Biomeditsinskaya Khimiya, 2025 vol. 71, issue 6, pp. 441–453.

CLINICAL-DIAGNOSTIC STUDIES

METABOLOMIC PROFILING OF PATIENTS WITH SEPSIS-ASSOCIATED ENCEPHALOPATHY

E.D. Kessenikh[1], K.M. Bykova[1], E.A. Murashko[1]*, Ya.A. Dubrovskii[1], V.V. Dorofeykov[1,2], I.A. Savvina[1,3]

[1]Almazov National Medical Research Centre,
2 Akkuratova str., Saint Petersburg, 197341 Russia; *e-mail: kate.murashko@xmail.ru
[2]Lesgaft National State University of Physical Education, Sport and Health,
35 Dekabristov str., St. Petersburg, 190121 Russia
[3]North-Western State Medical University named after I.I. Mechnikov,
41 Kirochnaya str., Saint-Petersburg, 191015 Russia

Sepsis-associated encephalopathy (SAE) is a condition characterized by acute brain dysfunction developed in the absence of a primary infection in the central nervous system. The aim of this study was to perform a pilot, untargeted metabolomic profiling of the blood plasma of SAE patients to identify metabolic changes potentially associated with the pathological condition and to generate hypotheses for further studies of its pathogenesis, as well as to the search for promising biomarkers, and the assessment of the severity of the patient's condition. Metabolomic profiling was performed using HPLC-HR-MS, followed by statistical analysis of the obtained data. This blinded, randomized, controlled clinical trial revealed significant differences in the metabolic profiles of the study and control groups. Functional analysis showed the metabolic pathways most affected by pathological processes in SAE patients. These included metabolism of acylcarnitines, lysophosphatidylcholines, and taurine, folate biosynthesis, and the drug metabolism involving the cytochrome P450 pathway. In SAE patients with impaired consciousness, including delirium and coma, decreased levels of long-chain acylcarnitines and lysophosphatidylcholines were observed. The metabolomic profiles of SAE patients differed significantly between the groups of deceased and surviving patients: concentrations of sulfur-containing amino acids were significantly lower in the group of deceased than in the group of survivors. Our study identified 64 candidate biomarkers that could potentially be used to predict sepsis outcomes. However, further study is needed using an expanded and independent cohort of patients.

Keywords: metabolomics; metabolites; sepsis-associated encephalopathy; sepsis

DOI: 10.18097/PBMCR1599

INTRODUCTION

Sepsis-associated encephalopathy (SAE) is an acute brain dysfunction occurring in the absence of a primary source of infection in the central nervous system (CNS) [1]. In recent years, advanced metabolite profiling of body fluids such as serum and plasma, cerebrospinal fluid, and urine, known as metabolomics, has emerged as a powerful and promising tool for identification of new biomarkers or “metabolic fingerprints” [2]. In critically ill sepsis patients numerous studies identified abnormal metabolic pathways, including amino acid metabolism, fatty acid oxidation, phospholipid metabolism, glycolysis, and the tricarboxylic acid cycle [3–5]. In our study, we explored how informative the initial biochemical profile of the disease before the initiation of specific sepsis therapy or its changes in SAE patients might be.

Metabolomics is a branch of omics science that provides information about small-molecule chemical compounds in human biological samples. Metabolomics aims to qualitatively and quantitatively evaluate low-molecular-weight compounds (less than 1500 Da) found in a studied biological system and enables the identification and quantification of metabolites present in a biological sample. The metabolome can be considered a product of cellular biological regulatory processes involving the genome, epigenome, transcriptome, and proteome, as well as environmental factors.

Metabolomics employs three approaches: the targeted approach quantifies and identifies a limited group of metabolites; the semi-targeted approach is used to evaluate a specific class of metabolites (e.g., amino acids, organic acids, or acylcarnitines); the untargeted approach is aimed at identification and quantification of all metabolites in a sample using single-platform or multi-platform approaches [6].

Targeted and untargeted metabolomics approaches can be used to identify metabolic fingerprints, representing limited sets of metabolites that distinguish phenotypes and can be used as multi-biomarkers for various diseases, for patient stratification, and for monitoring disease progression or

© 2025 by the authors. Licensee IBMC, Moscow, Russia. This article is an open access and distributed under the terms and conditions of the Creative Commons Attribution (CC BY-SA 4.0) license (http://creativecommons.org/licenses/by-sa/4.0/).

treatment efficacy [7]. A targeted study is limited to a specific compound or class of compounds, whereas untargeted analysis provides comprehensive chemical characterization of a complex biochemical system [8].

The most widely used analytical techniques in metabolomics studies are gas chromatography (GC) or liquid chromatography (HPLC) coupled with mass spectrometry (MS). The choice of the optimal metabolomics analysis strategy depends on the studied object, biological matrix, physicochemical properties of the detected metabolites, their stability, the sample preparation methods used in the laboratory, and the analytical characteristics of the equipment used.

The aim of our pilot study was to identify plasma metabolites in SAE patients before the initiation of sepsis-specific therapy as candidate biomarkers for further analysis of metabolic pathways associated with disease pathogenesis.

