, fumaric acid, and pseudouridine were significantly upregulated in patients with AKI. A random forest model constructed with selected clinical parameters and metabolites exhibited excellent discriminative ability (area under curve, 0.939; 95% CI, 0.879–0.998). In the AKI swine model, plasma levels of the 3 discriminating metabolites increased in a time-dependent manner (R^2 , 0.480–0.945). Use of this AKI predictive model was then confirmed in the validation cohort (area under curve, 0.972; 95% CI, 0.947–0.996). The predictive model remained robust when tested in a subset of patients with early-stage AKI in the validation cohort (area under curve, 0.943; 95% CI, 0.883–1.000).

CONCLUSIONS: High-resolution metabolomics is sufficiently powerful for developing novel biomarkers. Plasma levels of 3 metabolites were useful for the early identification of CSA-AKI.

Key Words: acute kidney injury ■ biomarkers ■ cardiac surgery ■ machine learning ■ metabolomics

Acute kidney injury (AKI) is the most frequent complication following cardiac surgery, with an incidence of 5% to 43%.¹ Patients with AKI have increased postoperative mortality and mid- to long-term morbidities, including heart failure, myocardial infarction, and accelerated progression to chronic kidney

disease.^{2–4} Early detection of potentially significant AKI in patients following cardiac surgery is important for the timely administration of appropriate clinical care.^{5,6} Serum creatinine levels have served as a gold standard for the diagnosis of AKI; however, serum creatinine concentrations may be affected by age, sex,

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CLINICAL PERSPECTIVE

WHAT IS NEW?

- Three plasma metabolites, gluconic acid, fumaric acid, and pseudouridine, were significantly upregulated in patients with cardiac surgery–associated acute kidney injury.
- A random forest model with 3 plasma biomarkers (gluconic acid, fumaric acid, and pseudouridine) and 2 clinical parameters (preoperative creatinine and estimated glomerular filtration rate) achieved high accuracy for cardiac surgery–associated acute kidney injury prediction.
- In an acute kidney injury swine model, plasma levels of the 3 discriminating metabolites increased in a time-dependent manner.

What Are the Clinical Implications?

- A panel of 3 metabolites (gluconic acid, fumaric acid, and pseudouridine) in plasma taken within 24 hours following cardiac surgery is useful for predicting cardiac surgery–associated acute kidney injury.

Nonstandard Abbreviations and Acronyms

ACCT	aortic cross-clamp time
AKI	acute kidney injury
C&P	clinical parameters and plasma biomarkers
CPB	cardiopulmonary bypass
CSA-AKI	cardiac surgery–associated acute kidney injury
FC	fold change
FDR	false discovery rate
LR	logistic regression
ML	machine learning
m/z	mass-to-charge ratio
OPLS-DA	orthogonal projections to latent structures-discriminant analysis
RF	random forest
SVM	support vector machine

muscle mass, and medications, and abnormal creatinine levels may not be detectable until patients suffer a significant loss of kidney function.⁷ The practice of measuring only serum creatinine may miss important opportunities for early treatment because of the time lag between kidney damage and the detection of elevated creatinine levels. In addition, AKI is associated with significantly increased inpatient costs of \$5000

to \$30 000.^{1,8} Therefore, it is important to identify biomarkers for the early detection of AKI following cardiac surgery to reduce the burden of AKI-associated morbidity and mortality.

In recent years, several novel biomarkers, such as NGAL (neutrophil gelatinase–associated lipocalin), cystatin C, and KIM-1 (kidney injury molecule-1), have been associated with renal tubular injury. These biomarkers may be useful for the early diagnosis and screening of patients at high risk for AKI.^{9–11} Although these biomarkers may prove to be useful, alone or in combination, for improving clinical diagnosis, the sensitivity, specificity, and repeatability remain insufficient for clinical implementation.^{12,13} Hence, developing biomarkers and assessing their clinical applicability for predicting AKI following cardiac surgery are urgently needed.

High-throughput metabolomics is an efficient approach for identifying plasma small molecules (<1500 Da) that have significant potential for providing insight into pathological states.¹⁴ The ability to measure metabolic alterations at the molecular level has become a powerful tool for defining the underlying pathophysiology and identifying novel treatment targets.¹⁵ Plasma metabolites may, therefore, provide a relevant molecular signature for identifying cardiac surgery–associated AKI (CSA-AKI). To date, only a few metabolomic studies have been published in the area of pediatric and adult AKI^{16–18}; however, these studies were primarily discovery-phase investigations with relatively small sample sizes and unsatisfactory predictive use.

In this study, we performed a liquid chromatography tandem mass spectrometry–based metabolomic analysis to identify CSA-AKI–related plasma metabolic signatures. We screened and identified metabolites in a discovery cohort and subsequently validated their potential as biomarkers for the early detection of CSA-AKI using machine learning (ML) algorithms in a validation cohort. An AKI swine model was developed to dynamically monitor these biomarkers. The results of this study may be useful for improving early prediction and may offer insights into the pathophysiology of CSA-AKI.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request. A detailed description of methods can be found in Data S1.

Study Design

This study was approved by the ethics committee of Fuwai Hospital and was conducted in accordance with

the 1964 Declaration of Helsinki. Written informed consent was obtained from all participants included in this study. Plasma samples were prospectively collected from patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) in the surgical intensive care unit of Fuwai Hospital. In total, 122 patients with CSA-AKI and 92 patients without CSA-AKI were eligible to participate in this study.

Outcomes

AKI was diagnosed according to the Acute Kidney Injury Network criteria¹⁹ in patients who stayed in the intensive care unit longer than 48 hours.

Liquid Chromatography Tandem Mass Spectrometry Analysis

Metabolomic profiling analysis was performed using a Vanquish ultraperformance liquid chromatography system coupled to a Q-Exactive HF mass spectrometer (Thermo Fisher Scientific). A Hypersil GOLD C18 column (100 × 2.1 mm, 1.9 μm; Thermo Fisher Scientific) was used for metabolite separation. For untargeted investigation, analysis was performed in the full-MS and data-dependent MS² scan modes. For targeted determination, analysis was performed in the multiple reaction monitoring modes. Metabolite concentrations were calculated by area response from independent calibration curves.^{20,21}

AKI Swine Model

The experimental animal research protocol was approved by the institutional review board of Fuwai hospital. A swine model of AKI was developed using a modified method according to a published study.²² Following surgical preparation, all swine (n=3) were subjected to midline laparotomy. Cross-clamp (1 hour) of the right renal artery and vein was performed followed by left nephrectomy. Then the clamp was released, and the right kidney was observed for reperfusion. All swine were monitored for 8 hours after surgery, and 9 blood samples were collected at 1-hour intervals from each swine via the venous catheter.

Machine Learning

We attempted 3 ML methods, namely random forest (RF), support vector machine (SVM), and logistic regression (LR) to develop the predictive models. All of the analyses were performed in R (version 4.0.5; R Foundation for Statistical Computing). RF models were fitted with the randomForest package, and SVM models were fitted with the e1071 package. The receiver operating characteristic (ROC) curve analysis

was performed to evaluate the models' performance, which was measured based on the area under the curve (AUC).

Statistical Analysis

Differences in metabolite levels between patients with AKI and without AKI were assessed with Mann-Whitney *U* tests. A *P* value <0.05 was considered statistically significant. ROC analyses were used for individual biomarkers, and their predictive abilities were assessed using the AUC.

RESULTS

Of the 214 subjects enrolled in our study, 122 patients were clinically diagnosed with AKI in the first 48 hours following cardiac surgery, and 92 patients without AKI were used as controls. To validate the potential applicability of the metabolomic biomarkers, patients were divided into a discovery cohort and a validation cohort as follows: (1) Patients who underwent only coronary artery bypass grafting or valve replacement surgery were assigned to the discovery cohort (47 patients with AKI and 48 patients without AKI). (2) To extend applicability of the biomarkers, patients who underwent other cardiac surgeries, such as aortic aneurysm repair, were assigned to the validation cohort (75 patients with AKI and 44 patients without AKI) (Table 1). Untargeted metabolomics was performed on the discovery cohort, followed by the discovery, identification, and verification of differentially expressed metabolites (Figure 1A). Potential biomarkers were combined into an ML model for improved predictive ability. Finally, the selected biomarkers were quantitatively analyzed in the independent validation cohort (Figure 1B).

Distinguishing Patients With AKI From Patients Without AKI Using Plasma Metabolomics

A total of 4004 and 3648 mass-to-charge ratio (*m/z*) features were detected in the positive and negative ion modes, respectively. In the positive mode, 1427 *m/z* features were significantly different between patients with AKI and patients without AKI; 156 *m/z* features showed a fold change (FC) >1.5, and 88 *m/z* features showed an FC <0.67, between patients with AKI and patients without AKI (Figure 2A). In the negative mode, 1342 *m/z* features were significantly different between patients with AKI and patients without AKI; 403 *m/z* features had an FC >1.5, and 145 *m/z* features had an FC <0.67, between patients with AKI and patients without AKI (Figure 2B). Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) were used to identify potential

Table 1. Clinical Characteristics of Patients

	Discovery cohort			Validation cohort		
	AKI, n=47	Non-AKI, n=48	<i>P</i> value	AKI, n=75	Non-AKI, n=44	<i>P</i> value
Demographics						
Male sex, n (%)	36 (76.6)	35 (72.9)	0.495	57 (76.0)	30 (68.2)	0.395
Age, y	57 (51–66)	57 (51–64)	0.483	57 (41–68)	55 (40–63)	0.116
BMI, kg/m ²	25.5±2.8	25.0±3.4	0.414	25.0±3.6	24.8±3.2	0.797
Surgery type, n (%)			0.218			0.273
CABG	18 (38.3)	25 (52.0)		0	0	
Valve	29 (61.7)	23 (48.0)		0	0	
Aorta	0	0		27 (36.0%)	12 (27.3%)	
CABG + valve	0	0		6 (8.0%)	7 (15.9%)	
CABG + aorta	0	0		7 (9.3%)	4 (9.1%)	
Valve + aorta	0	0		12 (16.0%)	8 (18.2%)	
CABG + valve + aorta	0	0		6 (8.0%)	0	
Other	0	0		17 (22.7%)	13 (29.5%)	
Surgery time, min						
CPB	118.5±37.8	107.5±31.4	0.123	172.6±79.1	123.7±53.6	<0.001
ACCT	78.7±28.2	77.2±26.2	0.654	107.8±41.7	87.2±44.0	0.012
Preoperative renal function						
Preoperative creatinine, μmol/L	89.7±17.7	83.7±13.5	0.066	90.3±17.7	86.9±19.6	0.330
Preoperative eGFR, mL/min per 1.73 m ²	84.2±24.6	87.9±15.6	0.385	83.4±22.5	88.6±24.1	0.246

Fisher exact test for sex. Mann-Whitney *U* test for age. Student *t* test for BMI, CPB, ACCT, preoperative creatinine, and eGFR. χ^2 test for surgery type. ACCT indicates aortic cross-clamp time; AKI, acute kidney injury; BMI, body mass index; CABG, coronary artery bypass graft; CPB, cardiopulmonary bypass; and eGFR, estimated glomerular filtration rate.

biomarkers with the ability to distinguish patients with AKI from patients without AKI. The 4004 and 3648 *m/z* features detected in the positive and negative ion modes, respectively, were entered into SIMCA 15.0 (Sartorius) to establish an OPLS-DA model. Optimum separation of the metabolic profiles for patients with AKI and patients without AKI was achieved from the OPLS-DA score plots for the positive ion mode (R^2Y , 0.996; Q^2 , 0.788) and the negative ion mode (R^2Y , 0.991; Q^2 , 0.838) (Figures 2C and 2D). Two hundred permutation tests and cross-validated residual ANOVA analyses were used to determine the risk of overfitting (positive mode: perm Q^2 , -0.257; cross-validated ANOVA *P* value=2.83 × 10⁻²³; negative mode: perm Q , -0.278; cross-validated ANOVA *P* value=4.70 × 10⁻²⁹; Figure S1).

