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Original Article

Prognostic biomarkers for sepsis mortality based on the literature and LC-MS-based metabolomics of sepsis patients

Shi Feng[1,2,3,4*], Nannan Cui[5*], Wenjun Zhao[1,2,3,4], Haige Zhao[6], Cuili Wang[1,2,3,4], Junnan Zheng[5], Tingting Zhu[1,2,3,4], Jianghua Chen[1,2,3,4], Hong Jiang[1,2,3,4], Qun Su[5]

[1]Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang, China; [2]Key Laboratory of Nephropathy, Zhejiang University, Hangzhou 310003, Zhejiang, China; [3]Institute of Nephropathy, Zhejiang University, Hangzhou 310003, Zhejiang, China; [4]Zhejiang Clinical Research Center of Kidney and Urinary System Disease, Zhejiang University, Hangzhou 310003, Zhejiang, China; [5]Department of ICU, The First Affiliated Hospital, Zhejiang University, Hangzhou 310003, Zhejiang, China; [6]Department of Cardiothoracic Surgery, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang, China. [*]Equal contributors and co-first authors.

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Abstract: Objectives: The management of sepsis, a potentially lethal overreaction to infection, is limited by the lack of prognostic tools to guide its treatment. Our aim is to identify a novel metabolic biomarker panel for predicting sepsis mortality based on a literature review and liquid chromatography-mass spectrometry (LC-MS)-based metabolomics. Methods: In the literature, we found metabolomics biomarkers reported to predict sepsis mortality. We determined the classifications, reported frequency, and KEGG pathway enrichment of these markers. Using serum samples from 20 sepsis survivors and 20 non-survivors within 28 days after admission to the intensive care unit (ICU), we performed LC-MS-based metabolomics. Based on the literature review and metabolomics, a prognostic biomarker panel for sepsis was identified and its area under the curve (AUC) values was assessed. Results: Kynurenate, caffeine, and lysoPC 22:4 were selected as a prognostic biomarker panel for sepsis. The panel had an area under the curve (AUC) of 0.885 (95% CI, 0.694-1) evaluated by linear support vector machine (SVM) and 0.849 (0.699-1) by random forest (RF), which was higher than that of the Sequential Organ Failure Assessment (SOFA). A combination of kynurenate, caffeine, and lysoPC 22:4 and SOFA provided the best discriminating performance, with AUCs of 0.961 (0.878-1) for SVM and 0.916 (0.774-1) for RF. Conclusions: The prognostic biomarker panel consisting of kynurenate, caffeine, and lysoPC 22:4 may aid in the identification of sepsis patients at a high risk of death, leading to personalized therapy in clinical practice that will improve sepsis survival.

Keywords: Sepsis, metabolomics, prognosis

Introduction

Sepsis, a life-threatening condition caused by the dysregulated host response to infection, is a major global public health concern [1]. Despite advances in clinical treatment, mortality and morbidity from sepsis are high, with a great global economic cost [2-4]. Early diagnosis, accurate prognosis, and timely and appropriate therapies are essential for the successful management of sepsis. However, identifying patients with a high risk of progression is still a formidable challenge [5, 6]. Many prognostic biomarkers have been proposed in the past decade, but none are specific and sensitive enough for clinical use [7]. Therefore, there is a pressing need to identify specific and sensitive biomarkers for predicting sepsis.

The technological advances in metabolomics have led to an increased interest in identifying metabolic biomarkers of sepsis. Metabolomics, the systematic identification and quantification of small metabolites, is a powerful tool to identify the modulators of biological processes and novel disease biomarkers [8]. Unlike the genome or the proteome, the metabolome directly reflects biochemical activities and cel-

lular phenotypes. Metabolites can report on the physiological and pathological processes of the body and also serve as regulators by interacting with and modulating the activity of other molecules [9, 10]. Several metabolomics studies have identified alterations in metabolic profiles in sepsis and reported many prognostic biomarkers [11, 12]. Although changes in various metabolites in plasma or serum, including carbohydrates, lipids, and amino acids, are associated with sepsis progression [13], these novel sepsis biomarkers have not been used in a clinical setting because they lack well-designed validation studies.

Here we reviewed the literature and summarized the blood-based biomarkers of sepsis mortality. Using liquid chromatography-mass spectrometry (LC-MS)-metabolomics, we also identified metabolites that were differentially expressed in sepsis survivors vs non-survivors. Combining the results of the literature review and LC-MS-based metabolomics, we created a metabolic biomarker panel for predicting 28-day mortality in sepsis patients. We aimed to identify previously reported metabolic biomarkers and the pathways associated with sepsis mortality and to develop a new metabolic biomarker panel to enhance our understanding and improve prognostication of the disease.

Methods

Literature review

Search strategy: Literature searches of MEDLINE (PubMed), EMBASE, Web of Science, and the Cochrane Library were conducted until July 14, 2022. Database-specific subject headings and text word synonyms for the subjects of sepsis and metabolomics were used. The references of the identified articles were also examined. The detailed search strings can be found in the Supplementary Methods.

Inclusion and exclusion criteria: Studies that identified blood concentrations of metabolites by metabolomic profiling in septic patients and assessed their associations with sepsis mortality were included. Reviews, editorials, conference abstracts, and studies on drug therapy response were excluded.

Data extraction and pathway analysis: Two reviewers (SQ and ZW) extracted data independently from eligible studies, including information on study design (year of publication, study region, analytical platform, sample type, and validation method), population characteristics (number of cases, gender and age distribution, mortality, and follow-up time), and significant differences. The major metabolites identified in these studies were summarized. The pathway enrichment analysis was performed using the software MBROLE 2.0 (http://csbg.cnb.csic.es/mbrole2/index.php).

LC-MS-based metabolomics

Patients and sample collection: Serum samples were provided by 20 sepsis survivors and 20 non-survivors within 28 days of intensive care unit (ICU) admission. These patients were enrolled at the First Affiliated Hospital, College of Medicine, Zhejiang University, between 1 March 2020 and 30 May 2021. Sepsis was diagnosed according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [1]. Serum samples were obtained before clinical intervention in the first 24 h following admission. Clinical data at the time of admission were also collected. The research was approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. Written informed consent was obtained from all enrolled participants.

Metabolite extraction and MS analysis: Serum samples were thawed and each sample was mixed thoroughly before combining an equal aliquot from each patient sample to create the pooled QC sample. Multiple aliquots of the QC sample were then prepared exactly as were the patient samples and used for injection to monitor the stability of the instrument and avoid bias. Patient samples and QC samples were extracted using 80% methanol by vortexing for 1 min, and centrifuging for 15 min at 16,000 × g at 4°C. The patient sample supernatants were randomized and equal volumes were placed in vials. The same volume of the QC sample supernatants was placed in vials before the patient samples and after every 10 patient samples to assess the stability of the instrument. Samples were examined using a TripleTOF 5600 Plus high-resolution tandem mass spectrometer (SCIEX, UK). The chromatographic separation was carried out using ultra-performance liquid chromatography (UPLC) equip-

ment (SCIEX, UK). For reversed-phase separation, an ACQUITY UPLC T3 column (100 mm × 2.1 mm, 1.8 m, Waters, UK) was used. A mobile phase containing solvent A (water, 0.1% formic acid) and solvent B (acetonitrile, 0.1% formic acid) was added to separate the metabolites. The gradient elution conditions were 5% solvent B for 0-0.5 minutes, 5%-100% solvent B for 0.5-7 minutes, 100% solvent B for 7-8 minutes, 100%-5% solvent B for 8-8.1 minutes, and 5% percent solvent B for 8.1-10 minutes, all with a flow rate of 0.4 ml/min. The column was held at a constant temperature of 35°C. The TripleTOF 5600 Plus instrument was used to detect the metabolites eluted from the column. The ion source gases 1 and 2 were kept at 60 pounds per square inch (PSI), while the curtain gas pressure was adjusted to 30 PSI. The interface heater reached a temperature of 650°C. The ion spray floating voltage of the positive-ion mode was set at 5 kV, while the negative-ion mode was set at 4.5 kV. Information-dependent acquisition (IDA) mode was used to acquire MS data. The mass range of the TOF instrument was 60-1200 Da. Every 150 ms, survey scans were conducted, and if the threshold of 100 counts/s was exceeded with a 1+ charge state, up to 12 product ion scans were obtained. The total cycle time was set to 0.56 s. Monitoring the 40 GHz multichannel thermal conductivity (TDC) detector with four anode/channel detection totaled four-time bins for each scan at an 11 kHz pulse frequency. The duration of the dynamic exclusion was set to 4 s. For every 20 samples, the mass accuracy was calibrated during the acquisition procedure. For every ten samples, a QC sample was evaluated to assess LC stability.

