

Construction of the Metabolomics-Based Prognosis-Prediction Models for ICU Septic Patients

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Research

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Abstract

Background: Global mortality related to sepsis remains unacceptably high in intensive care units (ICUs). Accurate prognostic evaluation of sepsis could effectively reduce the mortality of septic patients. Our goal is to present an effective and rapid method to assess the prognosis of sepsis.

Methods: We included 96 septic patients according to the sepsis 3.0 in ICU, who were grouped into survival and death groups according to 28-day, hospital, and 90-day prognosis. Liquid chromatography/mass spectrometry was performed to detect the metabolite changes in plasma. Multivariate logistic regression models, using differential metabolites and clinical indicators within 24 h after diagnosis of sepsis, were used to construct the prediction models for 28-day, hospital, and 90-day prognosis in sepsis.

Results: Metabolic profiles related to 28-day, hospital, and 90-day prognosis were significantly different between the survival and the death group. Specifically, 13, 4, and 29 primary differential metabolites related to amino acid metabolism and fatty acid metabolism were identified between the survival and death group at 28-day, hospital, and 90-day prognosis, respectively. Further, we found that model 1 including indoleacetic acid, 3-methylene-indolenine, heart rate, respiratory support, and application of pressure drugs; model 2 including lymphocyte count, alkaline phosphatase, SOFA, and L-alpha-amino-1H-pyrrole-1-hexanoic acid; model 3 including pyrrolidine, dopamine, heart rate, respiratory support, and application of pressure drugs, could predict 28-day, hospital, and 90-day prognosis of sepsis with a sensitivity of 75.51%, 73.58%, and 83.33%, specificity of 78.72%, 72.09%, and 76.19%, the area under the receiver operating characteristics curve of 0.881, 0.830, 0.892, respectively.

Conclusions: This research could be used to predict the 28-day, hospital, and 90-day prognosis of septic patients based on differential metabolites and clinical parameters, and could also be used to develop novel sepsis-treatment methods.

Introduction

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to pathogens [1]. It is the primary cause of morbidity and mortality in critically ill patients and is a substantial healthcare issue worldwide [2, 3]. Rapid progress has been made in clinical treatment, such as antibiotic treatment, in the past decade, but sepsis incidence and patients mortality are still rising in intensive care units (ICUs) [4, 5]. The heterogeneous manifestations and pathogenic complexities of sepsis challenge early diagnosis, accurate prognosis, timely sepsis-progression control, and appropriate treatment measures [4, 6]. An effective predictive method for prognosis is necessary to reduce the mortality [7].

Metabolomics is an emerging discipline, which measures and researches the endogenous small molecular compounds or metabolites in the body [8]. It provides information on metabolite-concentration changes, data on metabolic alterations that reflect further downstream from genomics, and reveals disease-related biomarkers or potential mechanisms [9, 10]. Unlike other omics techniques, metabolomics could directly

reflect the potential biochemical activities and a cell's state in a given time. Various analytical methods, such as nuclear magnetic resonance (NMR), gas chromatography/mass spectrometry (GC/MS), and liquid chromatography/mass spectrometry (LC/MS), have been used in metabolomics research. Among these, LC/MS is perceived to be the most appropriate approach for analyzing biological samples [11]. Metabolic disorders, which describe a patient's state, are critical in sepsis development. Although some inflammatory markers such as procalcitonin (PCT) and C-reactive protein (CRP) are widely used in identifying sepsis severity and death risk, they are limited in their specificity or sensitivity to predict outcomes and the measurement results vary with age and other factors [12, 13]. Altered plasma metabolism is a significant hallmark of sepsis [8]. Animal research has shown that changes in serum metabolic profiles started earlier than organ dysfunction [14]. Metabolomics analysis of severe patients may reveal new metabolisms that can contribute to potential pathophysiology and treatments. Therefore, it is an urgent medical need to identify and validate reliable biomarkers for sepsis prognosis [15].

In recent years, researchers have demonstrated the ability of some metabolites such as lipids, carbohydrates, and amino acids in predicting sepsis prognosis [7, 16]. Jaurila et al. reported six metabolites that distinguished survivors from non-survivors at the early-stage sepsis [7]. Mogensen et al. noted seven metabolites related to the nutritional conditions that could predict the 28-day mortality [17]. To our knowledge, most models predicting the prognosis of sepsis were based only on some metabolites, whereas a few models used both metabolites and clinical indicators to predict the prognosis at multiple time points. In this research, we proposed a LC/MS-based non-targeted metabolomic method to analyze the metabolic differences between the survival and death group at 28-day (28d), hospital, and 90-day (90d) to screen out potential metabolic biomarkers. Further, we constructed the logistic regression models using differential metabolites and clinical indicators to predict the 28d-, hospital-, and 90d-prognosis in patients with sepsis. Our goal is to develop an effective method for evaluating sepsis prognosis and providing a novel sepsis-treatment solution.

Material And Methods

Patients and Information Collection

The research was conducted in the First Affiliated Hospital of Zhengzhou University. We selected 110 patients with severe infection, hospitalized in the general ICU between March and September 2019. This research was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was acquired from all patients or their agents. Patients that met the sepsis 3.0 diagnostic criteria by the Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM) during their first 48-hour stay in ICU were included in our research. The diagnostic criteria of sepsis 3.0 include the following factors: (1) Suspected or confirmed infection diagnosed by clinicians; (2) Evidence of acute organ dysfunction: patients without previous chronic organ dysfunction (assuming the baseline sequential organ failure assessment score (SOFA) is 0) – $SOFA \geq 2$; patients with previous chronic organ dysfunction (SOFA should be based on the baseline situation) – $SOFA$ increased ≥ 2 . The exclusion standards were as follows: length of stay < 24 h, age < 18 years, terminal malignant tumor, pregnancy, data missing, and failure to obtain informed consent or authorization.