Identification of Potential Biomarkers

We screened the biomarkers through the following 4 steps: (1) False discovery rate (FDR) adjusted *P* value <0.05: A total of 1427 and 1342 *m/z* features met this criterion in the positive and negative ion modes, respectively. (2) FC >1.5 and OPLS-DA variable importance in projection >1.5 (AKI versus non-AKI): After this step, 66 and 183 *m/z* features were left in the positive and negative ion modes, respectively. These features

were considered to be significantly different between the AKI and non-AKI groups. (3) Selecting features that have been validated with database and standard compounds: The above 66 features (positive mode) and 183 features (negative mode) were then annotated using Compound Discoverer (Thermo Fisher Scientific) and their fragmentation spectra. Then, a total of 20 features in the positive mode and 58 features in the negative mode were successfully identified. (4) Selecting endogenous metabolites with AUC >0.8: We further excluded compounds that were not endogenous metabolites, such as pharmaceuticals and their metabolites. Only 3 metabolites with AUC >0.8 were left for further analysis (Figure 1A). Using strict screening criteria to assure the reliability of the selected compounds, we identified gluconic acid, fumaric acid, and pseudouridine as potential biomarkers with the AUC values (95% CIs) >0.8 (Figure 3A, 3B, S2). These 3 biomarkers also successfully recognized patients with early-stage AKI (n=16) who had not been diagnosed with AKI at the time of plasma collection but progressed to AKI within 48 hours following cardiac surgery (AUC of gluconic acid, 0.730; 95% CI, 0.592–0.869; AUC of fumaric acid, 0.848; 95% CI, 0.750–0.945; and AUC of pseudouridine, 0.866; 95% CI, 0.750–0.982) (Figure 3C).

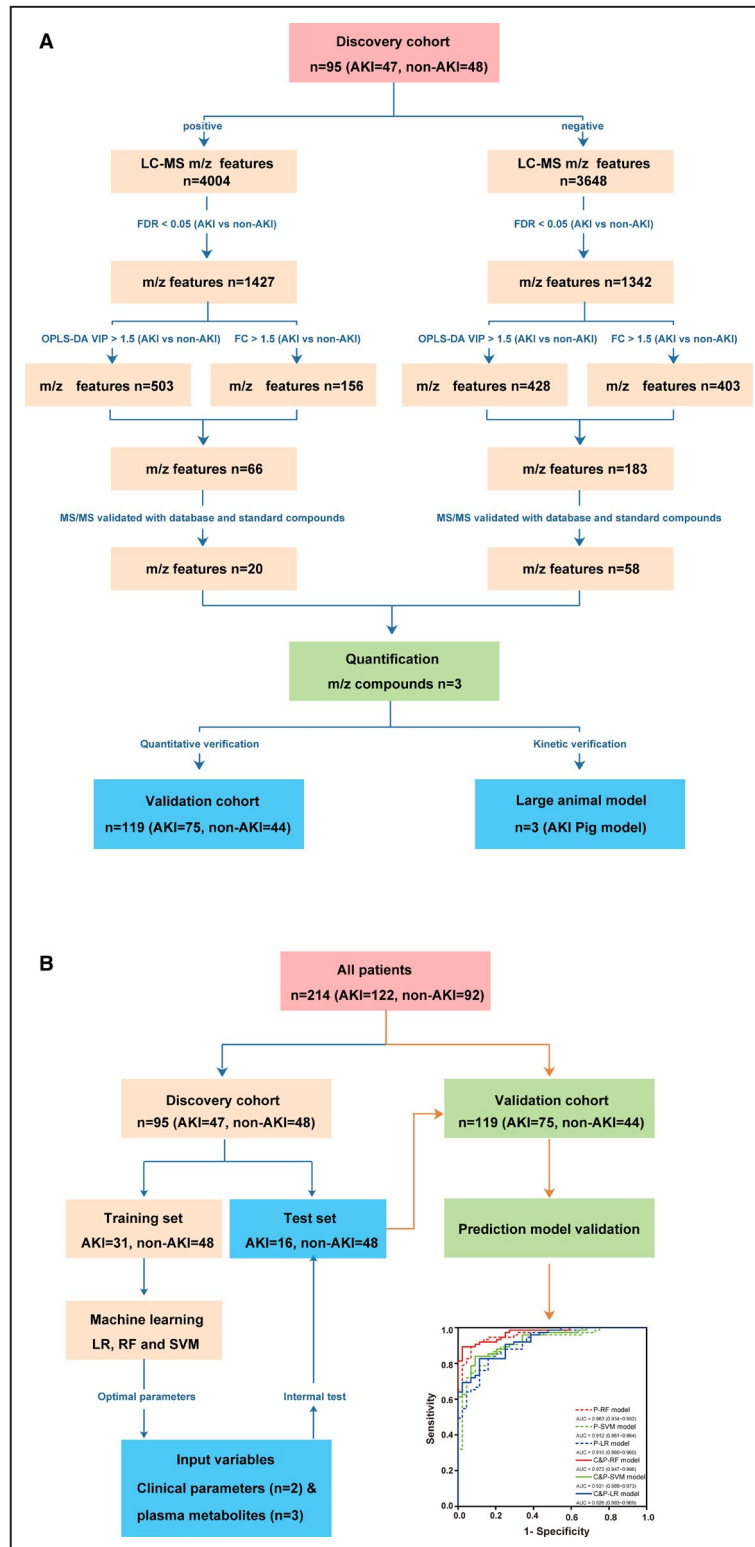


Figure 1. Schematic workflow of the present study. (A) Biomarker identification. (B) The construction of predictive models. AKI indicates acute kidney injury; AUC, area under the curve; C&P, clinical parameters and plasma metabolites; FDR, false discovery rate; FC, fold change; FDR, false discovery rate; LC, liquid chromatography; LR, logistic regression; MS, mass spectrometry; m/z, mass-to-charge ratio; OPLS-DA, orthogonal projections to latent structures–discriminant analysis; P, plasma metabolites; RF, random forest; SVM, support vector machine; and VIP, variable importance in projection.

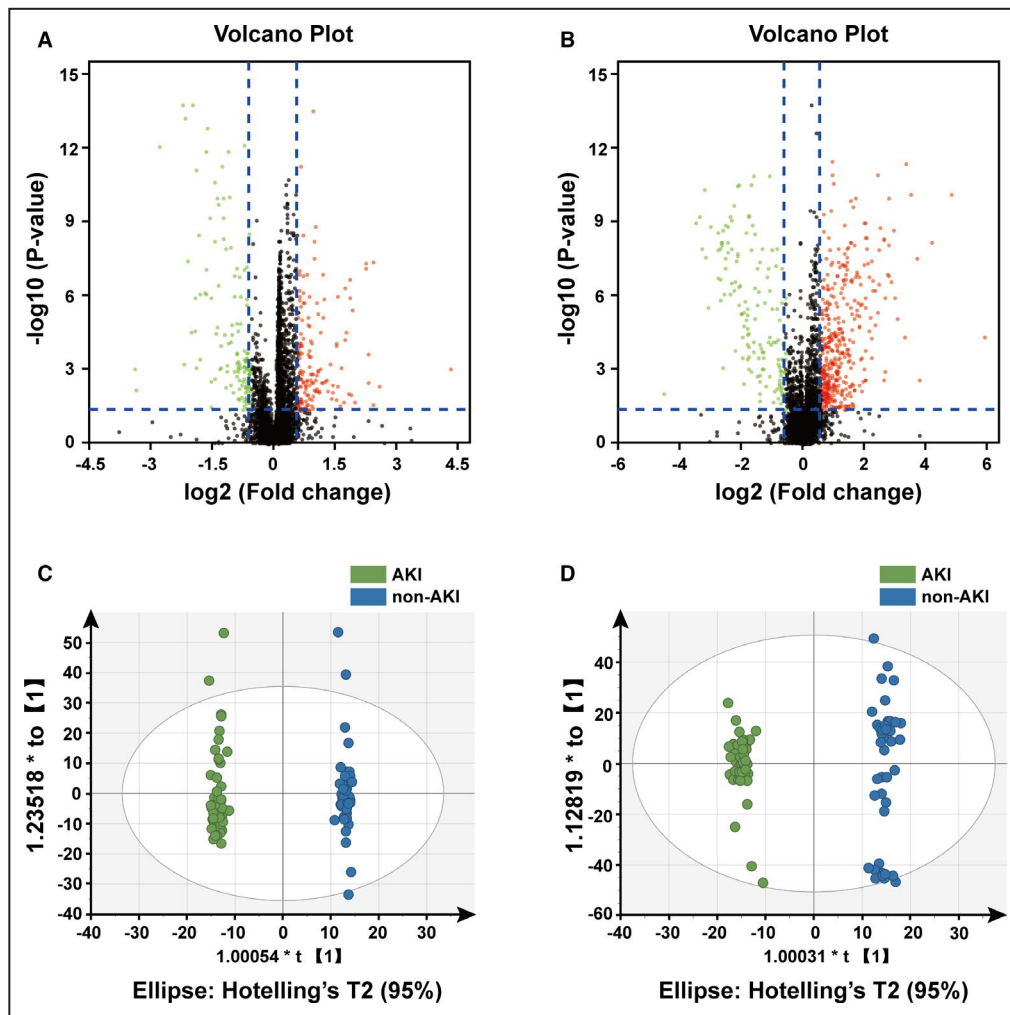


Figure 2. Separation and classification of metabolic profiles in plasma from patients with AKI and patients without AKI.

(A) Volcano plot of positive-ion-mode results. (B) Volcano plot of negative-ion-mode results. (C) OPLS-DA score plots based on the positive ion mode. (D) OPLS-DA score plots based on the negative ion mode. AKI indicates acute kidney injury; and OPLS-DA, orthogonal projections to latent structures-discriminant analysis.