Metabolomics data processing: The LC-MS data were preprocessed using the XCMS program. Before analysis with R package XCMS, CAMERA, and metaX toolboxes, the raw data files were transformed to mzXML format. All the information on retention duration and m/z was used to identify each ion. The intensity of each peak was determined, and a three-dimensional matrix was created with randomly assigned peak indices (retention time/z pairings), sample names (observations), and ion intensity data (variables). The information was then compared to internal and public databases. The metabolites were annotated by matching the exact molecular mass data (m/z) to those in the database within a 10-ppm threshold using the open-access databases KEGG and the Human Metabolome Database (HMDB). The peak intensity data was further preprocessed with MetaX. To improve data quality, characteristics that were detected in 50% of QC samples and 80% of test samples were removed, and missing peak values were extrapolated using the nearest neighbor technique. Using the preprocessed dataset, we used principal component analysis (PCA) to identify outliers and batch effects. The QC data were fitted with a robust QC-based locally estimated scatterplot smoothing (LOESS) signal correction that suggested the order of injection to minimize signal intensity drift over time. We determined the relative standard deviations of the metabolic parameters across all QC samples, and we eliminated those with standard deviations greater than 30%. Before the analysis, the group datasets were standardized. The probabilistic quotient normalization procedure was used to normalize all the samples. Then, we performed QC-robust spline batch correction using the QC samples. The *P*-values for the metabolites selected were calculated using student t-tests and then adjusted for multiple tests using a false discovery rate (FDR) (Benjamini-Hochberg). We conducted supervised partial least squares discriminant analysis (PLS-DA) using metaX to identify specific differences between the groups using the discriminant profiling statistical method. We used the variable important of projection (VIP) cutoff value of 1.0 to choose important characteristics. The pathway enrichment analysis was performed using the software MBROLE 2.0 (http://csbg.cnb.csic.es/mbrole2/index.php).

Creation and validation of a prognostic biomarker panel: We identified metabolites and KEGG pathways that were common to both the reviewed literature and our LC-MS-based metabolomics. These shared metabolites and differential metabolites belonging to shared KEGG pathways were defined as the metabolites selected by the literature review. Next, the top 10 features discriminating sepsis survivors and non-survivors were selected using random forest (RF) or linear support vector machine (SVM). The metabolites that were common to those selected by the literature review and the top 10 features selected by the two algorithms were chosen as the potential prognostic bio-

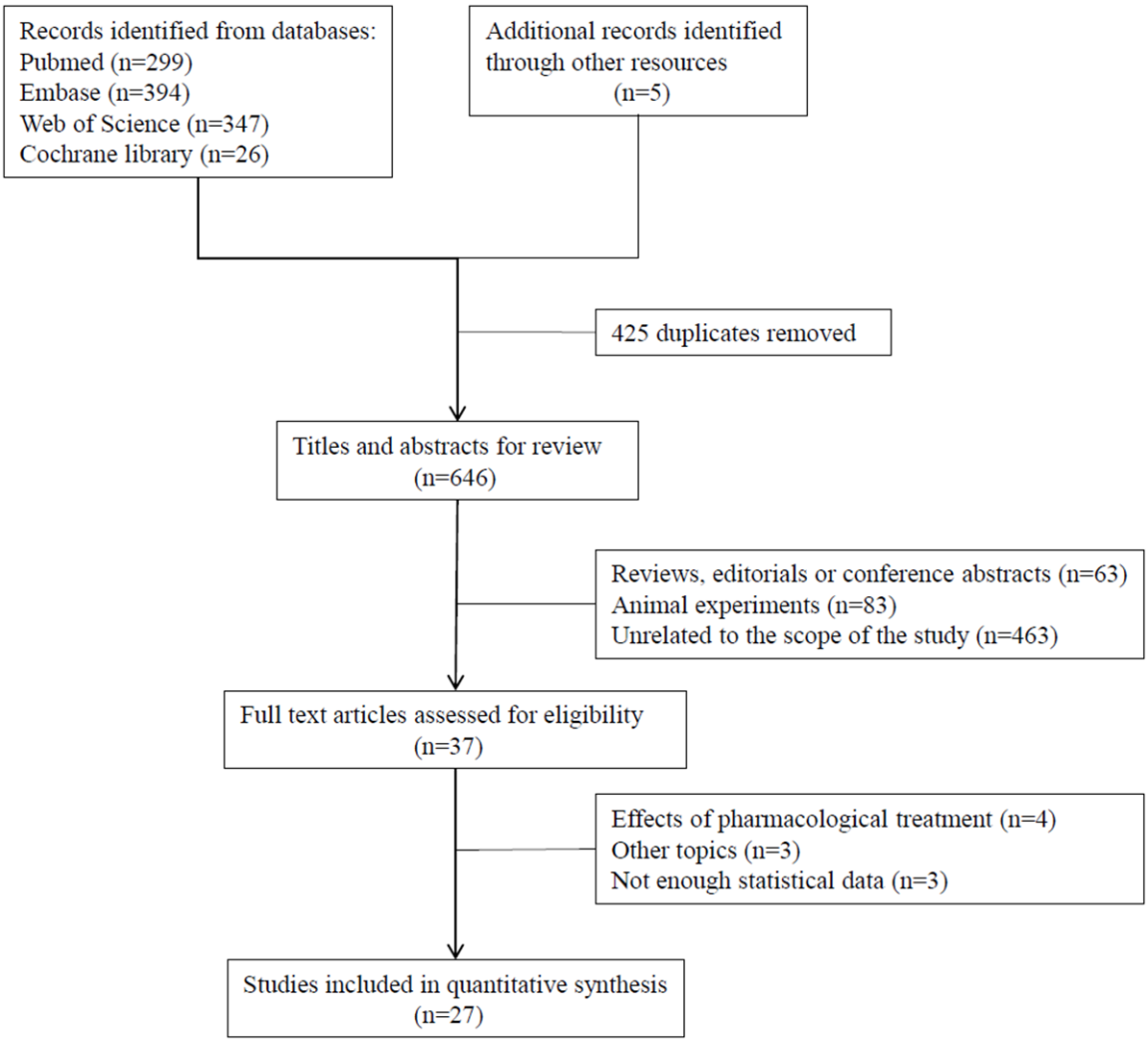

Figure 1. Flow diagram of literature search and study selection process.

marker panel. Receiver operating characteristic (ROC) analyses to assess the predictive value of this panel generated a ROC curve that was based on Monte Carlo cross-validation of SVM and RF models. The above analyses were performed by MetaboAnalyst 4.0 (https://www.metaboanalyst.ca).