The following clinical and laboratory information were collected within 24 h of sepsis diagnosis: gender, age, smoke, alcohol, SOFA, Glasgow Coma score (GCS), Acute Physiology And Chronic Health Evaluation II score (APACHE II), temperature, respiratory rate, heart rate, blood pressure, blood routine test (red blood cell count, white blood cell count, hemoglobin, platelet count, neutrophil percent, lymphocyte percent, monocyte percent, eosinophil percent, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell volume distribution width, mean platelet volume, platelet crit, and platelet volume distribution width), nucleated red blood cell count, nucleated red blood cell percent, glucose, lactic acid dehydrogenase, creatine kinase, creatine kinase isozyme, lactate, C-reactive protein, procalcitonin, α -amylase, lipase, serum lipid parameters (total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein), liver function indexes (total protein, albumin, globulin, alanine (Ala) aminotransferase, aspartate aminotransferase, γ -glutamyl transferase, alkaline phosphatase (ALP), serum cholinesterase, total bilirubin, direct bilirubin, indirect bilirubin), renal function indicators (glomerular filtration rate, serum urea, serum creatinine, uric acid, 24h urine volume), coagulation function indexes (prothrombin time, prothrombin activity, international normalized ratio, activated partial thromboplastin time, fibrinogen, thrombin time, D-Dimer, fibrin or fibrinogen degradation products), the application of respiratory support, and pressor drugs.

Sample Collection and Grouping

Plasma samples were obtained from septic patients within 24 h of sepsis diagnosis. A small amount (5 mL) of venous blood from each participant was collected with ethylenediaminetetraacetic acid (EDTA) vacuum anticoagulant tube (purple head cover). After shaking gently, these anticoagulant tubes were stored at 4 °C in a refrigerator until sample preparation. A telephone follow-up was conducted in December 2019 to record the 28d-, hospital-, and 90d-prognosis, based on which the sample patients were divided into the 28d-survival group (28dS) and 28d-death group (28dD), hospital-survival group (HOS-survival) and hospital-death group (HOS-death), 90d-survival group (90dS) and 90d-death group (90dD). Additionally, we collected plasma samples of 66 healthy people as the healthy control group.

Instruments and Reagents

Ekspert nanoLC 400 series® liquid chromatography system (SCIEX, USA), a TripleTOF® 6600 mass spectrometer (SCIEX, USA), a high-speed refrigerated centrifuge 5810R (Eppendorf, Germany), and the - 80 °C refrigerator (Thermo, USA) were the instrument used for this study. The reagents used included mass spectrometry grade acetonitrile, methanol (Thermo Fisher Scientific, USA), mass spectrometry grade formic acid (Sigma-Aldrich, USA), and purified water (Mass spectrometric grade).

Plasma Samples Pretreatment

After thawing the plasma samples on ice at 4 °C, 50 μ L plasma of each sample was injected into the Eppendorf (EP) tube, followed by the addition of 150 μ L methanol and 10 μ L internal standard (0.5 μ m/L CA-d4, 0.5 μ m/L CDCA-d4). Subsequently, the mixtures were vortexed for 30 s and centrifuged at 14,000 rpm for 10 min. The supernatant (150 μ L) was transferred into an injection bottle. Finally, 10 μ L of each sample was mixed to obtain the quality control (QC) sample; a measured amount of the QC sample (200 μ L) was used for metabolomics QC analysis.

Chromatography Conditions

The chromatographic column (ChromXP C18, 3 μm 120 \AA , 0.3 mm \times 150 mm, SCIEX, USA) was maintained at 30 $^{\circ}\text{C}$. The positive-ion mobile phase consisted of water with 0.1% formic acid (phase A) and acetonitrile (phase B); the negative-ion mobile phase comprised water (phase C) and acetonitrile (phase D). The gradient elution method was used for 18 min with a flow rate of 5 $\mu\text{L}/\text{min}$ in the positive-ion mode. The linear gradient of elution started at 5% B, increased linearly to 25% in 1 min, ramped up linearly to 95% in the next 9 min, and maintained 95% for 2 min. Subsequently, phase B was recovered to 5% within 1 min and held for another 5 min. The negative-ion mode followed a 15-min gradient elution method at a flow rate of 5 $\mu\text{L}/\text{min}$. Similarly, the linear gradient elution began from 5% B, increased linearly to 30% in 1 min, further increased linearly to 95% in the next 8 min, and then remained constant for 3 min. Afterward, phase B was restored to 5% in 1 min and maintained for 2 min. The injection volume was 2.0 μL .

Mass Spectrometry

Data acquisition and processing were conducted using the Peakview 2.0 software (AB, Milford, MA). Electrospray ionization (ESI) was used as the ion source in this research. The ion source temperature and spray voltage were set at 350 $^{\circ}\text{C}$ and 5500 V, respectively, for the positive-ion scanning modes, while they were set at 350 $^{\circ}\text{C}$ and -4500 V, respectively, in the negative-ion scanning mode. The declustering voltage, atomization gas 1, atomization gas 2, and the curtain gas were 80 V, 25 psi, 15 psi, and 30 psi, respectively. A full scan measured the samples with a range of mass spectral (m/z) 50–1000 Da in the positive and negative modes. The collision energy was at 35 ± 15 eV. Additionally, dynamic background subtraction-dependent data acquisition was used to gather the LC/MS data of low-level components.

Data Processing and Statistical Analysis

The preprocessing procedures, including data extraction, retention-time correction, peak recognition, peak extraction, peak integration, and peak alignment, were performed using the Software MarkerView TM (version 1.4.1, Waters Co., Milford, MA, USA). The data matrix comprising mass spectral (m/z), retention time (RT), and peak intensity was generated after total area normalization pretreatment. The orthogonal partial least squares discriminant analysis (OPLS-DA) was used for the multi-dimensional complex data (SIMCA 14.1 software). Further, variable importance in projection (VIP) of the OPLS-DA model was computed to identify differential metabolites that distinguish the survival group from the death group. Metabolites with $\text{VIP} > 1.0$ were selected for statistical analysis using the student's t -test (SPSS software version 21.0, Chicago, IL). The metabolites with $\text{VIP} > 1.0$ and $P < 0.05$ were the expected differential markers. Finally, the identification results combined with Kyoto Encyclopedia of Genes and Genomes (KEGG) (<http://www.genome.jp/kegg/>), Human metabolite database (HMDB) (<http://www.hmdb.ca/>), MetaboAnalyst 4.0, other databases, and literature reports were used to identify the structural biomarkers. The pathway enrichment analyses were explained by secondary mass spectrometry fragment information, while the R software (R version 3.5.3, heatmap package) was used to generate the heat maps of differential metabolites and display the trend variations.