Biomarker Panel Improves the Prediction of CSA-AKI Over a Single Metabolite

To further improve the predictive value of the 3 selected metabolites, we trained and validated several ML methods that could combine them into a predictive panel. We allocated 48 patients without AKI and 31 patients who had been diagnosed with AKI at the time of plasma collection to the training set, whereas 48 patients without AKI and the remaining 16 patients with early-stage AKI were allocated to the test set. The former was used to tune parameters of ML algorithms, whereas the latter was used to evaluate the algorithms' performance. Three ML algorithms, including RF, SVM, and LR were used to assess the 3 metabolites' predictive performance of CSA-AKI, and RF exhibited the larger AUC (0.922; 95% CI, 0.855–0.988) than SVM (0.884; 95%

CI, 0.793–0.976) and LR (0.883; 95% CI, 0.803–0.963) (Figure 3D). The calibration plot for each of the plasma biomarkers-based ML models is shown in Figure 3E. Model fit was acceptable for the RF and SVM model (Hosmer-Lemeshow test: RF model, $P=0.345$; SVM model, $P=0.206$) but was poor for the LR model (Hosmer-Lemeshow test: LR model, $P<0.001$). The partial dependence plots showed gluconic acid, fumaric acid, and pseudouridine have a strong impact on CSA-AKI, and the probability of CSA-AKI occurrence presented a nonlinear increase with 3 plasma biomarkers in the RF model but a linear increase in the SVM and LR models (Figure S3A).

Then we investigated the AUC of clinical parameters to assess their predictive performance for CSA-AKI. First, the predictive use of plasma creatinine was assessed: AUC of creatinine, 0.764; 95% CI, 0.667–0.860

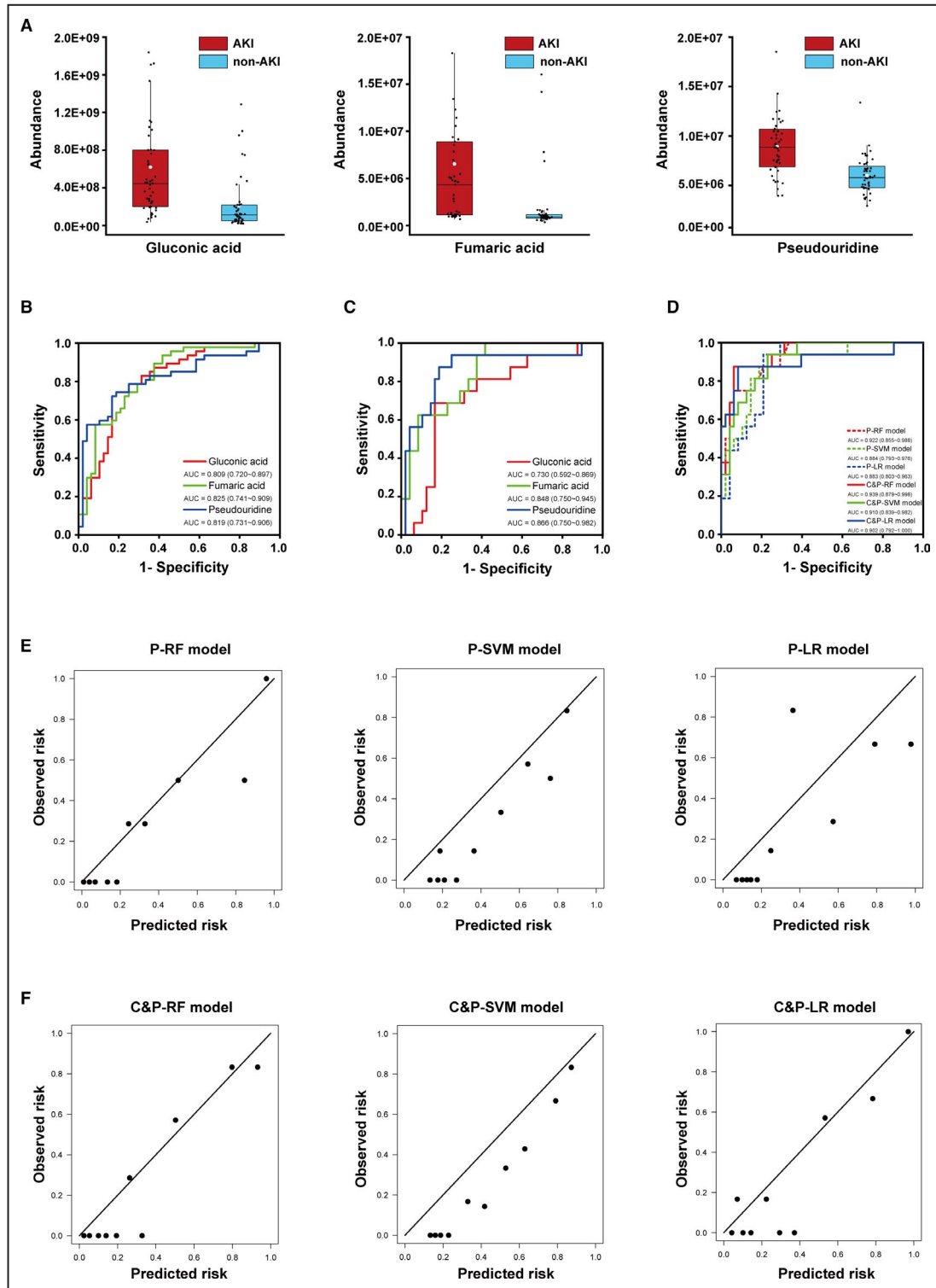


Figure 3. Abundance and use of selected metabolites for predicting CSA-AKI in the discovery cohort. (A) Box plots visualizing the abundance of each selected metabolite in patients with AKI and patients without AKI. (B) ROC curve generated by the individual metabolites for differentiating patients with AKI from patients without AKI. (C) ROC curve generated by the individual metabolites for differentiating patients with early-stage AKI from patients without AKI. (D) ROC curve generated by RF-, SVM-, and LR-based predictive models that used different input variables. (E) The calibration plot for each of the plasma biomarkers-based ML models. (F) The calibration plot for each of C&P-based ML models. AKI indicates acute kidney injury; AUC, area under the curve; C&P, clinical parameters and plasma metabolites; LR, logistic regression; P, plasma metabolites (gluconic acid, fumaric acid, and pseudouridine); RF, random forest; ROC, receiver operating characteristic; and SVM, support vector machine.

(Figure S4). Conversely, the CPB and aortic cross-clamp time (ACCT) exhibited a small AUC (0.612; 95% CI, 0.498–0.727 for CPB and 0.535; 95% CI, 0.418–0.653 for ACCT), indicating that CPB and ACCT were not predictive of CSA-AKI. We next used RF, SVM, and LR models with the 7 clinical parameters (sex, age, body mass index, CPB, ACCT, preoperative creatinine, and estimated glomerular filtration rate) as input variables, and these predictive models exhibited a smaller AUC (RF: 0.549; 95% CI, 0.397–0.720; SVM: 0.534; 95% CI, 0.355–0.713; LR: 0.569; 95% CI, 0.391–0.747) than the predictive models conducted by 3 plasma markers (Table 1, Figure S5). Plasma gluconic acid, fumaric acid, pseudouridine, preoperative creatinine, and estimated glomerular filtration rate remained as independent predictors ($P < 0.05$) for differentiating patients with AKI from patients without AKI after adjusting for age, sex, body mass index, CPB, and ACCT in the logistic regression analysis ($P < 0.05$). Finally, we combined the aforementioned 2 clinical parameters and 3 plasma biomarkers (C&P) to improve the accuracy of the predictive model because they were also independent predictors of the occurrence of CSA-AKI. The AUC of the RF, SVM, and LR model conducted by C&P was increased to 0.939 (95% CI, 0.879–0.998), 0.910 (95% CI, 0.839–0.982), and 0.902 (95% CI, 0.792–1.000), respectively (Figure 3D). Of note, the RF model showed satisfied sensitivity (0.875; 95% CI, 0.604–0.978) and specificity (0.938; 95% CI, 0.818–0.984), both of which were important for CSA-AKI because it progressed rapidly, and interventional measures would largely increase the inpatient costs, respectively. The calibration plot for each of C&P-based ML models is shown in Figure 3F. Model fit was acceptable for all 3 models (Hosmer-Lemeshow test: RF model, $P = 0.458$; SVM model, $P = 0.188$; LR model, $P = 0.248$). The partial dependence plot of each predictor in the C&P-based ML models are shown in Figure S3B. For the discovery cohort, all of the results of ROC analysis including AUC value, sensitivity, specificity, and their 95% CIs were summarized in Table S1. The predictive ability of the panel constructed by C&P was further improved, with the C&P-RF panel showing an optimal AUC value.

Variations in Potential Plasma Biomarkers Over Time in an AKI Swine Model

We constructed an AKI swine model and collected hourly plasma samples to investigate early-stage kinetic changes in potential biomarkers. Plasma levels of gluconic acid, fumaric acid, and pseudouridine rose in a linear fashion over time (R^2 for linear fit, 0.823, 0.480, and 0.945, respectively) (Figure 4). The increased multiples of gluconic acid, fumaric acid, and pseudouridine relative to baseline levels were 1.59 per hour, 0.17 per hour, and 0.11 per hour, respectively. The increased multiple of creatinine relative to baseline was 0.06 per hour (R^2 , 0.561). Using 1.5 times the baseline

level as the diagnostic threshold (Acute Kidney Injury Network),¹⁹ the time to reach the diagnostic criteria of gluconic acid, fumaric acid, and pseudouridine was 0.31, 2.94, and 4.55 hours, respectively, which was significantly earlier than that of creatinine (8.33 hours).

Predictive Ability of the Potential Biomarkers in an Independent Validation Cohort

The potential biomarkers were subsequently confirmed by comparing their fragmentation spectra with standards using a quantitative analysis in an independent validation cohort. Gluconic acid, fumaric acid, and pseudouridine were measured by multiple reaction monitoring using the calibration curve of an external standard. Similar to the results from the discovery cohort, the concentrations of all 3 biomarkers were significantly different between the AKI and non-AKI groups (Figure 5A). Results of the ROC analysis of plasma concentrations for each biomarker are shown in Figure 5B. The AUC of gluconic acid, fumaric acid, and pseudouridine was 0.878 (95% CI, 0.815–0.941), 0.747 (95% CI, 0.661–0.833), and 0.883 (95% CI, 0.823–0.943), respectively. The AUC of the 3 plasma

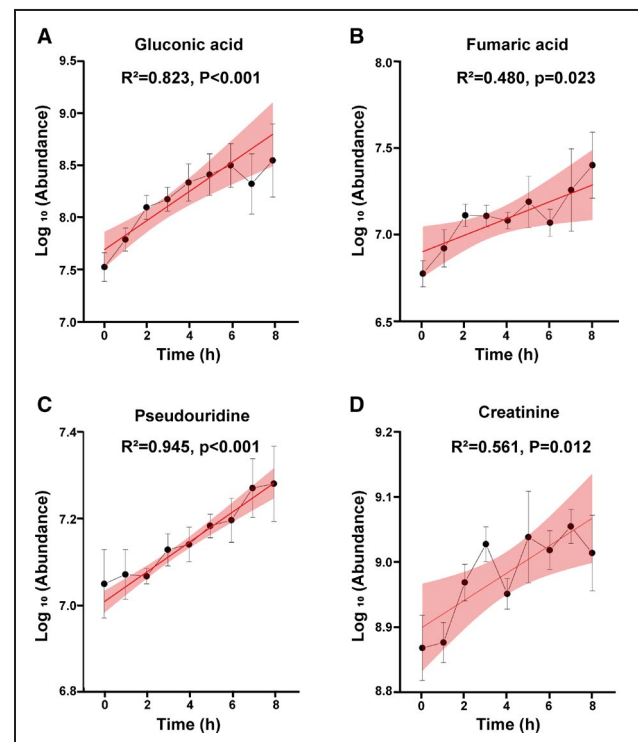


Figure 4. Time-dependent variation in plasma concentrations of the selected metabolites in an acute kidney injury swine model.