Results

Literature review

Characteristics of the included studies: After an eligibility assessment of the 1071 articles we identified in our initial search, 27 were included in our study (**Figure 1**). **Table 1** gives an overview of the characteristics of the 27 studies, each with 16-234 cases of sepsis mortality, that had reported blood-based metabolic biomarkers, with 18 studies that used plasma samples and 9 that used serum samples. Metabolite detection methods included MS-based metabolomics (n = 21) and nuclear magnetic resonance (NMR) (n = 6).

Metabolic biomarkers in sepsis mortality: Most of the potential metabolic biomarkers predicting the survival outcome of sepsis identified in these studies were lipids and lipid-like molecules (n = 66) and organic acids and derivatives (n = 47) at the superclass level (Supplementary Table 1). Lipids and lipid-like molecules were mainly fatty acyls (n = 28) and glycerophospholipids (n = 26), and the majority of organic acids and derivatives were carboxylic acids and their derivatives (n = 39) (**Figure 2A**). We also

Table 1. Characteristics of the included studies

PMID	Year	Study region	Analytical platform	Metabolite targets	Biological sample	Patient no. (F/M)	Age (years, median range, or SD) (NS vs S)	Population	Follow-up time	Validation
12562829	2003	Germany	LC-MS/MS	Targeted	Plasma	102 (31/71)	54.9 (17-80) vs 53.8 (20-91)	NS (n = 39) vs S (n = 63)	30 days	NA
23673400	2013	USA	LC-LTQ-orbitrap-MS and DSQ GC-MS	Untargeted	Plasma	30 (14/16)	79 (76-82) vs 78 (73-83)	NS (n = 15) vs S (n = 15)	90 days	mouse model
23884467	2014	USA	LC-Q-orbitrap-MS, DSQ GC-MS and LC-MS/MS	Untargeted and targeted	Plasma	121 (51/70)	68.8 ± 16.7 vs 56.4 ± 19.2	NS (n = 31) vs S (n = 90)	28 days	NS (n = 18) vs S (n = 34) NS (n = 36) vs S (n = 25)
	2014	USA	LC-Q-orbitrap-MS, DSQ GC-MS and LC-MS/MS			52 (18/34)	58.0 ± 18.8 vs 58.9 ±18.1	NS (n = 18) vs S (n = 34)		
	2014	USA	LC-Q-orbitrap-MS, DSQ GC-MS and LC-MS/MS			61 (28/33)	58.7 ± 16.5 vs 54.8 ± 13.1	NS (n = 36) vs S (n = 25)		
24498130	2014	USA	LC-Q-orbitrap-MS and DSQ GC-MS	Untargeted	Plasma	90 (51/39)	58 ± 15 vs 53 ± 14	NS (n = 34) vs S (n = 115)	28 days	NA
25553245	2014	China	LC-Q-orbitrap MS	Untargeted	Serum	35 (5/30)	67 ± 15 vs 63 ± 18	NS (n = 9) vs S (n = 26)	48 hours	NA
25849571	2015	China	LC-MS/MS	Targeted	Serum	35 (10/25)	61 ± 21 vs 54 ± 23	NS (n = 15) vs S (n = 20)	28 days	NA
25928796	2015	Canada	NMR	Targeted	Serum	16	63 (59.8-77)	NS (n = 8) vs S (n = 8)	NA	NA
26847922	2016	Italy	LC-MS/MS	Targeted	Plasma	20 (7/13)	69.9 ± 12 vs 61.3 ± 15.2	NS (n = 9) vs S (n = 11) [NS (n = 11) vs S (n = 9)]	28 days	NA
27406941	2017	USA	LC-Q-orbitrap-MS and DSQ GC-MS	Untargeted	Plasma	58	NA	NS (n = 30) vs S (n = 28)	28 days	NA
27614981	2016	France	LC-Q-orbitrap-MS	Untargeted	Serum	50 (23/27)	11.3 ± 0.9 vs 10.0 ± 0.8	NS (n = 29) vs S (n = 21)	7 days	NA
27632672	2017	USA	LC-MS/MS	Targeted	Plasma	22 (9/13)	60 (36-80) vs 60 (27-84)	NS (n = 9) vs S (n = 13)	Hospital stay	NA
28345042	2017	USA	LC-MS/MS	Targeted	Plasma	121 (51/70)	68.8 ± 16.7 vs 56.4 ± 19.2	NS (n = 31) vs S (n = 90)	28 days	mouse model
	2017	USA	LC-MS/MS			36 (9/27)	58 ± 16.7 vs 52 ± 21.5	NS (n = 16) vs S (n = 20)	28 days	mouse model
30379669	2019	China	UHPLC-MS	Targeted	Plasma	90 (36/54)	71.1 ± 14.8	NS (n = 21) vs S (n = 69)	28 days	NS (n = 24) vs S (n = 96)
	2019	China	UHPLC-MS			120 (40/80)	70.0 ± 14.2	NS (n = 24) vs S (n = 96)	28 days	NS (n = 24) vs S (n = 96)
31088568	2019	France	NMR	Untargeted	Serum	70 (30/40)	72.1 ± 0.4 vs 68.5 ± 0.3	NS (n = 30) vs S (n = 40)	7 days	NA
32075299	2020	Finland	1H NMR	Targeted	Serum	44 (15/29)	71 (61-75) vs 61 (55 to 67)	NS (n = 11) vs S (n = 33)	30 days	NA
32290837	2020	China	LC-MS/MS	Targeted	Plasma	188 (69/119)	67 ± 14.4 vs 61.8 ± 18.5	NS (n = 54) vs S (n = 134)	28 days	NA
32712289	2020	Poland	LC-MS/MS	Targeted	Serum	20 (12/8)	68.1 (56-76) vs 68.5 (48-86)	NS (n = 7) vs S (n = 13)	5 days	NA
33304464	2020	UK	LC-MS/MS	Targeted	Plasma	20 (4/16)	68 ± 13 vs 68 ± 16	NS (n = 8) vs S (n = 12)	ICU stay	NA
33868224	2021	China	LC-MS	Untargeted	Plasma	18 (5/13)	58.33 ± 11.5 vs 56.75 ± 14.03	NS (n = 6) vs S (n = 12)	7 days	NA
34345827	2021	USA	UHPLC-MS	Untargeted	Plasma	197 (90/107)	NA	NS (n = 74) vs S (n = 123)	60 days	NA
34460840	2021	Mexico	LC-MS/MS and DI-MS/MS	Untargeted	Plasma	45 (16/29)	58 (46-64)	NS (n = 28) vs S (n = 17)	Hospital stay	NA
34578983	2021	Austria	NMR	Untargeted and targeted	Plasma	53 (21/32)	66 (50-75)	28 days: NS (n = 25) vs S (n = 28)	28 days	NA
34578983	2021	Austria	NMR	Untargeted and targeted	Plasma	53 (21/32)	66 (50-75)	ICU stay: NS (n = 19) vs S (n = 34)	ICU stay	NA
34620961	2021	Poland	LC–MS/MS	Targeted	Serum	15 (8/7)	64.7 (56-72) vs 65.8 (48-84)	NS (n = 4) vs S (n = 11)	ICU stay	NA
35176448	2022	India	NMR	Targeted	Serum	31 (8/23)	42 ± 17.3 vs 46.7 ± 13.7	NS (n = 17) vs S (n = 14)	7 days	NA
35576846	2022	China	LC-MS	Untargeted	Plasma	96 (39/57)	57.41 ± 2.17 vs 50.34 ± 2.90	NS (n = 49) vs S (n = 47)	28 days	NA
							55.55 ± 2.26 vs 51.98 ± 2.98	NS (n = 53) vs S (n = 43)	Hospital stay	NA
							56.52 ± 2.14 vs 50.64 ± 3.09	NS (n = 54) vs S (n = 47)	90 days	NA
35710638	2022	USA	LC/MS and GC/MS	Targeted	Plasma	60 (27/33)	62 (48-67) vs 53 (46-63)	NS (n = 25) vs S (n = 35)	28 days	NA

Abbreviations: S, survivors; NS, non-survivors; NA, not available.