The Kolmogorov–Smirnova test was employed to analyze the conformity of continuous variables to normal distribution. Basic characteristics (gender, age, smoke, alcohol, and indicators within 24 h after diagnosis) of the survival- and death groups were compared using student's *t*-test for normal distribution, the Mann Whitney U test for not conforming to the normal distribution, and the Chi-square test for the classified data. Furthermore, logistic regression prognostic models were constructed using statistically significant variables at 28d-, hospital-, and 90d-prognosis in the survival and death groups. The receiver operating characteristic curves (ROC) were drawn to evaluate the predictive value. All statistical analyses were two-sided tests, and $P < 0.05$ was considered statistically significant.

Results

Patient Sets and Baseline Characteristics

We selected 100 septic patients from the 110-patients sample with severe infection. Of these, 96 were followed up and included in the study. **Supplementary Fig. 1** shows a detailed flowchart of subject selection. Among the 96 patients, 57 (59.4%) were male with an average age of 50.23 ± 16.24 years; and 39 (40.6%) were female with an average age of 59.38 ± 18.53 years. Furthermore, 49 patients (51.0%) died at 28d-sepsis, 53 patients (55.2%) died during the hospital-sepsis, and 54 patients (56.3%) died at 90d-sepsis. **Supplementary Tables 1–3** present the baseline characteristics and clinical parameters of septic patients. It was observed that the heart rate (HR), serum cholinesterase, SOFA, and APACHE II values were lower in 28dS than those in 28dD, while the 24h urine volume was significantly higher ($P < 0.05$). The levels of HR, monocyte percent, lymphocyte count (lymph#), red blood cell volume distribution width, SOFA, and APACHE II in the HOS-survival patients were significantly lower ($P < 0.05$) than the HOS-death patients. However, the levels of hematocrit, mean corpuscular hemoglobin, and ALP were significantly higher ($P < 0.05$) in the HOS-survival group than in the HOS-death group. Also, it was observed that the results of indicators such as HR, red blood cell volume distribution width, thrombin time, SOFA, and APACHE II decreased significantly, while serum cholinesterase and lipase significantly increased in the 90dS group than those in the 90dD ($P < 0.05$) group. The classified data showed a significant decrease in the possibility of applying respiratory support and pressor drugs in the survival group ($P < 0.05$) than in the 28dD- and 90dD groups. Additionally, the respiratory support probability in the HOS-survival group reduced significantly compared to the HOS-death ($P < 0.05$).

Plasma Chromatography Analysis

The plasma metabolites of the sepsis- and healthy control groups were analyzed by SCIEX OS (version 1.5) software. Each sample had two data sets established by positive- and negative-ion models. **Supplementary Fig. 2** shows the total ion chromatograms between the sepsis- and healthy control groups. The results suggested that the plasma metabolic profiles were markedly different in the sepsis- and healthy control groups.

Multivariate Statistical Analysis

Figure 1 shows the visual and intuitive results of the supervised OPLS-DA analysis on metabolic profiles. Based on the scatter plots of OPLS-DA score, it was apparent that the survival- and the death groups at 28d-, hospital-, and 90d-prognosis in positive- and negative-ion models were classified. All outcomes indicated significantly altered metabolite profiles between the two groups at the 28d-, hospital- and 90d-prognosis.

Identification of Differential Metabolites

The VIP scores in the OPLS-DA model, *P* values and fold change (FC) values in volcano plot (**Supplementary Fig. 3**) were used to evaluate the potential biomarkers. The biomarkers with $VIP > 1.0$, $|FC| > 1.50$ or $< 2/3$ ($\log_2(FC) \geq 0.585$ or $\log_2(FC) \leq -0.585$) and $P < 0.05$ ($-\log_{10}(P\text{-value}) > 1.30$) were considered as the main differential metabolites. The differential metabolites thus obtained were matched with the HMDB, KEGG, and MetaboAnalyst databases, and 13, 4, and 27 differential metabolites were identified at the 28d-, hospital-, and 90d-prognosis, respectively (Table 1, **Supplementary Table 4–5**). It was further noted that compared with 28dD, the levels of plasma L-aspartic acid, indoleacetic acid, \pm 5-hydroxy-4-octanone, 3-methyleneindolenine, 3-methoxytyramine, acetaminophen, dopamine, dihydro-4,6-dimethyl-2-(1-methylpropyl)-4H-1,3,5-dithiazine, histidinyl-tryptophan, alpha-linolenic acid, docosapentaenoic acid (22n-3) were up-regulated in differential metabolites of 28dS group, and those of indole-3-carbinol and 5-hydroxyomeprazole were down-regulated (Fig. 2A). Also, the main metabolic pathways of the disorders were tyrosine metabolism, alpha-linolenic acid metabolism, alanine, aspartate, and glutamate metabolism (**Supplementary Fig. 4**). Pseudouridine, oryzalexin E, and L-alpha-amino-1H-pyrrole-1-hexanoic acid were elevated significantly, while traumatic acid was reduced significantly in the HOS-survival group compared to the HOS-death group (Fig. 2B). A comparison of differential metabolites between 90dS- and 90dD groups showed that norvaline, erythrabyssin II, oleamide, (2E)-3-(3,4-dihydroxyphenyl) prop-2-enal, p-aminobenzoic acid, dopamine, 2-heptanethiol, ovalicin, acetaminophen, perilloside A, pyrrolidine, omeprazole sulfone, L-arginine, delta-12-prostaglandin J2, hexylresorcinol was found to be higher in the 90dS group than the 90dD group. It was also noted that the levels of blennin B, 3',4'-dihydrodiol, N-desmethylaminopyrine, panaxynol, hydroxytyrosol 1-O-glucoside, harmalol, toluene, 6-methylquinoline, 3-mercaptoplactate-cysteine disulfide, batatasin IV, malonoben, meconine were significantly decreased in the 90dS group than the 90dD group (Fig. 2C). Altered metabolic pathways mainly involved arginine and proline metabolisms, folate biosynthesis, tyrosine metabolism, and arachidonic acid metabolism (**Supplementary Fig. 5**).

Table 1
Major differential metabolites in plasma between 28dS and 28dD.