(A) Gluconic acid. (B) Fumaric acid. (C) Pseudouridine. (D) Creatinine. Data points are means of independent samples, and error bars represent the SEM ($n = 3$). The red line is the trendline resulting from linear fitting, and the shadow is the 95% CI.

biomarkers-based RF, SVM, and LR models was 0.963 (95% CI, 0.934–0.992), 0.912 (95% CI, 0.861–0.964), and 0.910 (95% CI, 0.860–0.960), respectively (Figure 5C). The calibration plot for each of the plasma biomarkers-based ML models is shown in Figure 5D. Model fit was acceptable for the RF and SVM model (Hosmer-Lemeshow test: RF model, $P=0.283$; SVM model, $P=0.179$) but was not acceptable for the LR model (Hosmer-Lemeshow test: LR model, $P<0.001$). For the validation cohort, the partial dependence plot of each predictor in the plasma biomarkers-based ML models is shown in Figure S6A.

Compared with the discovery cohort, the AUC of both CPB (AUC, 0.705; 95% CI, 0.606–0.804) and ACCT (AUC, 0.656; 95% CI, 0.549–0.762) was increased in the validation cohort (Figure S7). The more complex composition of surgery species may have led to differences in CPB and ACCT between the AKI and non-AKI groups in the validation cohort (Table 1). The AUC of the RF, SVM, and LR model constructed with C&P was increased to 0.972 (95% CI, 0.947–0.996), 0.927 (95% CI, 0.882–0.972), and 0.926 (95% CI, 0.883–0.969), respectively (Figure 5C). It is noteworthy that the RF model still showed great sensitivity (0.893; 95% CI, 0.795–0.950) and specificity (0.977; 95% CI, 0.865–0.999) in the validation cohort. The calibration plot for each of C&P-based ML models is shown in Figure 5E. Model fit was acceptable for the RF and SVM model (Hosmer-Lemeshow test: RF model, $P=0.221$; SVM model, $P=0.187$) but was poor for the LR model (Hosmer-Lemeshow test: LR model, $P<0.001$). For the validation cohort, the partial dependence plot of each predictor in the C&P-based ML models was also shown in Figure S6b. Of note, the consecutive parameters have a definite threshold. It may be a reason that the RF model was better suited than the SVM model for probability of CSA-AKI occurrence.

The predictive performance of these biomarkers was also validated in patients with early-stage AKI (16 of 75 AKI patients) (Figure 5F). The AUC of the RF, SVM, and LR model with the 3-marker panel was 0.935 (95% CI, 0.855–1.000), 0.828 (95% CI, 0.720–0.936), and 0.811 (95% CI, 0.705–0.917), respectively (Figure 5G). The accuracy was further improved when using the RF, SVM, and LR model conducted by C&P (RF: AUC, 0.943; 95% CI, 0.883–1.000; SVM: AUC, 0.849; 95% CI, 0.737–0.962; LR: AUC, 0.830; 95% CI, 0.721–0.938). The selected biomarkers showed an increased predictive ability in the validation cohort compared with biomarkers that have been previously reported^{23–31} (Table 2). The AUC of CPB (0.589; 95% CI, 0.422–0.757) and ACCT (0.605; 95% CI, 0.444–0.766) showed neither of them could accurately predict the occurrence of AKI at an early stage. Gluconic acid, fumaric acid, and pseudouridine remained as significant predictors that could differentiate patients with AKI from patients without AKI after adjusting for

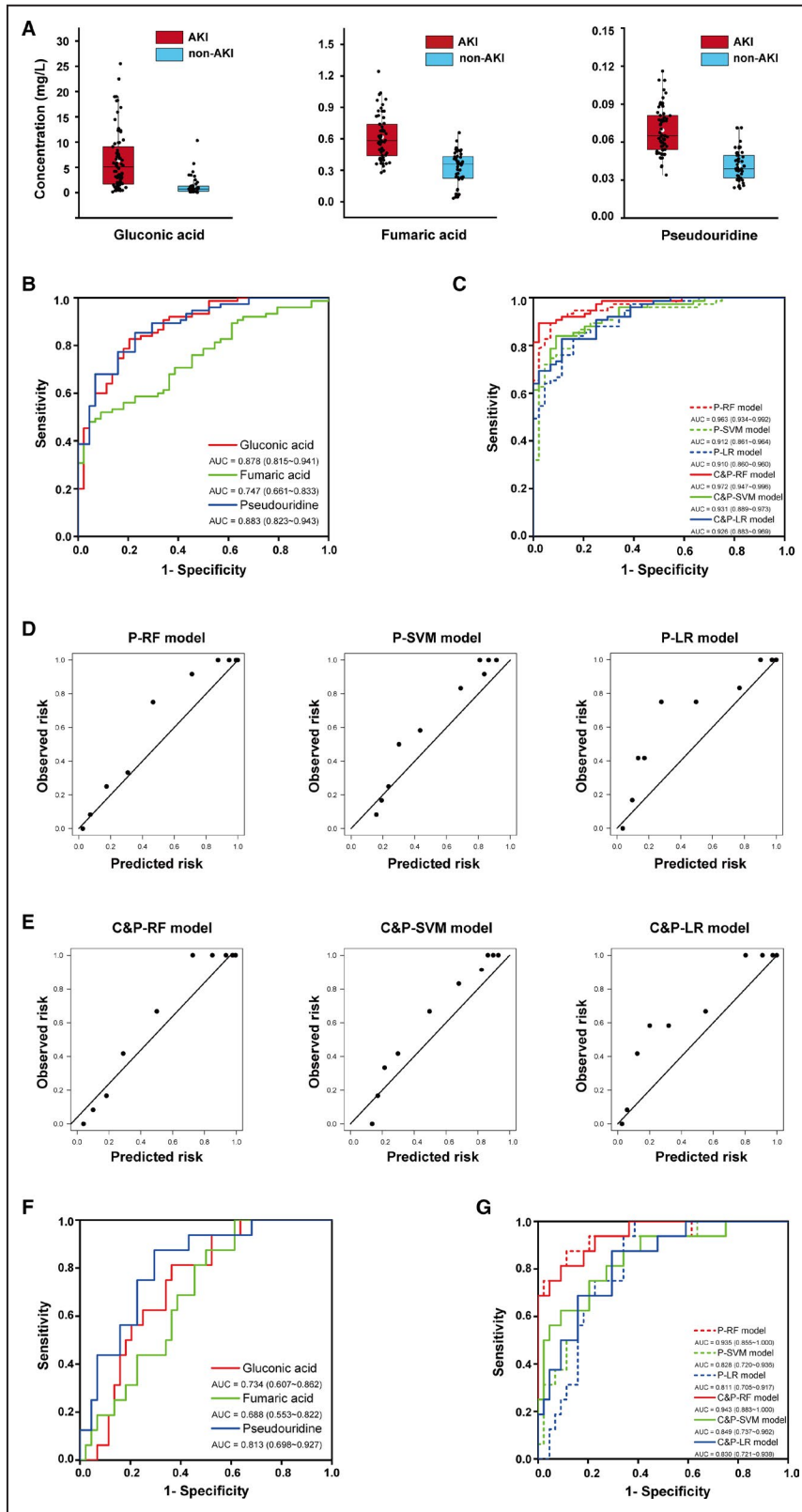
the clinical parameters in a logistic regression analysis ($P<0.05$). These results indicate that the potential biomarkers had excellent stability and reproducibility and therefore may be suitable for the early diagnosis of CSA-AKI. For the validation cohort, all of the results of ROC analysis, including AUC value, sensitivity, specificity, and their 95% CI are summarized in Table S2. The contribution of variables to the models are shown in Table S3, including the variable importance of the RF and LR model, and P values from the LR model.

DISCUSSION

In this observational study, we used untargeted and targeted metabolomic approaches to develop biomarkers of CSA-AKI. Plasma metabolic profiles were noticeably different between patients with AKI and patients without AKI. The targeted quantitative approach confirmed that levels of gluconic acid, fumaric acid, and pseudouridine were elevated in the plasma of patients with CSA-AKI. The predictive ability of these markers remained strong for patients with early-stage AKI, indicating that the 3-marker panel could predict the development of CSA-AKI in the early postoperative period.

A strength to our study was the prospective inclusion of patients who experienced cardiac surgery, which reduced potential bias attributable to medications. OPLS-DA analysis identified a significant difference between patients with AKI and patients without AKI and showed that patients with AKI had a unique metabolic pattern. Previous AKI-related studies reported a variety of potential metabolite biomarkers primarily in urine.^{18,32} Martin-Lorenzo and collaborators found that urinary levels of acetylneuraminic acid, phosphoethanolamine, and serine were significantly upregulated during AKI.³² Similarly, urinary levels of leucine and valine were elevated in pediatric patients with AKI.¹⁸ A dopamine metabolite named homovanillic acid sulfate has been identified as a potential urinary biomarker of pediatric patients with AKI undergoing CPB³³; however, homovanillic acid sulfate was not found in this study. Few studies based on plasma/serum metabolomics have investigated AKI biomarkers.³⁴ A pilot study showed that serum levels of acylcarnitines, methionine, homocysteine, pyroglutamate, asymmetric dimethylarginine, and phenylalanine were elevated in patients with AKI.¹⁶ Interestingly, there was no overlap between the metabolite biomarkers reported in previous studies and those identified in this study, which may be attributable to our use of patients without AKI as controls rather than healthy individuals or to the small sample size in the previous study.¹⁶

Potential biomarkers have rarely been identified in both human and animal studies. In this study, we



observed rapidly increasing plasma levels of the potential biomarkers, which were also present in a swine AKI model. Previous studies have shown that AKI may occur within hours following cardiac surgery,^{35,36}

necessitating continuous monitoring and dense sampling in the early phase following surgery, which is difficult for clinical implementation. Therefore, we constructed a large animal model (AKI swine model) to

Figure 5. Absolute quantification of selected metabolites and their predictive use for CSA-AKI in the validation cohort

(A) Box plots showing plasma concentrations of the 3 selected metabolites in patients with AKI and patients without AKI. (B) ROC curve generated by the individual metabolites for differentiating patients with AKI from patients without AKI. (C) ROC curve generated by RF-, SVM-, and LR-based predictive models for differentiating patients with AKI from patients without AKI. (D) The calibration plot for each of the plasma biomarkers-based ML models. (E) The calibration plot for each of C&P-based ML models. (F) ROC curve generated by the individual metabolites for differentiating patients with early-stage AKI from patients without AKI. (G) ROC curve generated by RF-, SVM-, and LR-based predictive model for differentiating patients with early-stage AKI from patients without AKI. AKI indicates acute kidney injury; AUC, area under the curve; C&P, clinical parameters and plasma metabolites; LR, logistic regression; P, plasma metabolites (gluconic acid, fumaric acid, and pseudouridine); RF, random forest; and SVM, support vector machine.

simulate the early phase of the AKI process. Following surgery in the AKI swine model, hourly kinetic changes in levels of these biomarkers were significantly greater than changes in creatine levels. These biomarkers may respond more rapidly to renal injury than creatine.