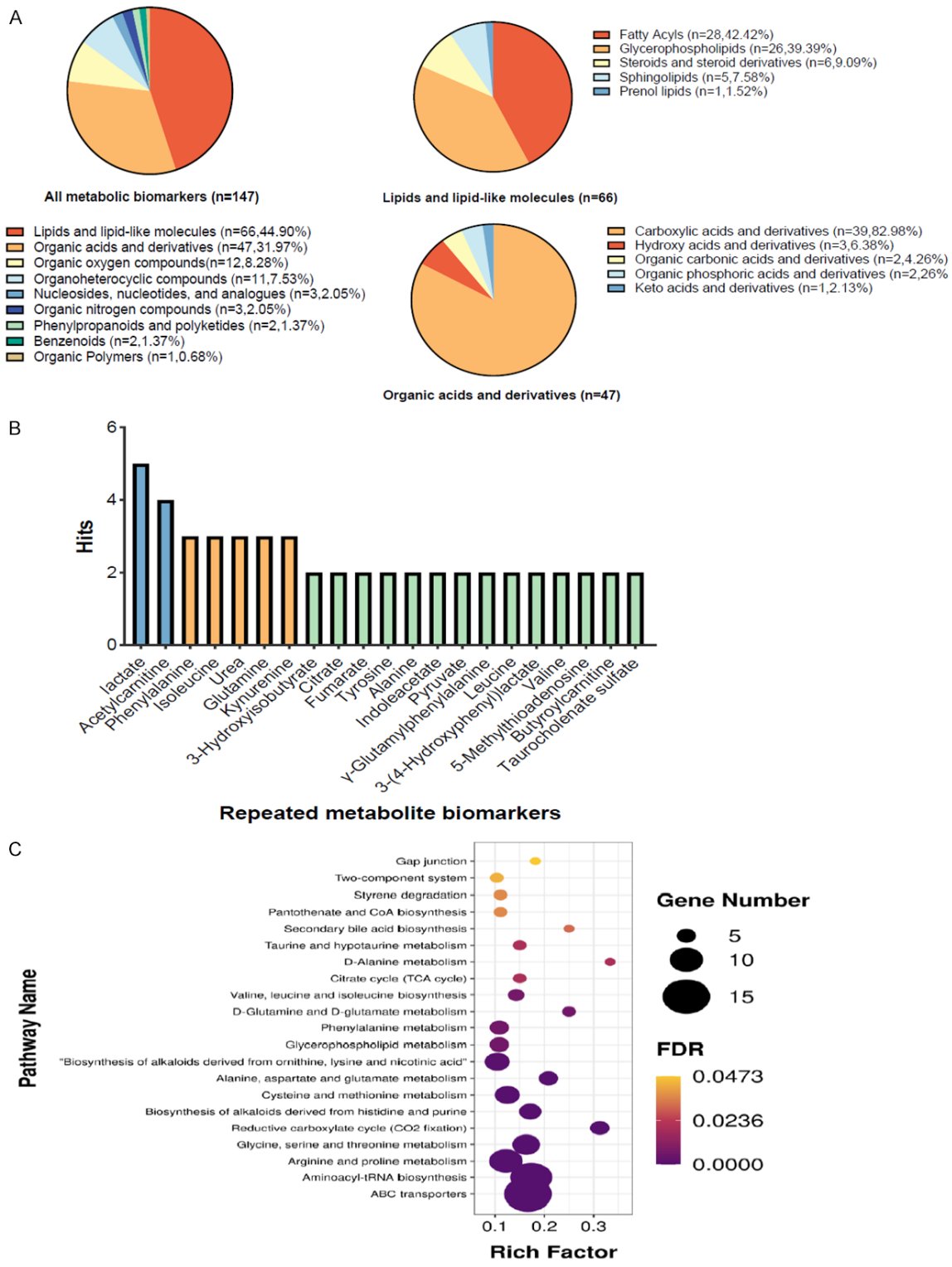

Figure 2. Descriptions of differentially expressed metabolites in the included studies. A. Classification of reported metabolic biomarkers by superclass level (left) and class level (right). B. Metabolic biomarkers reported in two or more studies. C. KEGG pathway analysis of reported metabolic biomarkers. Rich factor, the ratio of the number of reported genes enriched in the pathway to the number of annotated genes. Q-value, *P*-value after the multiple hypothesis test correction. FDR, false discovery rate.

Table 2. Characteristics of sepsis survivors and non-survivors

Characteristic[a]	non-survivor	survivor	*P* value
Patients, No.	20	20	
Age, years	72 (57-76)	63 (53-69)	0.292
Male	12.0 (60.0%)	13.0 (65.0%)	1
APACHE II	21 (15-23)	17 (12-20)	0.0628
SOFA	9.5 (7.8-12)	5.0 (3.0-7.0)	0.001
WBC	13 (7.9-15)	13 (8.3-16)	0.827
Neutrophil %	88 (85-94)	89 (85-91)	0.342
Lymphocyte %	5.7 (2.7-9.7)	5.5 (4.5-9.5)	0.526
Monocytes %	3.2 (2.1-6.0)	3.7 (2.5-5.6)	0.923
Heart failure	1.00 (5.0%)	3.00 (15.0%)	0.598
CKD	7.00 (35.0%)	3.00 (15.0%)	0.273
COPD	1.00 (5.0%)	0 (0%)	1
Diabetes	5.00 (25.0%)	4.00 (20.0%)	1
Hypertension	8.00 (40.0%)	11.0 (55.0%)	0.527
Smoke	11.0 (55.0%)	8.00 (40.0%)	0.527
CRP	91 (45-150)	58 (28-110)	0.384
PCT	1.9 (0.41-2.8)	0.94 (0.54-2.1)	0.666

[a]Continuous variables are expressed as mean ± standard deviation or median (25th percentile-75th percentile). Categorical variables are expressed as number (percent). Abbreviations: APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; WBC, white blood cell count; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; PCT, procalcitonin.

assessed overlap among the markers and found 21 metabolites were identified in two or more studies. The biomarkers reported most often were lactate and acetylcarnitine, with a frequency of five and four, respectively (**Figure 2B**). A KEGG pathway enrichment analysis for all the markers showed that 31 pathways were enriched at a *P*-value of 0.05 (Supplementary Table 2), and 21 had a rich factor > 0.1 (**Figure 2C**).

LC-MS-based metabolomics

Clinical samples: Serum samples were collected from 40 sepsis patients-20 sepsis survivors and 20 non-survivors. **Table 2** shows baseline demographics and clinical characteristics for each group. The sequential organ failure assessment (SOFA) score in the non-survival group was significantly higher ($P = 0.001$) than in the survival group. The two groups were well-balanced with respect to other variables.

Untargeted metabolite profiling and the reliability of the system: We measured global metabolic changes using untargeted metabolomics (n = 20). To assess the quality of the MS data, we created total ion chromatograms (TICs) for the samples and found a considerable overlap, which indicated the stability of the analytical system (Supplementary Figure 1A). We used the m/z width and retention-time width to verify that the instrument status and the sample preparation instrument status satisfied our requirements (Supplementary Figure 1B). A total of 24,098 annotated compounds were found in the serum samples, and 11,630 secondary metabolites in positive and negative-ion modes were identified in the Human Metabolome Database HMDB (Supplementary Figure 1C, 1D).