NO	Differential metabolites	m/z	RT(min)	VIP	Pvalue	FC(28dS/28dD)	Involved pathways
1	L-Aspartic acid	134.0186	183.862	1.31	0.03004	1.77	1,3,4,5,6,9,10,11,12,13,14,15,16
2	Indoleacetic acid	176.0706	483.7835	1.75	0.02512	1.57	2
3	± 5-Hydroxy-4-octanone	145.1194	652.734	2.03	0.02076	1.55	
4	3-Methylene-indolenine	130.0647	482.6875	1.61	0.00103	2.09	
5	3-Methoxytyramine	168.1016	541.522	1.04	0.02504	1.61	7,8
6	Histidiny-Tryptophan	342.1568	294.159	1.14	0.01623	1.84	
7	Acetaminophen	152.0704	347.44	1.28	0.00864	1.81	
8	Dopamine	154.0819	303.058	1.00	0.00037	1.82	7,8
9	Indole-3-carbinol	130.0646	524.279	1.33	0.03452	0.52	
10	Dihydro-4,6-dimethyl-2-(1-methylpropyl)-4H-1,3,5-dithiazine	206.1001	347.5935	2.13	0.04818	1.88	
11	Alpha-Linolenic acid	277.2161	813.787	2.45	0.04016	1.59	17,18
12	5-Hydroxyomeprazole	360.1006	522.938	2.13	0.00369	0.37	
13	Docosapentaenoic acid(22n-3)	329.2462	875.1265	2.39	0.00904	1.72	18

Compared with 28dD group, potential biomarker information was screened in 28dS group. 28dS, 28d survival group; 28dD, 28d death group; FC, fold change; VIP, contribution rate of different substances to orthogonal partial least squares discriminant analysis (OPLS-DA) model construction; RT, retention time. Involved pathways: 1. Arginine biosynthesis; 2. Tryptophan metabolism; 3. Alanine, aspartate and glutamate metabolism; 4. Glycine, serine and threonine metabolism; 5. Cysteine and methionine metabolism; 6. Lysine biosynthesis; 7. Tyrosine metabolism; 8. Betalain biosynthesis; 9. Histidine metabolism; 10. Beta-Alanine metabolism; 11. Cyanoamino acid metabolism; 12. Nicotinate and nicotinamide metabolism; 13. Pantothenate and CoA biosynthesis; 14. Aminoacyl-tRNA biosynthesis; 15. 2-Oxocarboxylic acid metabolism; 16. Biosynthesis of amino acids; 17. Alpha-Linolenic acid. metabolism; 18. Biosynthesis of unsaturated fatty acids.

Analysis of Metabolic Data

The changing trends of differential metabolites among various study groups were analyzed using heat maps (R version 3.5.3, heatmap package) (Fig. 3). Clear boundaries were observed between the survival and death groups at 28d, hospital, and 90d of sepsis.

Construction of prognosis model in patients with sepsis of ICU based on metabolomics

With 28d outcome as the dependent variable, 13 differential metabolites and 7 clinical indicators with significant differences in univariate analysis were selected as independent variables for multivariate logistic regression analysis. Model 1 included indoleacetic acid, 3-methylene-indolenine, HR, respiratory support, and application of pressor drugs—the optimal variable combination to predict 28d-prognosis. The prediction model equation for 28d prognosis was: $PRE = 1/(1 + EXP(-(-10.44 + 0.605*indoleacetic\ acid + 0.615*3\text{-methylene-indolenine} + 0.03*HR + 1.88*respiratory\ support + 1.835*application\ of\ pressor\ drugs)))$. The predictive accuracy of model 1 was 77.08%, with an area under the ROC curve (AUC) of 0.881 (Fig. 4A). Additionally, the sensitivity and specificity of model 1 for predicting the 28d-prognosis of sepsis patients were 75.51% and 78.72%, respectively. The results showed that indoleacetic acid, 3-methylene-indolenine, HR, respiratory support, and application of pressor drugs were correlated with 28d-prognosis of sepsis, and they were independent risk factors for the short-term prognosis of patients with sepsis.

For hospital prognosis, 4 differential metabolites and 10 significant clinical parameters were identified as the combination of prognostic variables. Model 2, attained by multivariate logistic regression analysis, included lymph#, ALP, SOFA, and L-alpha- amino-1H-pyrrole-1-hexanoic acid. The prediction-model equation for hospital prognosis was $PRE = 1/(1 + EXP(-(-3.424 + 1.307*lymph\# - 0.01*ALP + 0.182*SOFA + 0.551*L\text{-alpha-amino-1H-pyrrole-1-hexanoic acid})))$. The accuracy, sensitivity, and specificity of model 2 in predicting the hospital prognosis were 72.92%, 73.58%, and 72.09%, respectively. Furthermore, the AUC of model 2 was 0.830 (Fig. 4B), which indicated that the combination of lymph#, ALP, SOFA, and L-alpha-amino-1H-pyrrole-1-hexanoic acid had the potential to predict the hospital prognosis of septic patients.

We established model 3 to predict 90d-prognosis of sepsis based on 27 differential metabolites and 9 clinical indexes with a significant difference. Model 3 included pyrrolidine, dopamine, HR, respiratory support, and application of pressure drugs. The 90d prediction-model equation was $PRE = 1/(1 + EXP(-(-11.403 - 3.983*pyrrolidine + 5.613*dopamine + 0.025*HR + 2.499*respiratory\ support + 1.72*application\ of\ pressor\ drugs)))$. The accuracy, sensitivity, and specificity of model 3 were 80.21%, 83.33%, and 76.19%, respectively, with an AUC of 0.892 (Fig. 4C). These results showed that the combination of pyrrolidine, dopamine, HR, respiratory support, and application of pressor drugs was of great value in predicting 90d-prognosis of sepsis patients, which may be used to predict the long-term prognosis of sepsis.

Discussion

This research proposed a metabolomic approach based on LC/MS to evaluate the prognosis in patients with sepsis. We identified some potential new small-molecule biomarkers for predicting the prognosis of sepsis patients. We noted 13 differential metabolites related to 28d-prognosis, 4 differential metabolites

associated with hospital prognosis, and 27 differential metabolites associated with 90d-prognosis in plasma of septic patients. An analysis of these metabolisms showed altered amino acid and fatty acid metabolisms in the survival and death groups at 28d, hospital, and 90d. We also constructed three predictive prognosis models for the 28d-, hospital-, and 90d-sepsis. The three models, model 1—indoleacetic acid, 3-methyleneindole, HR, respiratory support, and application of pressure drugs; model 2—lymph#, ALP, SOFA, and L- α -amino-1H-pyrrole-1-hexanoic acid; and model 3—pyrrolidine, dopamine, HR, respiratory support, and application of pressure drugs, could effectively predict 28d-, hospital-, and 90d-prognosis of sepsis, respectively. To our knowledge, our research is the first to establish the multivariate logistic regression models at 28d, hospital, and 90d by combining differential metabolites with clinical factors.