Metabolites of the tricarboxylic acid cycle are common biomarkers of AKI.¹⁸ Previous studies have shown that urinary levels of tricarboxylic acid intermediates decreased significantly during AKI.³⁷ This decrease in levels of tricarboxylic acid intermediates was associated with the upregulation of fumaric acid levels in plasma, similar to our findings in this study. These observations suggest that carboxylic acid levels may reflect a reduction in renal clearance during AKI rather than tissue-specific production. Gluconic acid has been associated with a deterioration in renal function in patients with end-stage chronic kidney disease.³⁸ In a previous study of an aristolochic acid–induced nephrotoxicity rat model, gluconic acid exhibited time- and dose-dependent changes, which suggested that this metabolite may be associated with the extent of kidney impairment.³⁹ CSA-AKI has been related to oxidative stress because of unique characteristics such as CPB and ischemia-reperfusion injury.⁴⁰ One study has shown that gluconic acid may be an indicator of

oxidative stress in keratoconus.⁴¹ Gluconic acid is a byproduct of glucose oxidation; therefore, elevated plasma levels of gluconic acid may result from oxidative stress in patients with AKI. Pseudouridine is a modified nucleoside that originates from the catabolism of tRNA. When pseudouridine is released from tRNA, it is not involved in RNA synthesis and is gradually excreted from the body in the urine.⁴² Enhanced plasma levels and reduced urine levels of pseudouridine have been detected in many nephropathies, including chronic kidney disease and diabetic nephropathies.^{42–44} To our knowledge, our study is the first to report a marked increase in plasma levels of pseudouridine in patients with AKI. Pseudouridine may be useful as a marker of acute and chronic kidney damage. Pseudouridine was used as an endogenous renal filtration marker decades ago; however, its use was abandoned because of its reabsorption from renal tubules.⁴⁵ Consequently, the accumulation of pseudouridine in plasma may be attributable to decreased renal excretion and increased tubular reabsorption.

The ideal biomarker for AKI would have high specificity and sensitivity for detecting AKI in a rapid and stable manner. At present, however, few biomarkers fulfill these criteria. Although levels of serum creatinine may be

Table 2. Reported Plasma Biomarkers for Cardiac Surgery–Associated Acute Kidney Injury

Blood biomarkers	Time point	Sensitivity (95% CI)	Specificity (95% CI)	AUC* (95% CI)	References
Cyclophilin A	Within 6 h after cardiac surgery	0.76 (0.66–0.84)	0.59 (0.48–0.69)	0.69 (0.62–0.76)	23
SLPI	At 24 h after cardiac surgery	0.70(45.7–88.1)	67.6 (57.8–76.4)	0.69 (0.58–0.80)	24
SLPI	At 48 h after cardiac surgery	77.8 (52.4–93.6)	71.2 (61.4–79.9)	0.79 (0.69–0.90)	24
Alpha 1-antitrypsin	At 2 h after CPB	0.66 (–)	0.57(–)	0.628 (0.550–0.705)	25
NGAL	Preoperation	0.57 (0.54–0.61)	26
TIMP-2, IGFBP7	ICU admission after cardiac surgery	0.60 (–)	0.88 (–)	0.744 (–)	27
Creatinine	Immediate postoperation/preoperation	0.24 (0.16–0.24)	0.99 (0.99–0.99)	0.77 (0.75–0.79)	28
GDF-15	Preoperation	0.750 (–)	29
Syndecan-1	Within 2 h after cardiac surgery	0.757(–)	0.691 (–)	0.77 (0.68–0.85)	30
Endogenous ouabain	Preoperation	0.73 (0.65–0.81)	31

(–) indicates not mentioned; AUC, area under the curve; CPB, cardiopulmonary bypass; GDF-15, growth-differentiation factor-15; ICU, intensive care unit; IGFBP7, insulin-like growth factor-binding protein 7; NGAL, neutrophil gelatinase-associated lipocalin; SLPI, secretory leukocyte protease inhibitor; and TIMP-2, metalloproteinase-2.

*Logistic regression model includes other variables, including Euroscore, age, plasma creatinine, diabetes, and duration of cardiopulmonary bypass.

affected by multiple factors such as muscle mass, chronic disease, and drug use, creatinine is the most widely used biomarker for AKI diagnosis because it reflects changes in the glomerular filtration rate.⁴⁶ However, it requires many hours to many days to diagnose AKI after renal damage when using serum creatinine.⁴⁰ Our metabolites-based predictive model may be useful for early AKI prediction. These metabolites increased in a linear fashion and have a significantly steeper slope than creatinine in a renal ischemia-reperfusion swine model, indicating that they may be more sensitive for quantifying renal clearance in early-stage AKI. Other biomarkers have been examined for potential use in the early detection of AKI.⁴⁷ A previous study reported that the AUCs of plasma or serum NGAL, cystatin C, and uric acid were 0.62 to 0.83, 0.68 to 0.76, and 0.77 to 0.86, respectively, for the diagnosis of AKI.⁴⁸ The most frequently investigated biomarkers were proteins released from the kidney because of several theoretical advantages over serum creatinine. Because of the complexity of AKI pathogenesis, however, the predictive accuracy of these biomarkers varies extensively among different studies. Therefore, these biomarkers have not been widely used in clinical practice.⁴⁰

Although this study identified an independent predictive panel that may be useful for early and effective diagnosis of CSA-AKI and confirmed the generalizability of this panel in different types of cardiac surgery, limitations of the study warrant attention. First, our study was based on a small-sized patient population treated at a single center. Studies with larger patient populations from multiple treatment centers are needed. Second, we constructed an ischemic AKI pig model instead of a CSA-AKI model because of the technical barriers to construct the latter model. Therefore, future clinical studies are needed to determine if these markers are specific to CSA-AKI or for all kinds of AKI. Third, we identified 3 biomarkers in a CSA-AKI cohort and tested their predictive value of AKI in a porcine ischemic AKI model, but the metabolic kinetics of these markers is not clear and needs to be examined in either animal or clinical studies. Fourth, variations in the elapsed time between surgery and collection of patient samples may introduce bias. Although fasting plasma samples were uniformly collected in the morning of the day following surgery, different surgery times during the previous day led to variation in the time interval between surgery and sample collection. Future studies should collect samples at an earlier and more consistent time point following cardiac surgery to monitor the progress of early-stage AKI and assess the correlation between biomarkers and outcomes of patients with CSA-AKI. In addition, further studies are warranted to determine the 3 biomarkers' significance, such as the primary source for these markers, the correlation of these marker with the prognosis,

and the possibility of detection of these markers in the urine as a noninvasive diagnostic method.

CONCLUSIONS

In summary, the application of MS-based metabolomic techniques can reflect the specific metabolic perturbation in patients with CSA-AKI. The nontargeted metabolomic strategy is an effective approach that could be applied to screen potential differential features, which were subsequently identified by their fragment ion spectra and quantified by chemical standards in multiple reaction monitoring mode for validation of the diagnostic performance of CSA-AKI. Early dynamic monitoring of AKI can be achieved by a large animal model. Earlier diagnosis of AKI would enable intensive care unit physicians to administer supportive treatments, such as catheters and renal replacement therapy, in a timely manner. We hope to provide a framework that will guide development of novel biomarkers for postoperative complications.

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Disclosures

None.

Supplementary Material

Data S1, Tables S1–S3, Figures S1–S7

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Supplemental Material

Data S1.

Supplemental Methods

Study design and population

Postoperative plasma samples were prospectively collected in the surgical intensive care unit (ICU) of Fuwai Hospital from March 2018 to December 2018. Fasting blood samples were obtained by venipuncture from all patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) and immediately centrifuged at 2500 rpm for 30 min. The supernatant plasma was divided into aliquots and stored at -80°C. AKI was diagnosed according to the Acute Kidney Injury Network (AKIN) criteria¹⁹ in patients who stayed in the ICU longer than 48 h. The exclusion criteria included: age < 18 years, AKI prior to ICU admission, and chronic kidney disease (CKD). All enrolled patients received identical management and treatment practices.

Chemicals and reagents used for metabolomic analysis

LC-MS grade water with 0.1% formic acid (H₂O with 0.1% FA) and acetonitrile with 0.1% formic acid (ACN with 0.1% FA) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All other chemicals and reagents were purchased from Millipore Sigma (St. Louis, MO, USA).

Metabolite extraction from plasma samples

To extract metabolites from plasma samples, 50 µL aliquots of plasma were first mixed with 450 µL of acetonitrile:methanol (v/v, 1:1) by vortexing for 5 min. The mixture was then centrifuged at 18,400 g at 4°C for 30 min to precipitate proteins. The resulting supernatants were collected and stored at -80°C until used for LC-MS/MS analysis. The quality control (QC) sample was using the mixture composed of equal volume from each sample.

Untargeted LC-MS/MS based metabolomic analysis

Metabolomic profiling was performed using a Vanquish ultra-performance liquid chromatography system coupled to a Q-Exactive HF mass spectrometer (Thermo Fisher

Scientific). A Hypersil GOLD C18 column (100 × 2.1 mm, 1.9 μm, Thermo Fisher Scientific) maintained at 40°C was used for metabolite separation. Metabolites were separated using a 15 min gradient at a flow rate of 0.25 mL/min. Mobile phase A was H₂O with 0.1% FA and mobile phase B was ACN with 0.1% FA. The gradient was set as follows: 0-1.5 min, 5% B; 1.5-6.0 min, 5–95% B; 6.0-11.0 min, 95% B; 11.0-11.5 min, 95-5% B; 11.5-15.0 min, 5% B.

The mass spectrometer was operated in electrospray ionization (ESI) positive ion mode and negative ion mode. Analysis was performed in the full scan [mass-to-charge ratio (m/z) = 67~1000] and data-dependent scan (dd-MS²) modes (the parent ion ranked in the top five). The instrument settings for the full scan mode were: 120,000 resolution, 2×10^6 automatic gain control (AGC), and 200 ms maximum ion injection time (IT). The settings for the MS/MS mode were: 30,000 resolution, 1×10^5 AGC, 100 ms maximum injection time, 15 s dynamic exclusion, and a collision energy of 40. Source ionization parameters were: spray voltage set at 3.5 kV for positive ion mode and 4.0 kV for negative ion mode, capillary temperature set at 320°C, sheath gas set at 25, and aux gas set at 5.