Metabolites differentially expressed between sepsis survivors and non-survivors: To reduce data dimensionality and explore sample grouping, we created a PCA model and a partial least squares discriminant analysis (PLS-DA) (**Figure 3A**). PLS-DA analysis revealed a clear separation between survivors and non-survivors with a $Q^2 = 0.35$. This was validated by 200 permutations and was trustworthy without overfitting (Supplementary Figure 2). Further, we found greater dispersion of samples from non-survivors vs survivors. This may result from the variety of pathogenic bacteria that cause sepsis, thereby leading to the dysfunction of various organs.

We used fold change (FC) (FC ≥ 2 or ≤ 1/2), the *P*-value of t-tests ($P < 0.05$), and VIP scores (VIP > 1) to identify metabolites that were differentially expressed between the two groups. We found 420 substantially different features in the positive mode and 192 in the negative mode (**Figure 3B**, **3C**). These metabolites were enriched in 10 KEGG pathways (FDR < 0.05), with the greatest number of differentially expressed metabolites identified in the glycerophospholipid metabolism pathway (**Figure 3D**).

Biomarker panel for predicting sepsis mortality: To find a biomarker panel for predicting sepsis outcomes, we combined the results of the literature review with our LC-MS-based metabolomics data. We first identified the differentially expressed metabolites and KEGG path-

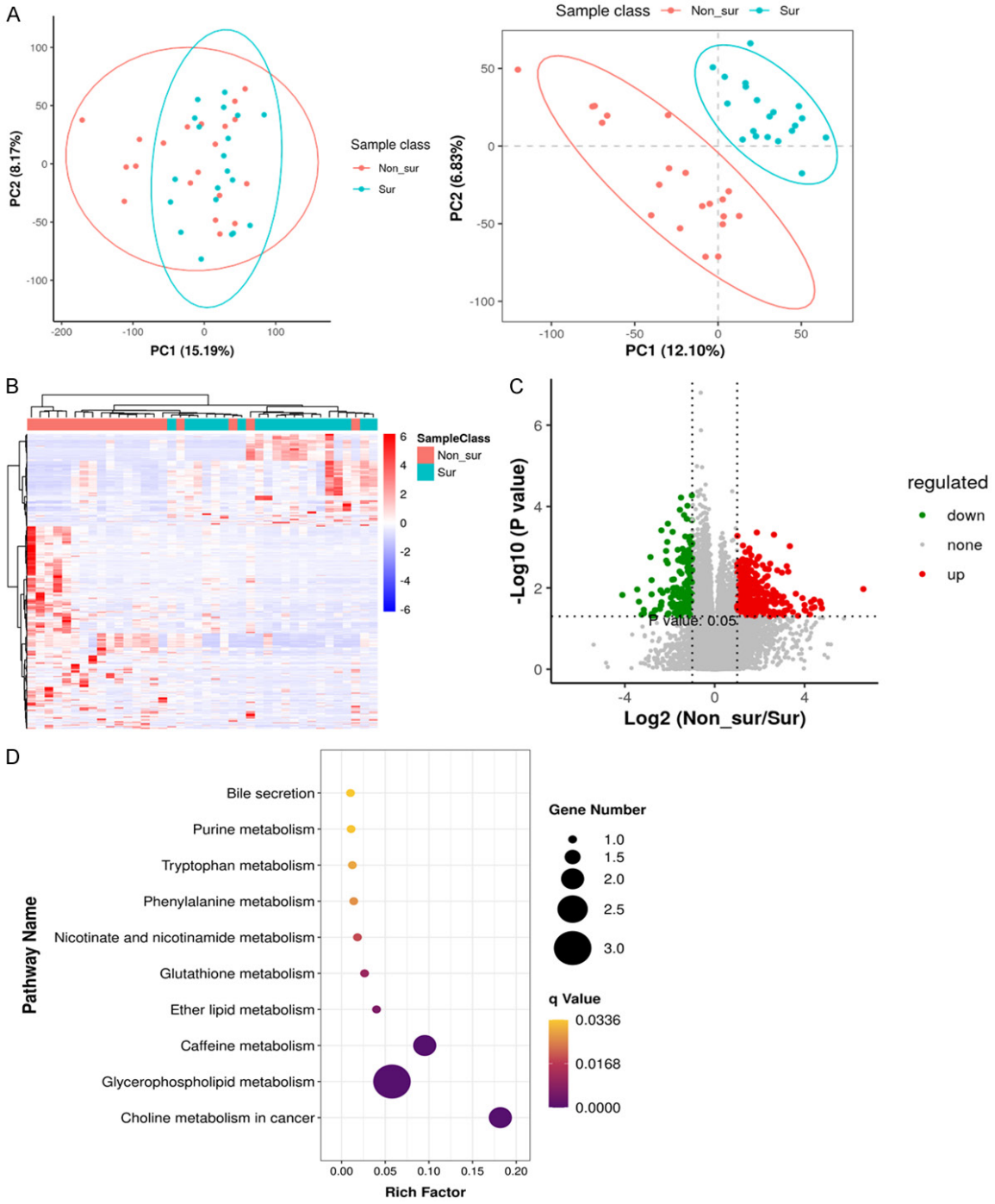

Figure 3. Untargeted metabolomics conducted in sepsis survivors and non-survivors. A. Principal component analysis (PCA; left) and partial least squares discriminant analysis (PLS-DA) demonstrating the distinction between sepsis survivors (Sur, blue dots) and non-survivors (Non-sur, red dots). B. Heatmap of differences in metabolites between survivors and non-survivors. C. Volcano plot of differences in metabolites between survivors and non-survivors. D. KEGG pathway analysis of differentially expressed metabolites between survivors and non-survivors.

ways shared between the literature review results and the metabolomics results of our cohort (Supplementary Table 3). We found that four differentially expressed metabolites, glycerophosphocholine, pyroglutamic acid, kynurenate, and caffeine, that we identified in our study were also reported previously. Phenylalanine metabolism and glycerophospholipid