Amino Acid Metabolism

Amino acid metabolism disorders are common in consumptive diseases such as sepsis [18]. In this research, tyrosine, alanine, aspartate, and glutamate metabolisms were the main pathways involved in the 28d-plasma amino-acid metabolic disorders (Fig. 5A). Hirose et al. reported that a minimum glutamate level was a critical prognostic factor for mortality in ICU patients [19]. Like phenylalanine, glutamate has been identified as a potential differentiation product of sepsis [20]. N-methyl-d-aspartate receptors (NMDAR) are receptors of glutamate; the blockade of NMDAR might be a promising therapeutic strategy for sepsis-associated acute lung injury (ALI) by regulating neuropeptides [21]. Alanine is closely related to glycolysis, gluconeogenesis, and the tricarboxylic acid (TCA) cycle. Recently, some animal research has shown that Ala's concentration in septic mice may increase or decrease compared to the control mice [22, 23]. Another research evaluated the risk factors related to the prognosis of septic children in the death and survival groups [24]; it was noted that the Ala level decreased in the death group presumably due to a significant increase in alanine aminotransferase, an enzyme that catalyzes the transfer of amino to α -ketoglutarate [24]. In contrast, some research suggested that the increase in Ala concentration in septic rats may be due to the enhanced pyruvate metabolism or transamination to Ala through the Cori cycle [25]. Our study found higher plasma aspartate concentrations in the 28dS group than in the 28dD group, which could be related to the high level of serum aspartate aminotransferase that increases the oxaloacetic acid synthesis, an intermediate of TCA cycle, and generates more energy via the TCA cycle. Histidine is an essential amino acid for human beings, which plays a significant role in enzyme-catalyzed methylation and antioxidant dipeptides formation [26]. Reduced plasma histidine levels are linked to inflammation, oxidative stress, protein-energy expenditure, and mortality in chronic kidney disease patients [27]. Besides, histidine is a histidine-rich glycoprotein (HRG) component that could be used for diagnosis, severity assessment, and sepsis prognosis prediction as a new biomarker [28]. We also observed elevated tryptophan concentration in the 28dS group compared to the 28dD group; tryptophan protects the liver by reducing the pro-inflammatory cytokine levels [29]. The immunomodulatory enzyme, indoleamine 2,3-dioxygenase (IDO), controls tryptophan metabolism and correlates with hypotension in human septic shock [30, 31]. The IDO-mediated tryptophan catabolism is associated with immune dysfunction and impaired microvascular reactivity in sepsis [32]. Interestingly, tryptophan's intestinal flora metabolism also seems to be related to metabolic abnormalities caused by sepsis [33].

In this research, the amino acid pathways of 90d-plasma-metabolic disorders included arginine and proline metabolisms, folate biosynthesis, and tyrosine metabolism (Fig. 5B). Arginine is a semi-essential amino acid, which plays an indispensable role in the immune reaction of sepsis [34]. The primary mediator of inflammation is inducible nitric oxide synthase (iNOS), which could produce nitric oxide (NO) from arginine. Overexpression or dysregulation of iNOS may be associated with various pathologies of sepsis [35]. Arginine deficiency in sepsis might impair the local perfusion of nitric oxide and increase the catabolic state [36]. Xu et al. stated that elevated arginine levels might be a clinical marker of sepsis in elderly patients [37]. However, our research indicated increased plasma concentrations of arginine in 90dS than in 90dD. The up-regulated arginase and urea cycle in sepsis of non-survivors inhibited the nitric oxide synthesis by the endothelium to relieve pulmonary hypertension and decrease arginine concentration [38, 39]. As a stress substrate, proline is essential for the inflammatory microenvironment [40]. As an energy storage unit, proline would be consumed excessively in sepsis, causing a sharp decline in its content. The low-proline concentration in plasma may increase oxidative stress deteriorating the disease [41, 42]. Elmassry et al. found that trans-4-hydroxyproline and four other metabolites could serve for early diagnosis of sepsis caused by *Pseudomonas aeruginosa* infection in burn patients [43]. Valine, leucine, and isoleucine, called branched-chain amino acids (BCAAs), act as substrates for energy production and protein synthesis [29]. Our study found significantly higher valine levels in 90dS than in 90dD. The benefits of BCAAs may be linked with their role as glutamine precursors. Glutamine is a critical factor in maintaining immune function and intestinal integrity, and affects the protein balance positively [44].

Fatty Acid Metabolism

Changes in fatty acid metabolism are the prominent components of different metabolomic phenotypes of survival and death in sepsis. We observed that alpha-linolenic acid metabolism was the fatty acid metabolism related to 28d-prognosis (Fig. 5A), and arachidonic acid was the fatty acid metabolism associated with 90d prognosis (Fig. 5B). Previous research has demonstrated a faster rate of fatty acid beta-oxidation and free fatty acid (FFA) clearance in non-survivors of sepsis [7, 14]. Omega-3 fatty acid supplementation could reduce the mortality of septic patients [45, 46]. Our research indicated that the plasma concentrations of alpha-linolenic acid and docosapentaenoic acid (22n-3) in the 28dS were higher than that in the 28dD. One possible reason could be the enhanced beta-oxidation of fatty acids in the death group. Second, the metabolites related to beta-oxidation might aggravate the metabolic pressure of the kidney and liver, impairing the two organs and further detoxification [47]. Third, an overactivated inflammatory reaction after sepsis causes the body to release a large amount of linolenic acid to inhibit the inflammatory reaction [48]. Arachidonic acid is a polyunsaturated omega-6 fatty acid with pro-inflammatory and anti-inflammatory activities [9, 49]. Arachidonic acid is metabolized to prostaglandins, an inflammatory medium, through cyclooxygenase-2 (COX-2). A specific inhibitor, PTUPB, could improve multiple organ dysfunction in septic mice by inhibiting COX-2 [50]. Another metabolic pathway of arachidonic acid is the production of leukotrienes (LTs) by lipoxygenase. Leukotrienes are critical inflammatory mediators that aggravate inflammatory response. To sum up, the arachidonic acid level may be associated with increased organ dysfunction and decreased survival rate in severe sepsis [51].