Data processing

The acquired raw data were processed using Compound Discoverer version 3.1 software (Thermo Fisher Scientific) with default settings to conduct peak area extraction and metabolite identification. The m/z features with spectral relative standard deviations (RSDs) < 30% for quality control and < 20% for missing values were included for subsequent statistical analyses. Orthogonal partial least squares discriminant analysis (OPLS-DA) was carried out using SIMCA[®] version 15.0 software (Umetrics, Umeå, Sweden) and included unit variance (UV) scaling prior to multivariate analysis. The performance of OPLS-DA models was assessed by the intercepts of R²Y (a measure of goodness of fit) and Q² (a measure of accuracy of fit). A permutation test was performed to prevent overfitting and a cross validated-analysis of variance (CV-ANOVA) was used to assess the consistency of fit.

Quantitative analysis of targeted metabolites in the validation cohort

Absolute quantification was performed for selected metabolites in the validation cohort. Using previously described LC-MS/MS methods, the concentrations of metabolites were calculated by area response from independent calibration curves^{20, 21}. For gluconic acid, fumaric acid, and pseudouridine, the precursor ions with a m/z of 195.07, 115.02, and 243.06

(M-H⁺), respectively, which yielded product ions with a m/z of 75.01, 71.01, and 153.01, respectively, were used for quantification.

Construction of an ischemia-reperfusion-induced AKI swine model

A swine model of AKI was developed using a modified method according to a published study²². All female Landrace swine (50~70 kg, n=3) were prepared with pre-medication, tracheal intubation, and inhalation anesthesia. Both the left jugular vein and right femoral artery were cannulated after local infiltration anesthesia. Following surgical preparation, all swine were subjected to midline laparotomy. A 60-min cross-clamp of the right renal artery and vein was performed followed by left nephrectomy. The clamp was released and the right kidney was observed for immediate reperfusion. The abdomen was then closed by a contained laparotomy. All swine were monitored for 8 h after surgery, and nine blood samples were collected at 1 h intervals from each swine via the venous catheter. The experimental animal research protocol was approved by the Institutional Review Board of Fuwai hospital.

Statistical analysis

Differences in metabolite levels between AKI and non-AKI patients were assessed with Mann-Whitney U-tests. False discovery rate (FDR) adjusted p-values < 0.05 were considered to be statistically significant. Spearman correlation analyses were performed using MetaboAnalyst version 4.0 (<http://www.metaboanalyst.ca/>). The Gephi version 0.9.2 network analysis and visualization software package were used to determine and visualize the force-directed layout-based correlation network. Receiver operating characteristic (ROC) curve analyses were used for individual biomarkers. Binary logistic regression models were constructed using the biomarkers and their predictive abilities were assessed using area under the curve (AUC). Linear fitting was performed using Origin[®] 2019b (OriginLab Corporation, Northampton, MA, USA) and all data were analyzed using IBM SPSS version 20.0 (International Business Machines Corporation, Armonk, NY, USA).

Table S1. The results of ROC analysis in the discovery cohort.

	AUC (95% [CI])	Sensitivity (95% [CI])	Specificity (95% [CI])
Whole dataset (47 AKI vs 48 non-AKI)			
Gluconic acid	0.809 (0.720~0.897)	0.745 (0.594~0.856)	0.813 (0.669~0.906)
Fumaric acid	0.825 (0.741~0.909)	0.936 (0.814~0.983)	0.583 (0.433~0.721)
Pseudouridine	0.819 (0.731~0.906)	0.745 (0.594~0.856)	0.813 (0.669~0.906)
Test set (16 AKI vs 48 non-AKI)			
Gluconic acid	0.730 (0.592~0.869)	0.688 (0.415~0.879)	0.833 (0.692~0.920)
Fumaric acid	0.848 (0.750~0.945)	1.000 (0.759~1.000)	0.583 (0.433~0.721)
Pseudouridine	0.866 (0.750~0.982)	0.875 (0.604~0.978)	0.813 (0.669~0.906)
P-RF	0.922 (0.855~0.988)	0.750 (0.474~0.917)	0.938 (0.818~0.984)
P-SVM	0.884 (0.793~0.976)	0.938 (0.677~0.997)	0.771 (0.623~0.875)
P-LR	0.883 (0.803~0.963)	0.938 (0.677~0.997)	0.792 (0.646~0.890)
C&P-RF	0.939 (0.879~0.998)	0.875 (0.604~0.978)	0.938 (0.818~0.984)
C&P-SVM	0.910 (0.839~0.982)	0.938 (0.677~0.997)	0.771 (0.623~0.875)
C&P-LR	0.902 (0.792~1.000)	0.875 (0.604~0.978)	0.917 (0.791~0.973)

The whole dataset included 47 AKI patients and 48 non-AKI patients, while the test set included 16 early-stage AKI patients and 48 non-AKI patients. The early-stage AKI patients indicated those who had not been diagnosed with AKI at the time of plasma collection but progressed to AKI within 48 h following cardiac surgery. C: Clinical parameters (pre-creatinine and estimated glomerular filtration rate); P: Plasma metabolites (gluconic acid, fumaric acid, and pseudouridine); C&P: Clinical parameters and plasma metabolites.

Table S2. The results of ROC analysis in the validation cohort.

	AUC (95% [CI])	Sensitivity (95% [CI])	Specificity (95% [CI])
75 AKI vs 44 non-AKI			
Gluconic acid	0.878 (0.815~0.941)	0.827 (0.718~0.901)	0.795 (0.642~0.897)
Fumaric acid	0.747 (0.661~0.833)	0.520 (0.402~0.636)	0.909 (0.774~0.970)
Pseudouridine	0.883 (0.823~0.940)	0.853 (0.748~0.921)	0.773 (0.618~0.880)
P-RF	0.963 (0.934~0.992)	0.893 (0.795~0.950)	0.932 (0.803~0.982)
P-SVM	0.912 (0.861~0.964)	0.867 (0.764~0.931)	0.818 (0.668~0.913)
P-LR	0.910 (0.860~0.960)	0.827 (0.718~0.901)	0.841 (0.693~0.928)
C&P-RF	0.972 (0.947~0.996)	0.893 (0.795~0.950)	0.977 (0.865~0.999)
C&P-SVM	0.931 (0.889~0.973)	0.840 (0.733~0.911)	0.909 (0.774~0.970)
C&P-LR	0.926 (0.883~0.969)	0.827 (0.718~0.901)	0.886 (0.746~0.957)
16 early-stage AKI vs 44 non-AKI			
Gluconic acid	0.734 (0.607~0.862)	0.813 (0.537~0.950)	0.636 (0.477~0.772)
Fumaric acid	0.688 (0.553~0.822)	0.813 (0.537~0.950)	0.545 (0.390~0.693)
Pseudouridine	0.813 (0.698~0.927)	0.875 (0.604~0.978)	0.705 (0.546~0.828)
P-RF	0.935 (0.855~1.000)	0.875 (0.604~0.978)	0.886 (0.746~0.957)
P-SVM	0.828 (0.720~0.936)	0.938 (0.677~0.997)	0.659 (0.500~0.791)
P-LR	0.811 (0.705~0.917)	1.000 (0.759~1.000)	0.614 (0.455~0.753)
C&P-RF	0.943 (0.883~1.000)	0.813 (0.537~0.950)	0.909 (0.774~0.970)
C&P-SVM	0.849 (0.737~0.962)	0.750 (0.474~0.917)	0.795 (0.642~0.897)
C&P-LR	0.830 (0.721~0.938)	0.875 (0.604~0.978)	0.705 (0.546~0.828)

There were 75 AKI patients and 44 non-AKI patients in the validation cohort. We also performed ROC analysis for 16 early-stage AKI patients and 44 non-AKI patients. C: Clinical parameters (pre-creatinine and estimated glomerular filtration rate); P: Plasma metabolites (gluconic acid, fumaric acid, and pseudouridine); C&P: Clinical parameters and plasma metabolites.

Table S3. The variable importance and statistical test for different predictive models.

	P-RF	P-LR	C&P-RF	C&P-LR
Variable importance				
Preoperative creatinine			1.380	1.737
eGFR			3.600	2.006
Gluconic acid	25.131	2.008	23.333	2.595
Fumaric acid	12.109	2.025	18.666	2.101
Pseudouridine	21.370	2.101	15.996	2.244
Wald test of independent variables				
Preoperative creatinine				0.042
eGFR				0.045
Gluconic acid		0.047		0.009
Fumaric acid		0.043		0.036
Pseudouridine		0.036		0.025
χ^2 test of model		0.028		0.0150

C: Clinical parameters (pre-creatinine and estimated glomerular filtration rate); P: Plasma metabolites (gluconic acid, fumaric acid, and pseudouridine); C&P: Clinical parameters and plasma metabolites; eGFR: estimated glomerular filtration rate.

Figure S1. Graphical representation of 200 permutation tests used to discriminate AKI patients from non-AKI patients. (a) positive ion mode; (b) negative ion mode.