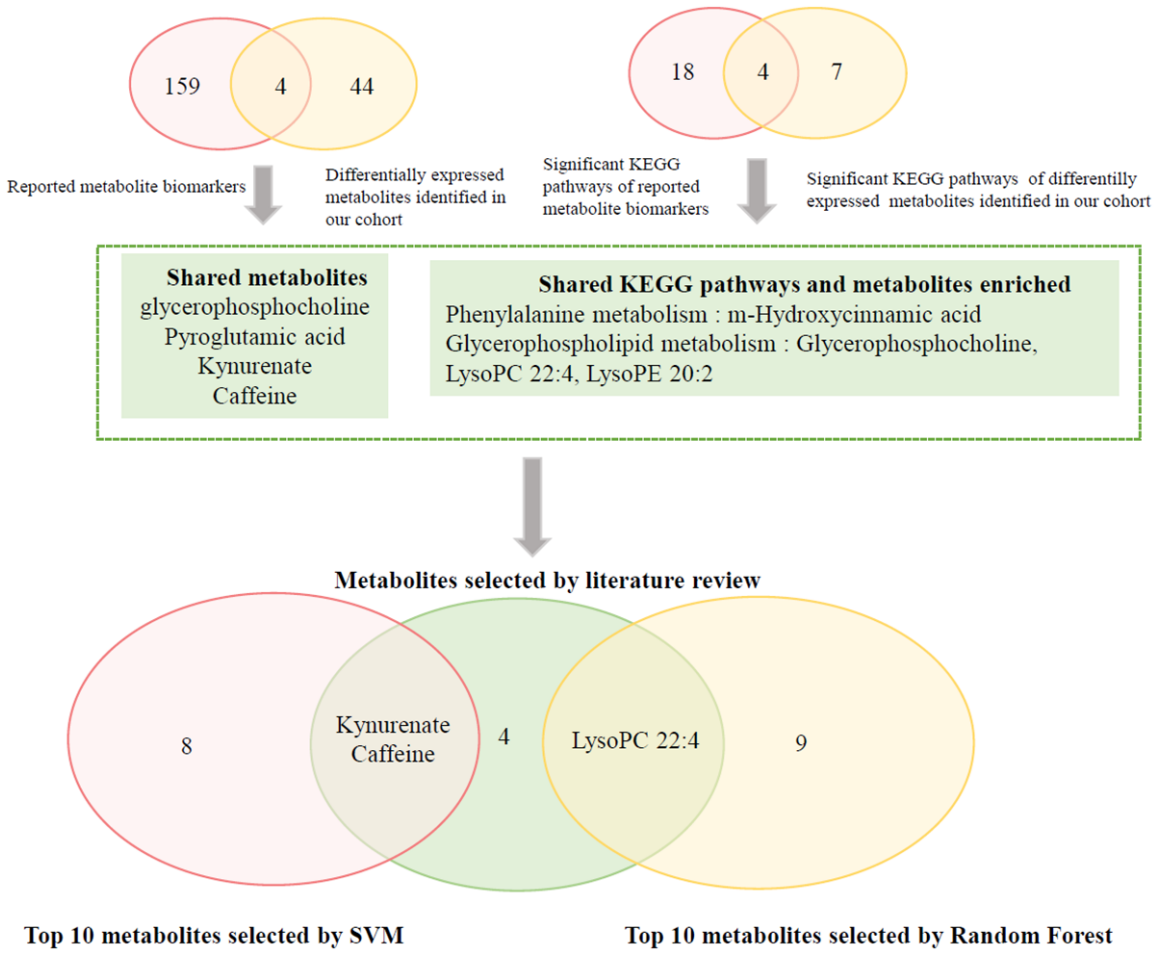

Figure 4. Identification of potential prognostic metabolic biomarkers. Prognostic metabolic biomarkers were chosen from the overlap between metabolites identified by the literature review and metabolites selected by linear support vector machine (SVM) and random forest (RF).

metabolism were the two KEGG pathways enriched in both the literature review and our metabolomics results. The differentially expressed metabolites m-hydroxycinnamic acid, glycerophosphocholine, lysoPC 22:4, and lysoPE 20:2 are involved in these two pathways. We chose seven metabolites based on the literature review. Using RF and SVM, we selected the top 10 features discriminating sepsis survivors and non-survivors (Supplementary Figure 3). Based on the overlap between the metabolites identified by the literature review and the top 10 features selected by the two algorithms, we identified kynurenate, caffeine, and lysoPC 22:4 as the potential prognostic biomarker panel (**Figures 4**, **5A**).

The predictive performance of this biomarker panel was evaluated by the SVM and RF algorithms using ROC curves and determining the areas under the receiver operating characteristic curves (AUC). The prognostic metabolite panel showed good predictive performance as assessed by SVM (AUC = 0.885, [95% CI], 0.694-1) or RF (AUC = 0.849, 0.699-1). A combination of the prognostic metabolite panel and the traditional biomarker SOFA score showed the highest AUC value of 0.961 (0.878-1) for SVM and 0.916 (0.774-1) for RF (**Figure 5B**, **5C**).

Discussion

Sepsis is a global healthcare problem affecting millions of people [14]. Metabolomics offers a promising approach for identifying novel metabolic sepsis biomarkers that could provide risk stratification, thereby improving clinical decision-making in sepsis. In this study, 27 research articles on sepsis were reviewed to identify potential metabolic biomarkers for predicting

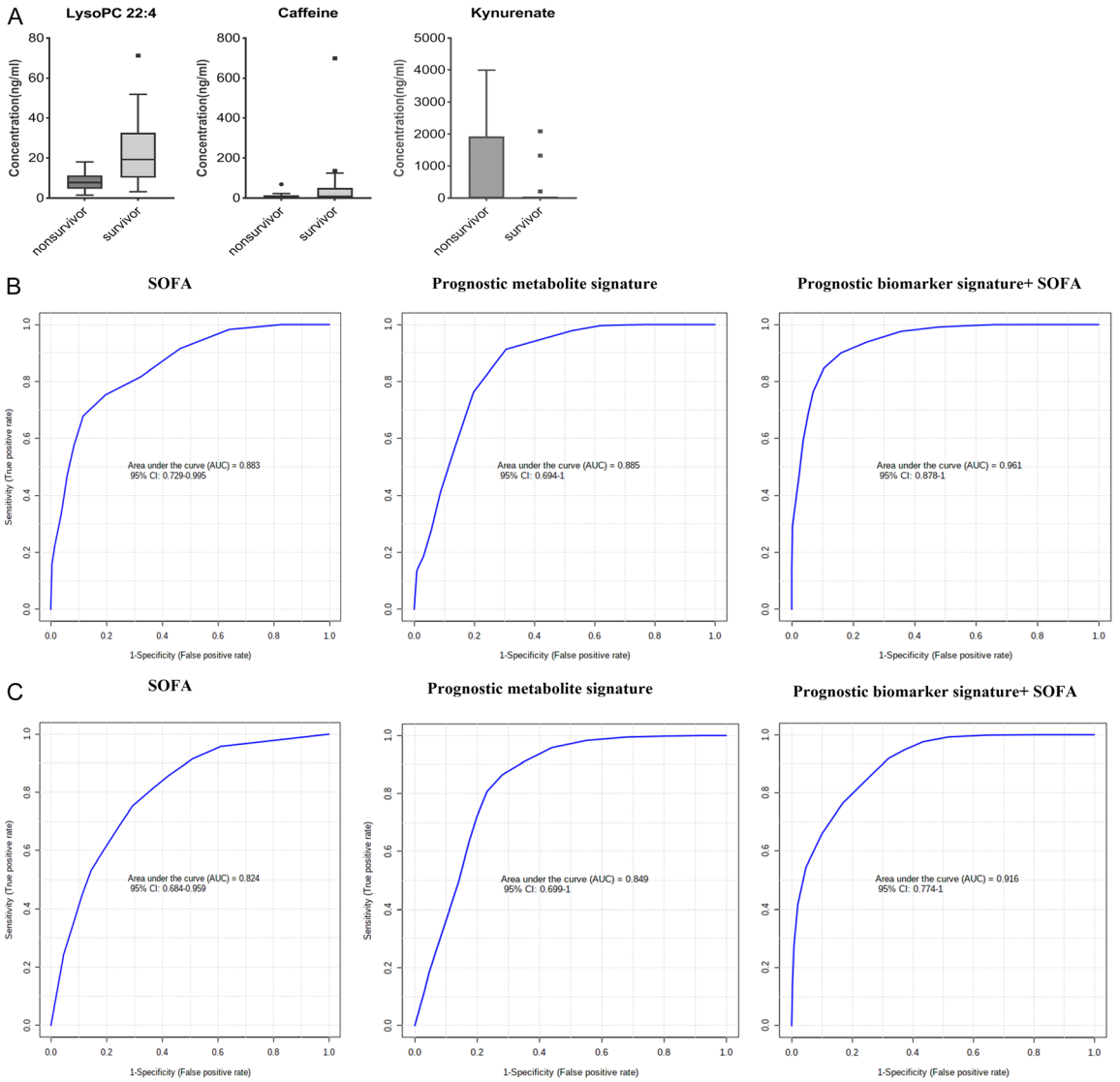

Figure 5. Serum levels of prognostic biomarkers and ROC analyses of the prognostic biomarker panel. A. Serum levels of the three metabolites selected for predicting sepsis mortality. B. Receiver operating characteristic (ROC) curves generated by linear support vector machine (SVM) showed the predicted performance of biomarkers for sepsis mortality. C. ROC curves generated by random forest (RF) showed the predicted performance of biomarkers for sepsis mortality. SOFA, sequential organ failure assessment.

sepsis mortality. Across these studies, we identified common biomarkers and several metabolic pathways enriched for differential biomarkers. Our untargeted metabolic profiling results using plasma samples from sepsis patients also identified unique metabolic signatures in sepsis survivors vs non-survivors. Based on these results, we identified a prognostic biomarker panel that showed good discriminating power for predicting sepsis mortality.