Prognosis Prediction Model

The differential clinical indicators and metabolites identified in our research were used to set up the multivariate logistic models to strengthen the accuracy of prognosis prediction. We found five metabolites (indoleacetic acid, 3-methylene-indolenine, L-alpha-amino-1H-pyrrole-1-hexanoic, pyrrolidine, dopamine) and six clinical indicators (HR, respiratory support, application of pressor drugs, lymph#, ALP, and SOFA) could predict the prognosis of patients with sepsis. High heart rate is a hallmark of sepsis and an important index of mortality [52]. It has been reported that HR and blood pressure (BP) dynamics of multiple time scales are independent predictors of sepsis [53]. We can say based on clinical experience that the application of pressure drugs and respiratory support indicate a poor prognosis. Research has indicated the most common cause of death was sepsis in patients with acute respiratory distress syndrome (ARDS), and 70% were on necessary respiratory support at the time of death [54]. Also, the SOFA is one of the indicators used widely by clinicians to evaluate the severity of the disease and the prognosis of critically ill patients; the score has a notable correlation with the mortality of patients with sepsis [55, 56]. A meta-analysis suggested that the ALP therapy might improve renal function and survival in patients with sepsis-related acute kidney injury [57]. Similarly, research has shown that intestinal ALP could detoxify lipopolysaccharide, a significant mediator of sepsis pathophysiology [58]. Our results showed that lymph# could be applied to predict the hospital prognosis of patients with sepsis. Earlier research has reported that neutrophil-to-lymphocyte ratio (NLR) may be a useful prognostic biomarker of septic patients, and higher the NLR value, the worse is the prognosis in these patients [59]. Dopamine inhibits cytokine production through dopamine type 1 (D1) receptor and suppresses systemic inflammatory response [60]. Feketeova et al. indicated that dopaminergic agonists might control systemic inflammation, hyperglycemia, and provide advantages for treating septic patients with diabetes [61]. Recent researches have reported a new semisynthetic glycopeptide antibiotic, eremomycin pyrrolidide (5) synthesized by eremomycin and pyrrolidine condensation, may be a promising antibiotic for further preclinical and clinical evaluation of infectious diseases, such as sepsis [62]. Our research used indoleacetic acid and 3-methylene-indolenine for the first time used to predict the prognosis of septic patients. Further research may be required to verify our findings.

Our research is not without underlying limitations. First, even though metabolomics represents the molecular phenotype of cells, it is necessary to note that it is difficult to directly compare the results of different metabolites without the risk of bias introduction due to biological and technical differences. Second, the metabolites were measured from a single biological fluid, plasma, at a single point of time, and included a relatively small number of patients. We assume that the initial variations in metabolites could predict the disease severity, while the variations in metabolites between the survival and death groups will change over time. Measuring the metabolites at multiple time points may be more beneficial in estimating the progress and prognosis of sepsis. It is expensive to carry out numerous metabolic and biochemical tests on multiple samples, such as plasma, tissue, and urine, of each patient on a larger scale; however, the exercise is essential to construct effective and beneficial sepsis prognosis models.

Conclusion

The prediction model constructed in our research using differential plasma metabolites and clinical parameters could be used for predicting the prognosis of septic patients and may provide a new idea for sepsis treatment. In this research, we found that the altered levels of plasma metabolites in patients with

sepsis were mainly related to amino acids and fatty acids metabolism, which could be considered as clues of the relevant pathophysiological mechanism. Hence, metabonomic is a promising method that could help us understand the pathophysiology of the host response. In the future, detecting multiple metabolites and clinical parameters in sepsis at different time points will hopefully help determine the prognosis and treatment of sepsis in time.

Abbreviations

ICUs, intensive care units; LC/MS, liquid chromatography /mass spectrometry; OPLS-DA, orthogonal partial least squares discriminant analysis; SOFA, sequential organ failure assessment score; APACHEII, acute physiology and chronic health scoring system score; GCS, Glasgow Coma Score; ROC, receiver operating characteristic curve; AUC, area under curve; HR, heart rate; Lymph#, lymphocyte count; ALP, alkaline phosphatase; Ala, alanine.

Declarations

Consent for publication

Not applicable.

Author Contributions

All the authors contributed substantially to the work presented in this article. TWS, XFD and RT conceived the study. DW, MC and XJZ contributed to data interpretation. GYS contributed to construct the prediction models. XFD and RT, HS contributed to the study protocol and wrote the article. YQC, HYL, JYS and TWS revised the article. The corresponding author had full access to all of the data and the final responsibility for the decision to submit this article for publication.

Availability of Data and Materials

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Ethical Approval and Consent to Participate

Yes, ethical standards statement included.

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Competing Interest

The authors have no conflicts of interest to disclose.