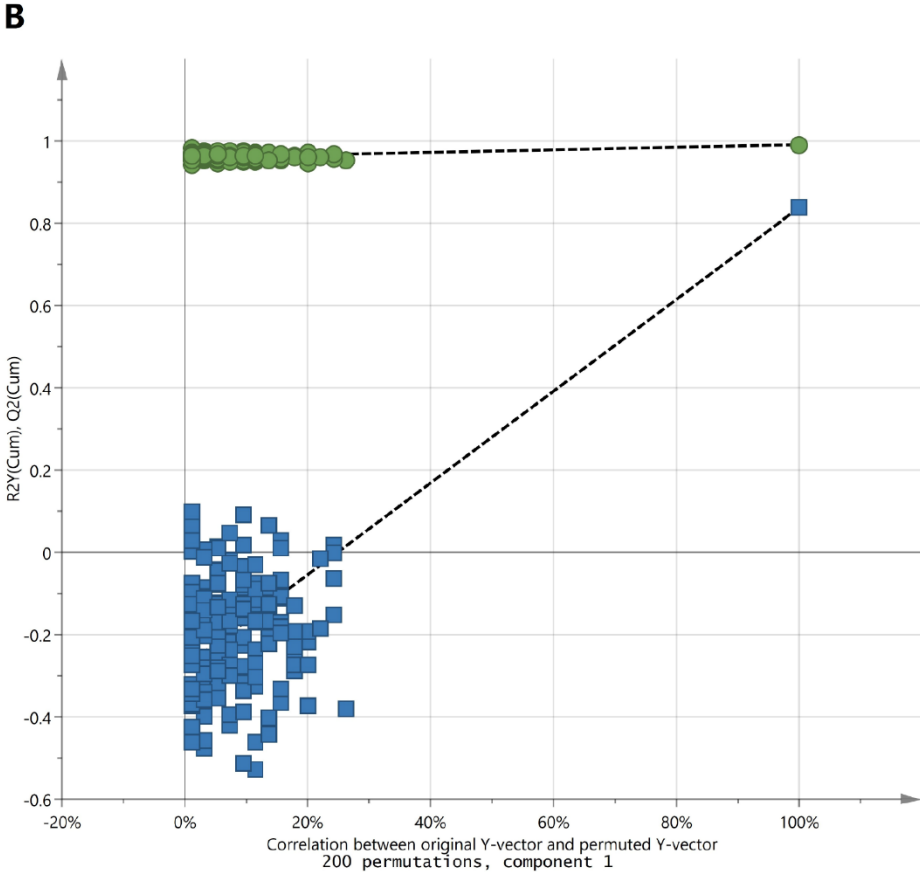
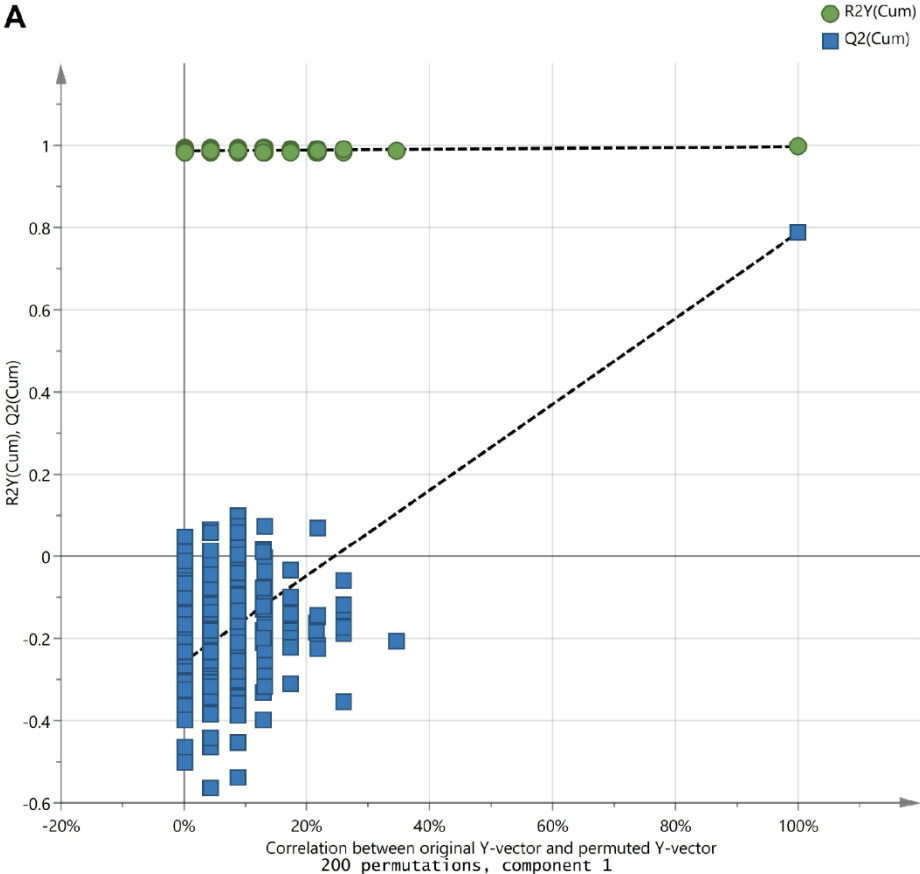


Figure S2. MS/MS spectra. (a) gluconic acid; (b) fumaric acid; (c) pseudouridine.

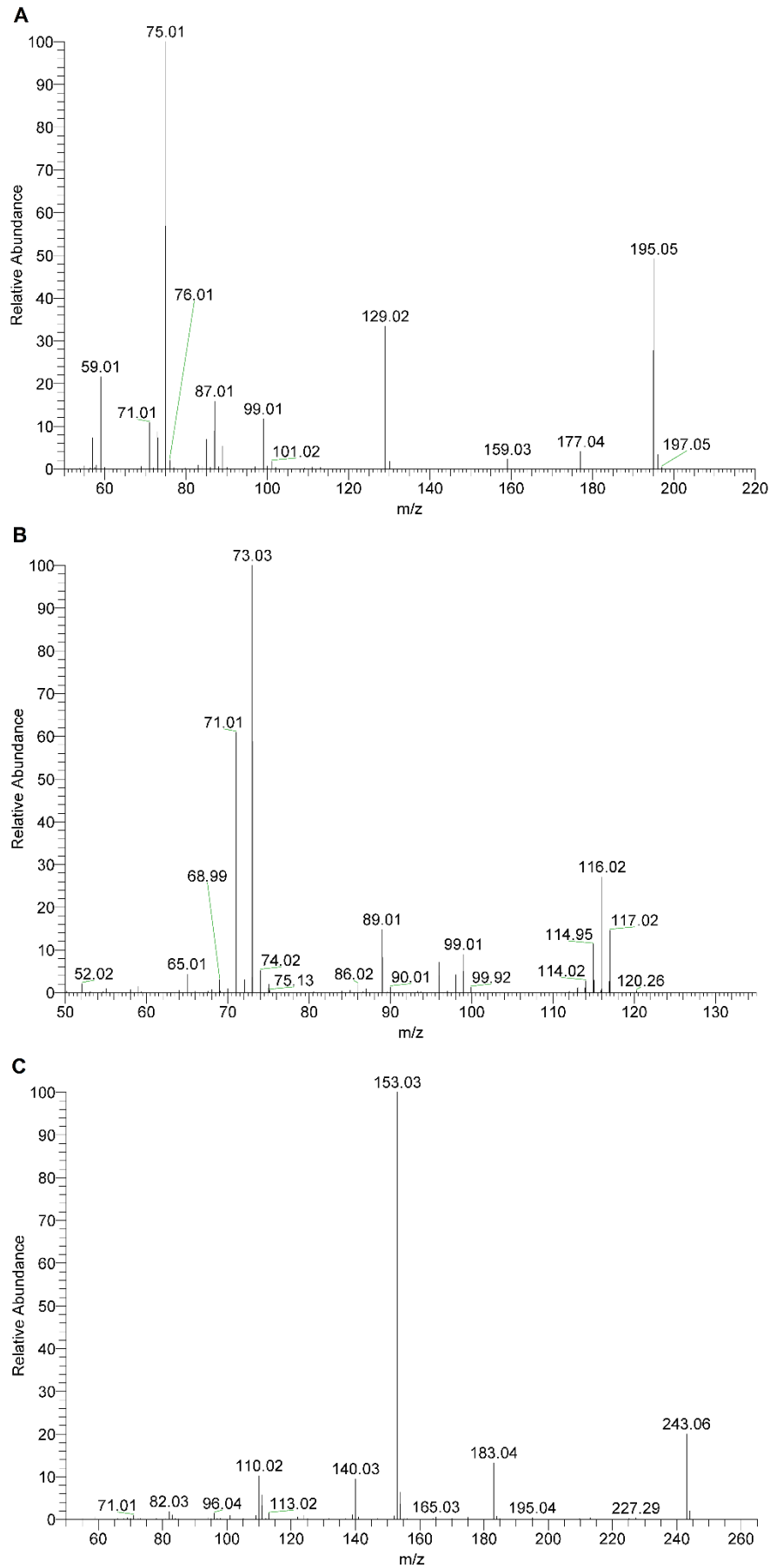
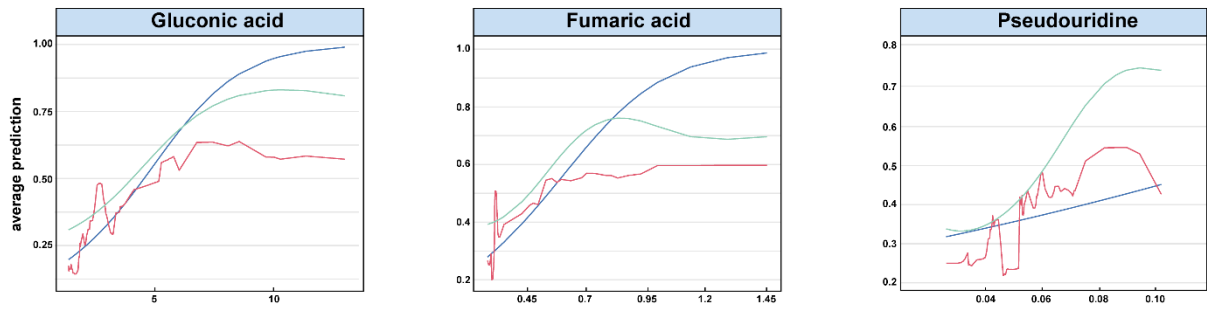


Figure S3. The partial dependence plot of the LR, RF, and SVM model in the test set. (a) Plasma metabolites-based models; (b) Clinical parameters & Plasma metabolites-based models.

a Plasma metabolites-based models: — LR — SVM — RF



b Clinical parameters & Plasma metabolites -based models: — LR — SVM — RF

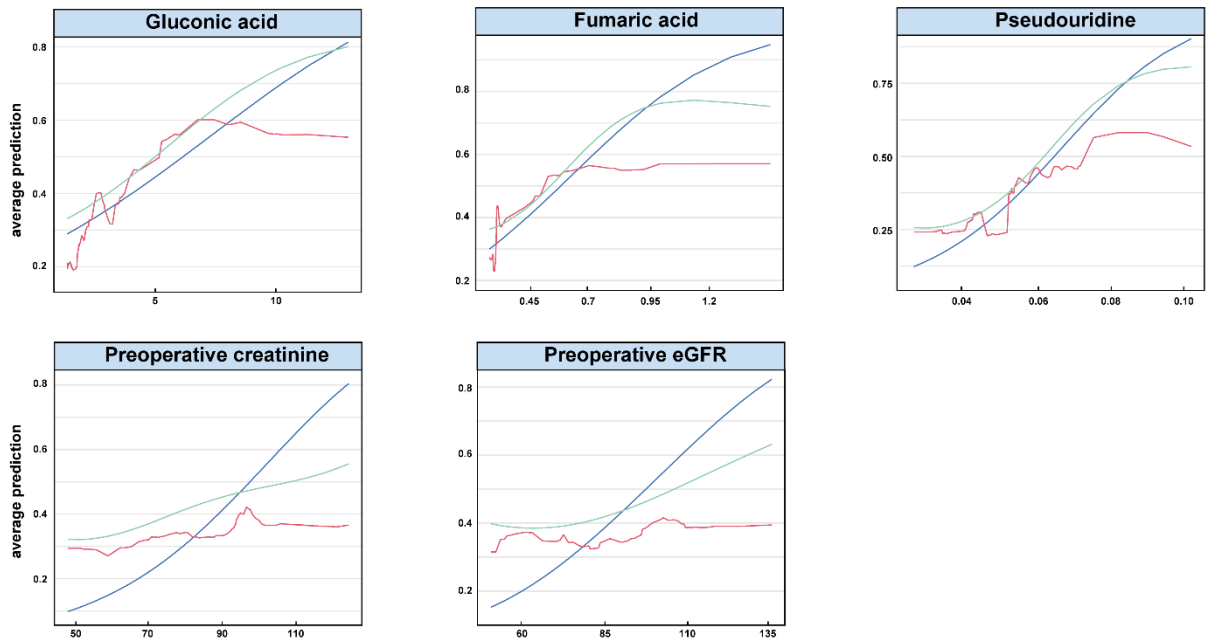


Figure S4. ROC analyses of creatine for discriminating AKI patients from non-AKI patients in the discovery cohort.