In 2020, Wang et al. [13] conducted the first meta-analysis of metabolomics for sepsis mortality prediction, which included 16 studies published before July 2019 and identified 122 metabolic biomarkers. However, in the last three years, there has been a large increase in the number of biomarkers identified by metabolomics. Our reappraisal of the literature showed that most metabolic biomarkers are lipids and lipid-like molecules (n = 66) and organic acids and derivatives (n = 47). Although the meta-analysis by Wang et al. [13] showed little overlap in the reported metabolites across studies, by analyzing more recent studies, we found several common biomarkers. Lactate (n

= 5) and acetylcarnitine (n = 4) were the most frequently reported metabolic biomarkers, followed by phenylalanine, isoleucine, urea, glutamine, and kynurenine (n = 3). The biomarkers were enriched in some metabolic pathways, with energy metabolism and amino acid metabolism showing the greatest difference between sepsis survivors and non-survivors (**Figure 2C**). Therefore, a poor outcome from sepsis might be associated with dysregulated energy production and uncontrolled proteolysis.

Based on the literature review and our metabolomics data from the 40 sepsis patients, we selected lysoPC 22:4, kynurenate, and caffeine as the candidates for the prognostic panel for sepsis mortality prediction. Kynurenate is a degradation product of tryptophan, an important modulator of the immune response [15], and kynurenate plasma levels may predict sepsis in critically ill patients [16]. Increased plasma kynurenate concentrations may also be associated with long-term mortality in patients with sepsis and pneumonia [17]. In septic shock patients with acute kidney injury treated by continuous venovenous haemofiltration, failure to reduce kynurenate levels correlated with death [18]. Consistent with the literature, we found higher levels of kynurenate in sepsis non-survivor patients, further supporting its prognostic value in sepsis mortality. Decreased levels of lysoPC species have also been found in sepsis non-survivors [19, 20], with lysoPC (16:1) and lysoPC (24:0) reported as potential predictive markers of sepsis mortality [13, 21]. Their effect may be explained by an excessive immune response caused by low lysoPC levels. We also found that five lysoPC were significantly decreased in sepsis non-survivors (Supplementary Table 1), with lysoPC (22:4) providing the best prognostic value for outcomes in sepsis patients. The prognostic panel showed better performance than the SOFA scoring system in predicting 28-day mortality in sepsis. However, because the combination of the SOFA score and our metabolite panel provided a higher AUC, this panel may increase the prognostic value of traditional clinical indicators of sepsis.

Our study was limited by methodological heterogeneity and incomplete quantitative data on metabolites, so we could not provide a quantitative meta-analysis of published studies, and the results of our literature review may have had some bias. Also, the retrospective and single-center nature of our sepsis patient metabolomics data may have introduced some selection bias. Therefore, the clinical role of these metabolites and the efficacy of the prognostic panel require further validation using large independent cohorts.

Conclusions

We identified frequently reported metabolites and metabolic pathways associated with sepsis mortality in published studies. We also conducted LC-MS-based metabolomics analysis on a patient cohort to determine the relationship between serum levels of metabolites and 28-day sepsis mortality. From these results, we identified a prognostic panel providing strong performance in classifying sepsis survivors and non-survivors. This work may add to our understanding of the pathogenesis of sepsis-induced death, offer a prognostic tool, and aid in identifying therapeutic targets of sepsis.

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Disclosure of conflict of interest

None.

Address correspondence to: Hong Jiang, Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang, China. Tel: +86-57187236992; Fax: +86-57187236189; E-mail: jianghong961106@zju.edu.cn; Qun Su, Department of ICU, The First Affiliated Hospital, Zhejiang University, No. 79, Qingchun Road, Hangzhou 310003, Zhejiang, China. Tel: +86-57187236992; Fax: +86-57187236189; E-mail: sqicu@zju.edu.cn

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Supplementary Methods

Search strategies used in databases

Pubmed-July, 14, 2022

("Sepsis" [Mesh] OR "shock, septic" [MeSH] OR sepsis [tiab] OR Pyemia* [tiab] OR Pyohemia* [tiab] OR Septicemia* [tiab] OR Blood Poisoning* OR Bacteremia [tiab] OR Viremia [tiab] OR Fungemia [tiab] OR Candidemia [tiab] OR Endotoxemia [tiab] OR Parasitemia [tiab] OR Urosepsis [tiab]) AND ("Metabolomics" [MeSH] OR "Metabolome" [MeSH] OR Metabolomic OR Metabolomics OR Metabonomic OR Metabonomics) AND (blood [tiab] OR serum [tiab] OR plasma [tiab])

Records identified: 299

Embase-July, 14, 2022

('sepsis'/exp OR 'septic shock'/exp OR sepsis: ti, ab, kw OR pyemia*: ti, ab, kw OR pyohemia*: ti, ab, kw OR septicemia*: ti, ab, kw OR 'blood poisoning*': ti, ab, kw OR bacteremia: ti, ab, kw OR viremia: ti, ab, kw OR fungemia: ti, ab, kw OR candidemia: ti, ab, kw OR endotoxemia: ti, ab, kw OR parasitemia: ti,ab,kw OR urosepsis: ti, ab, kw) AND ('metabolomics'/exp OR 'metabolome'/exp OR metabolomic: ti, ab, kw OR metabolomics: ti, ab, kw OR metabonomic: ti, ab, kw OR metabonomics: ti, ab, kw) AND (blood: ti, ab, kw OR serum: ti, ab, kw OR plasma: ti, ab, kw)

Records identified: 394

Web of Science-July, 14, 2022

((TS = (Sepsis OR Pyemia* OR Pyohemia* OR Septicemia* OR Blood Poisoning* OR Bacteremia OR Viremia OR Fungemia OR Candidemia OR Endotoxemia OR Parasitemia OR Urosepsis)) AND ALL = (Metabolomic OR Metabolomics OR Metabolome OR Metabonomic OR Metabonomics)) AND TS = (Blood OR Serum OR Plasma)

Records identified: 347

Cochrane library-July, 14, 2022

#1 MeSH descriptor: [Sepsis] this term only

#2 MeSH descriptor: [Shock, Septic] this term only

#3 (sepsis OR pyohemia* OR septicemia* OR blood posioning* OR Bacteremia OR Viremia OR Fungemia OR Candidemia OR Endotoxemia OR Parasitemia OR Urosepsis): ti, ab, kw