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Figures

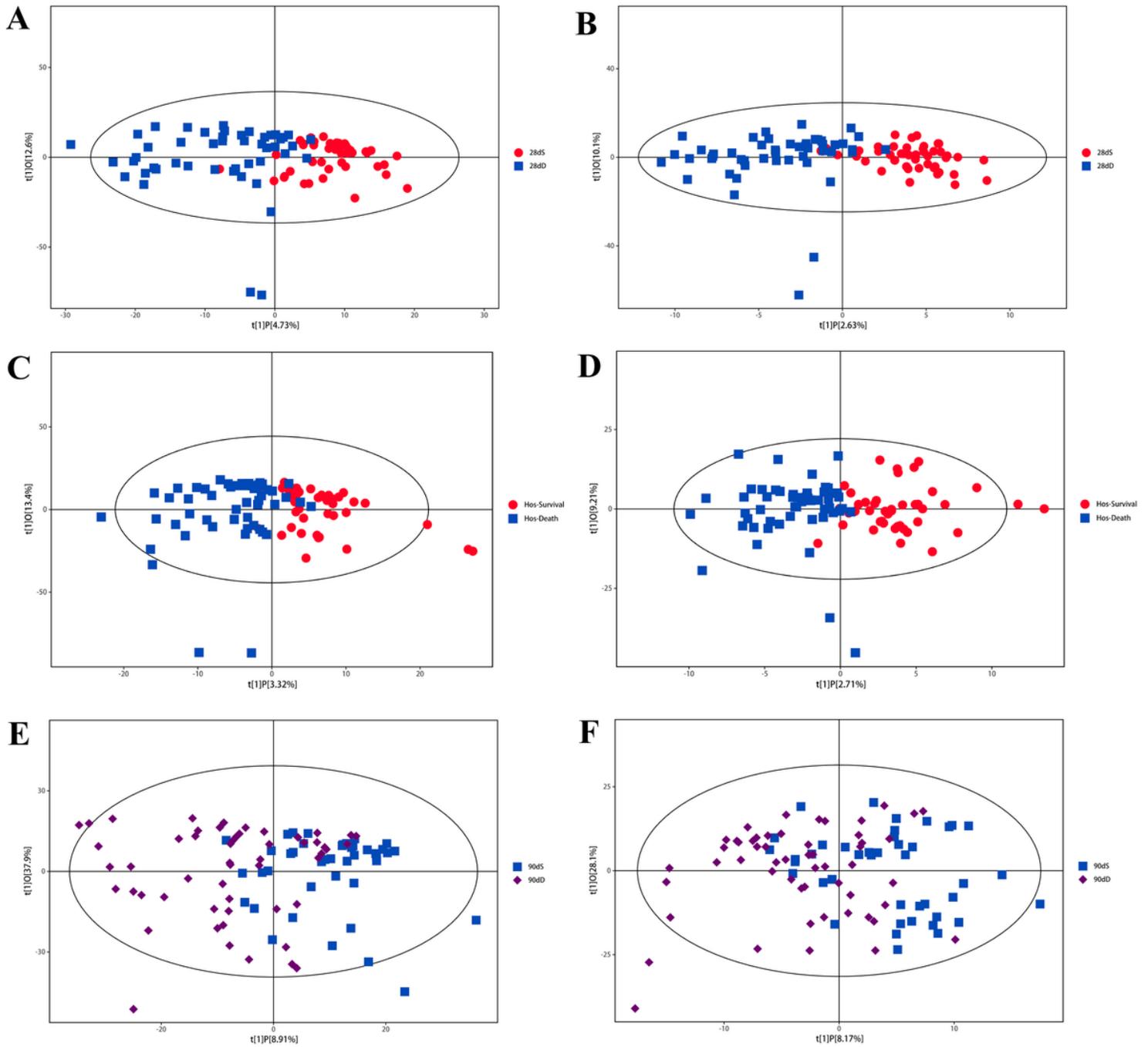


Figure 1

OPLS-DA analysis of metabolic profiles for plasma. (A, C, E) Scatter plots in positive-ion mode. (B, D, F) Scatter plots in negative-ion mode. Scatter plots. (A, B) between 28dS and 28dD groups; (C, D) between HOS-survival and HOS-death groups; and (E, F) between 90dS and 90dD groups.



Figure 2

Relative intensities of major differential metabolites in plasma (A) between 28dS and 28dD groups; (B) between HOS-survival and HOS-death groups; and (C) between 90dS and 90dD groups. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

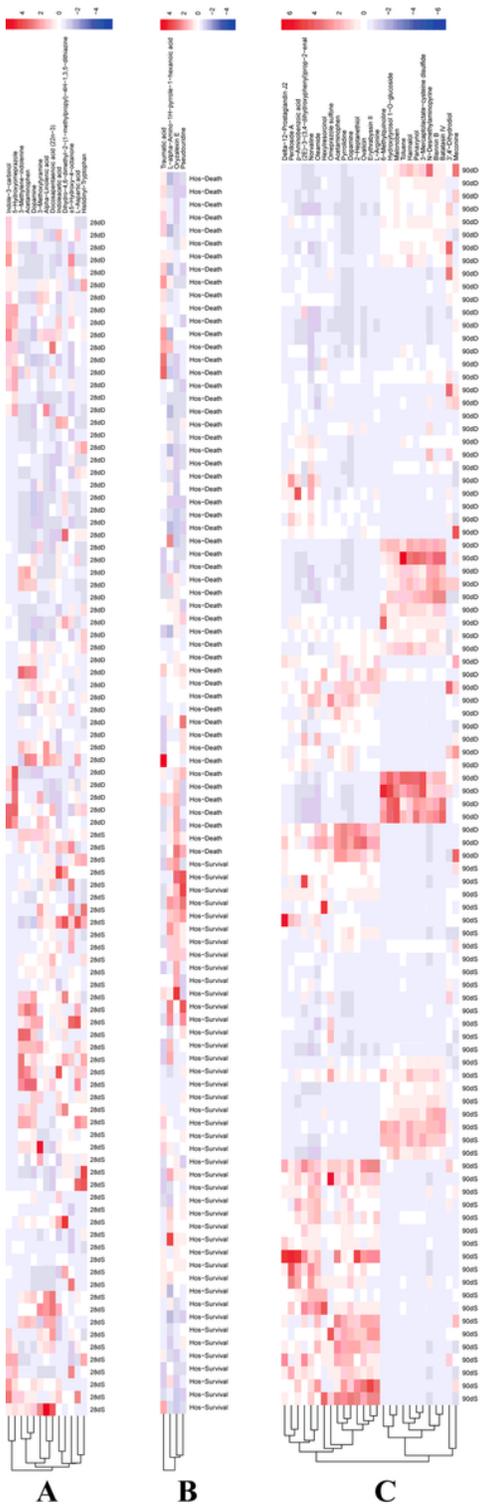


Figure 3

Heat maps of major differential metabolites in plasma (A) between 28dS and 28dD groups; (B) between HOS-survival and HOS-death groups; and (C) between 90dS and 90dD groups.