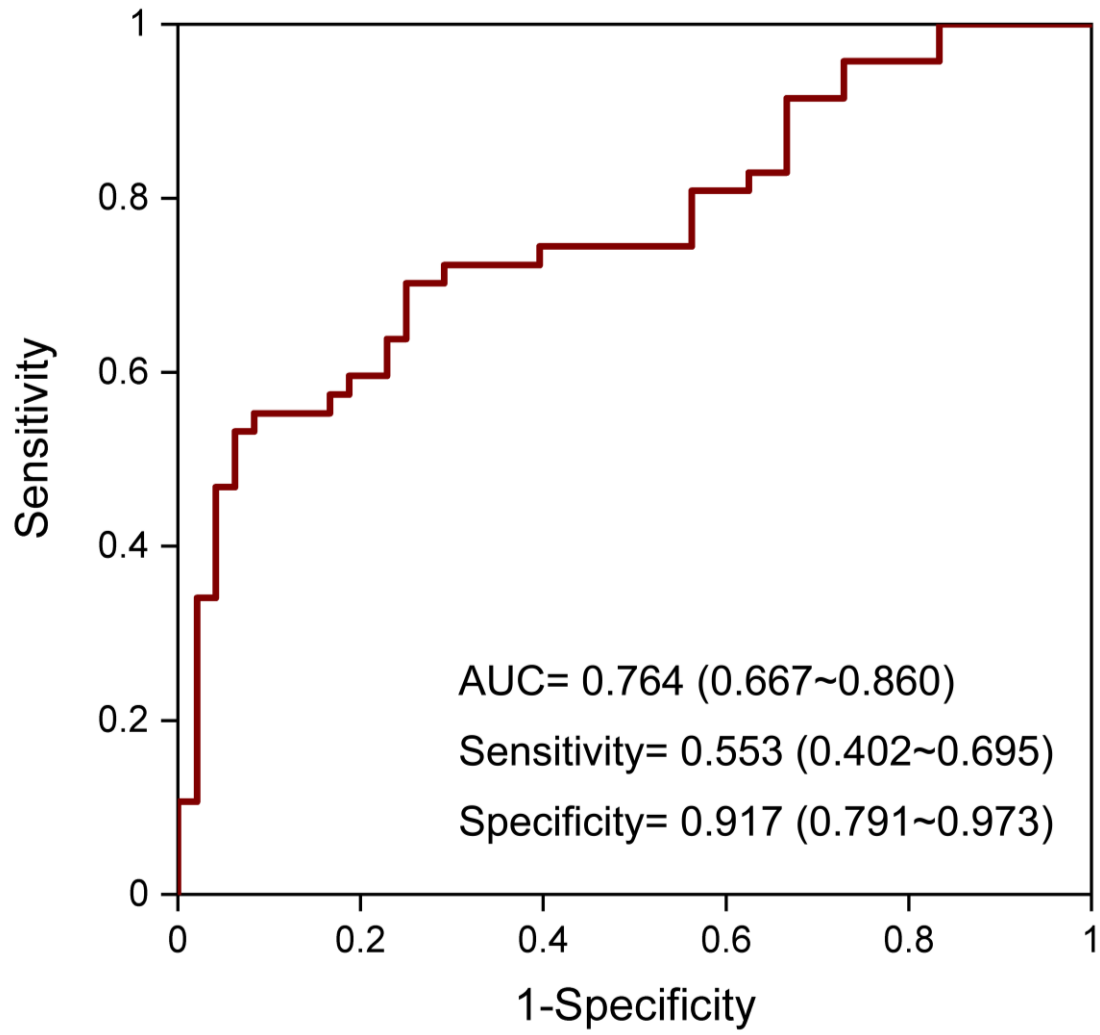


Figure S5. Comparison of the abilities in predicting CSA-AKI of clinical parameters-based and plasma metabolites-based ML models in the test set.

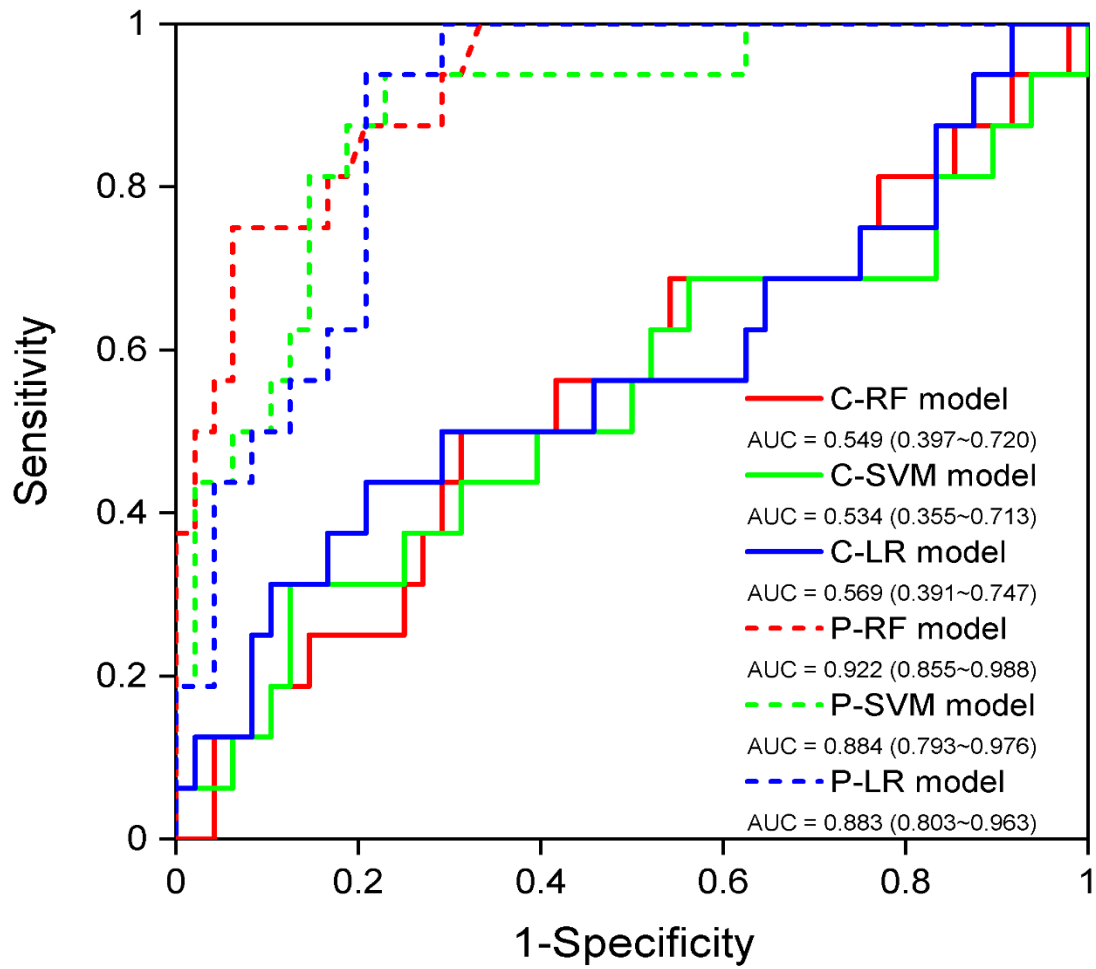
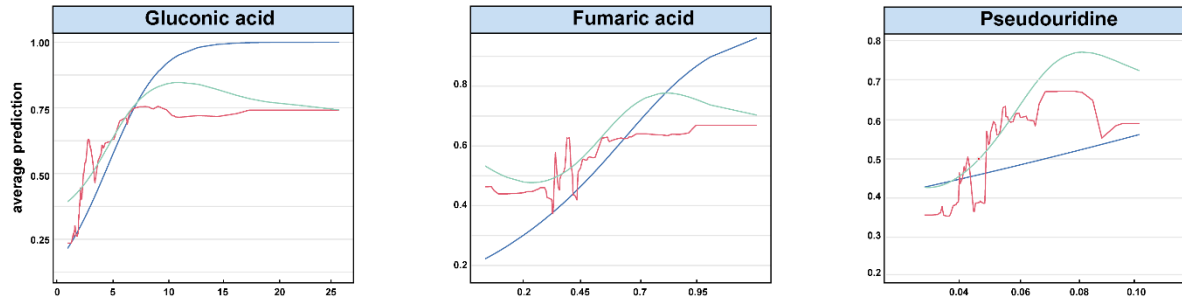


Figure S6. The partial dependence plot of the LR, RF, and SVM model in the validation cohort. (a) Plasma metabolites-based models; (b) Clinical parameters & Plasma metabolites-based models.

a Plasma metabolites-based models: — LR — SVM — RF



b Clinical parameters & Plasma metabolites-based models: — LR — SVM — RF

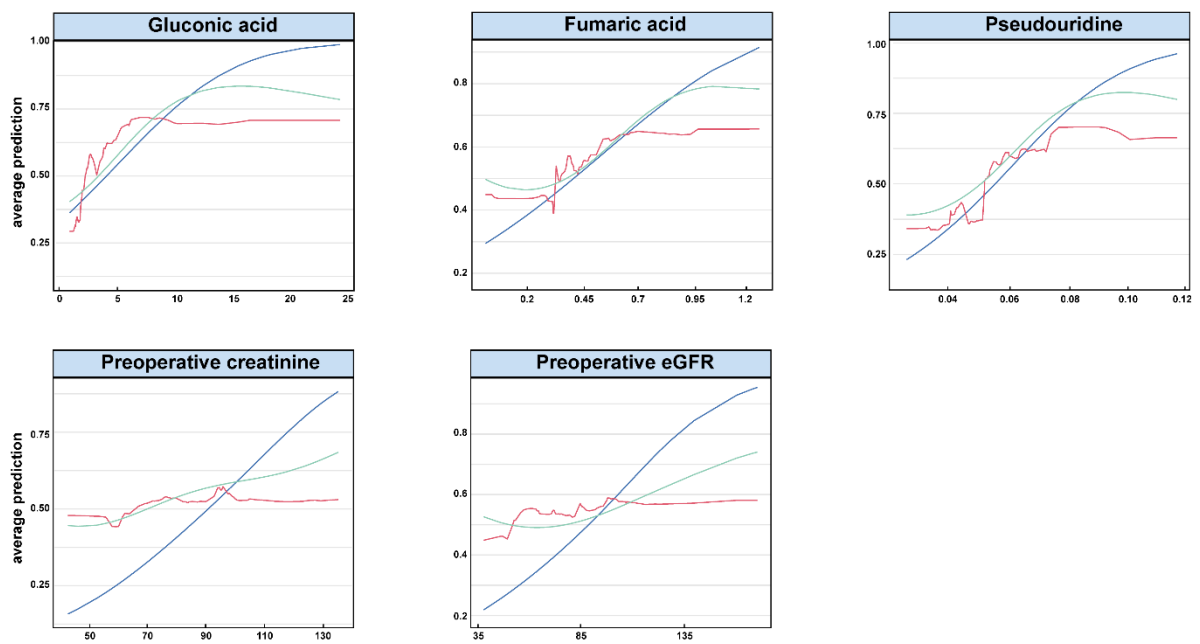


Figure S7. ROC curve analyses of CPB and ACCT for discriminating AKI patients from non-AKI patients in the validation cohort.