#4 MeSH descriptor: [Metabolomics] this term only

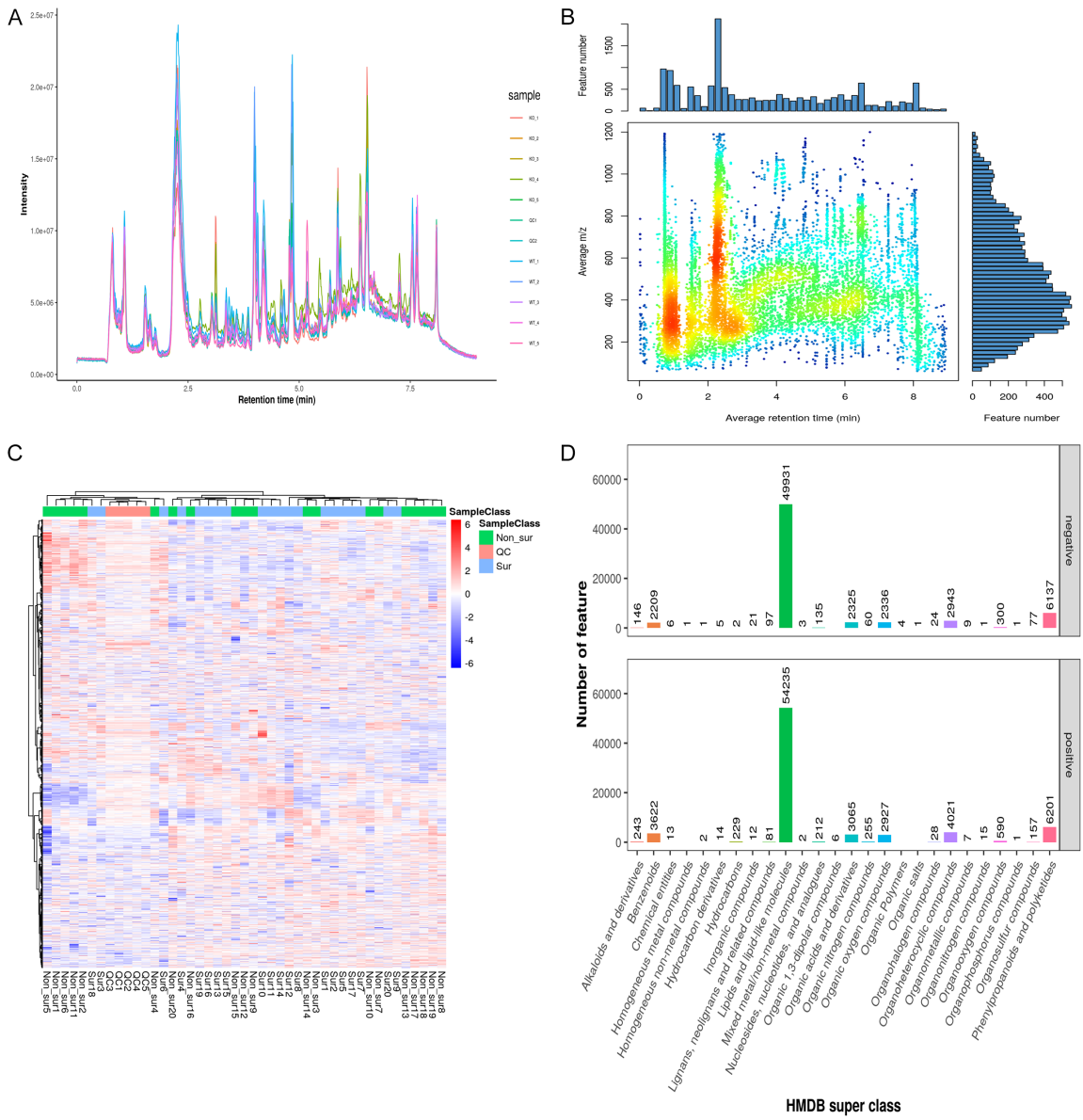
#5 MeSH descriptor: [Metabolome] this term only

#6 Metabolomic OR Metabolomics OR Metabonomic OR metabonomics

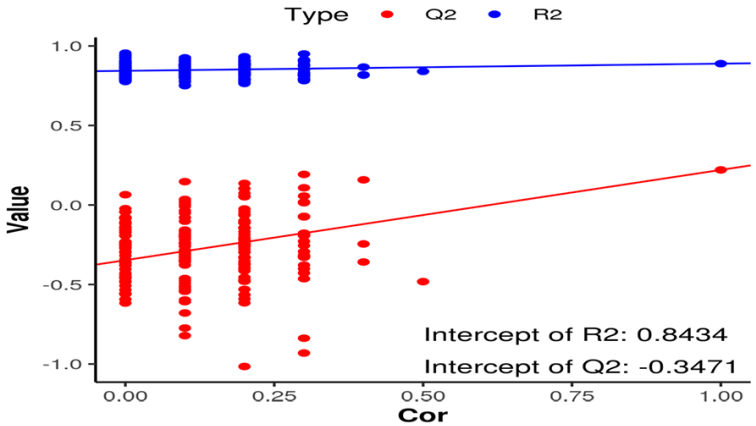
#7 (blood OR serum OR plasm): ti, ab, kw

#8 (#1 OR #2 OR #3) AND (#4 OR #5 OR #6) AND #7

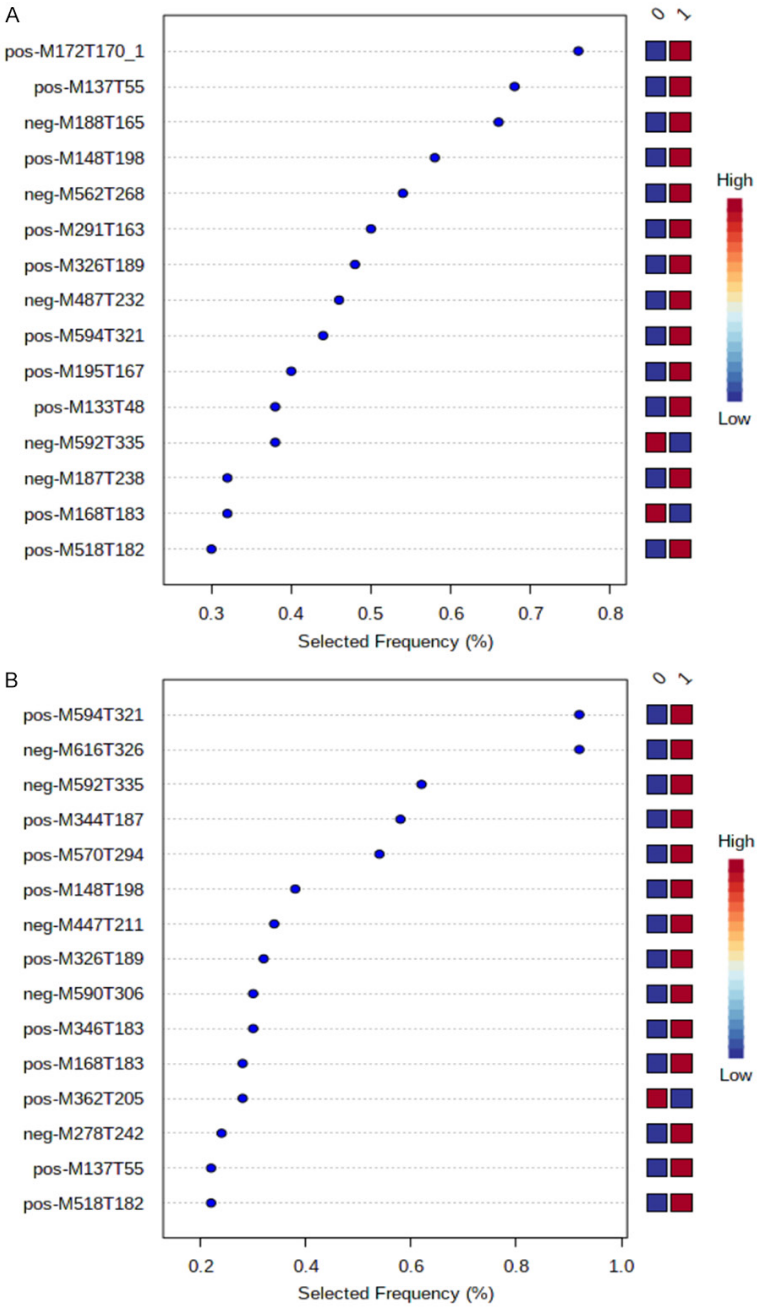
Records identified: 26



Supplementary Figure 1. The distinguishing metabolites between sepsis survivors and non-survivors.



Supplementary Figure 2. The PLS-DA model's validation plot reveals that it is not over-fitting.



Supplementary Figure 3. The most important features classifying sepsis survivors and non-survivors.