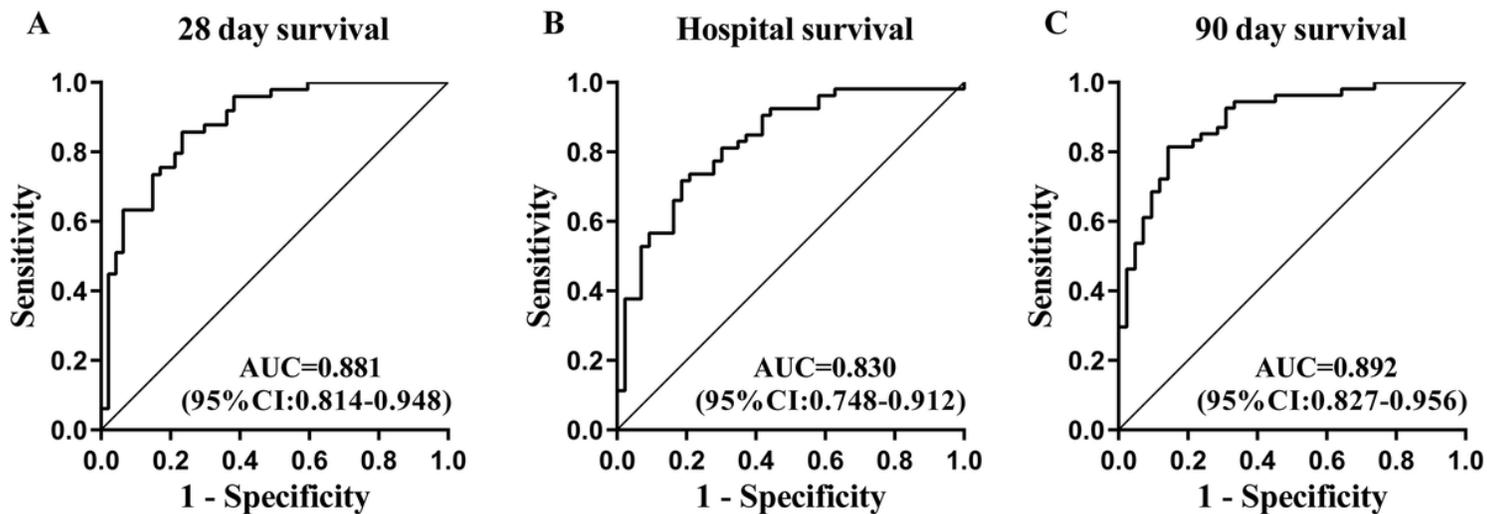


Figure 4

The receiver operating characteristics (ROC) curve for predicting (A) 28d, (B) hospital, and (C) 90d prognosis in patients with sepsis.

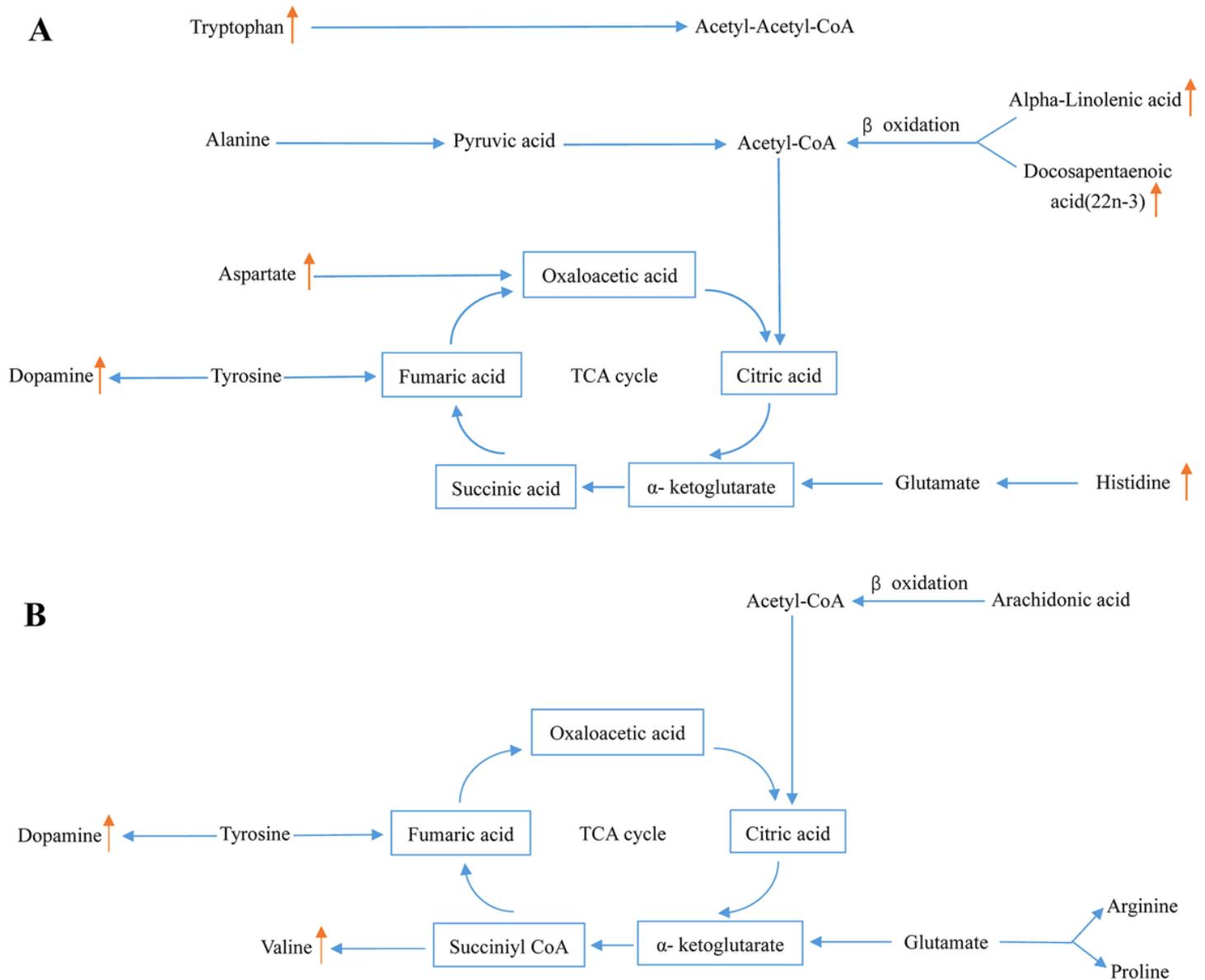


Figure 5

Metabolic network maps in plasma at (A) 28d, and (B) at 90d. The red arrow indicates an increased concentration of the metabolite in the survival group compared to the death group.

Supplementary Files

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