MATERIALS AND METHODS

This single-center, prospective, blinded, randomized, controlled clinical trial, conducted from April 2022 to December 2023, included 10 patients with a confirmed diagnosis of sepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3, 2016) (study group) and 10 volunteers (control group), matched for age, gender, and comorbidities with the study group (Table 1). The non-inclusion criteria for the study were a history of CNS infection, chronic intoxication (alcohol, drugs), viral hepatitis B or C, HIV infection, and current pregnancy. The exclusion criteria were the development of acute cerebrovascular accident and focal CNS infection during treatment of a patient included in the study. The primary sources of infection were nosocomial pneumonia (n = 5; 50%) and secondary peritonitis (n = 5; 50%). Patients in both groups received comparable long-term therapy for cardiovascular diseases, including β-blockers, ACE inhibitors, antiplatelet agents, diuretics, and gastroprotectors. Blood plasma samples for metabolomic analysis were collected on the first day of sepsis diagnosis and before the start of sepsis-specific therapy. Blood was collected into vacuum tubes containing EDTA anticoagulant, and samples were centrifuged for 15 min at 1600 *g* at room temperature. Plasma was aliquotted and stored at -80°C. Thus, both groups were comparable in terms of underlying medical conditions and background therapy, minimizing the influence of chronic diseases on intergroup differences in metabolomic profiles.

Preparation of Plasma Samples for HPLC-MS Analysis

Plasma samples prepared for analysis were gently thawed on ice. Quality control (QC) samples were prepared by pooling identical aliquots of all samples selected for metabolomic analysis. Plasma was deproteinized with ice-cold acetonitrile at a ratio of 1:3 and then centrifuged at 10,000 *g* for 10 min. For the HPLC-MS metabolomics study, 100 μl of supernatant from each of the obtained extracts, including quality control samples and a negative control sample (blank), were collected.

Untargeted Metabolomics Study

The metabolomics study of plasma samples from patients with sepsis was performed using high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS). An Elute UHPLC liquid chromatograph (Bruker Daltonics, Germany) was used for chromatographic separation in reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) modes using Intensity Solo 1.8 C18-2 100×2.1 mm analytical columns with an Acquity BEH C18 1.7 μm precolumn (Bruker Daltonics) and SeQuant® ZIC-cHILIC 3 μm, 100×2.1 mm precolumn with a ZIC-cHILIC 3 μm, 20×2.1 mm precolumn (Supelco

Table 1. Characteristics of patients with SAE and the control group

Parameter	Patients of the main group with SAE (n = 10)	Patients of the control group (n = 10)
Age, years	63.5±8.2	64.5±7.9
Females, n	4	4
Males, n	6	6
BMI, kg/m^2	27.5±3.2	25.5±4.5
Hypertension, n	9 (90%)	9 (90%)
IHD, n	8 (80%)	7 (70%)
DM 2, n	5 (50%)	4 (40%)
Heart rhythm disturbances, n	6 (60%)	5 (50%)
Digestive system diseases, n	8 (80%)	7 (70%)
Lung diseases, n	5 (50%)	4 (40%)

Abbreviations: BMI – body mass index; IHD – ischemic heart disease; DM 2 – Diabetes mellitus type 2.

Analytical Products, USA). A Q-TOF Maxis Impact high-resolution time-of-flight mass spectrometer (Bruker Daltonics) with atmospheric pressure electrospray ionization was used to detect metabolites in the samples. Mass spectra were acquired in positive ion mode over a scan range of *m/z* 50–1300. The ESI source parameters were set as follows: dry gas flow — 6.0 L/min; nebulizer pressure — 26 psi; dry gas temperature — 220°C; capillary voltage — 4500 V; scan rate — 2 Hz.

Statistical Data Processing

Data preprocessing, normalization, statistical analysis, and annotation were performed using Compass DataAnalysis software (Bruker Daltonics), ProteoWizard MSConvert, and the Metaboanalyst 5.0 online platform. The following steps were performed during the data preprocessing procedure: *m/z* detection, smoothing, chromatographic resolution, isotope filtering and exclusion, combining and aligning the resulting data set, filling missing values with half the minimum nonzero value within each variable, normalization by the median for each sample followed by reduction to the overall median, and $\log_2$ normalization. Variables were filtered according to three quality criteria: repeatability using QC samples (RSD% ≤ 30%), interindividual variability (RSD > 20% for samples), and prevalence in the data set as the ratio of the mean variable value to its median in QC ≤ 1.5.

Comparisons between study groups were performed using a two-tailed Student's *t*-test and the Mann-Whitney U-test. Effect sizes were assessed using the fold change in mean metabolite values across groups ($\log_2$(FC)) and Cohen's d. Multiple comparisons were controlled using the Benjamini-Hochberg method (FDR, q = 0.05).

Principal component analysis (PCA) was used to reduce data dimensionality, along with partial least squares discriminant analysis (PLS-DA), orthogonal projections onto latent structures (OPLS-DA), heatmapping, and a random forest classification algorithm.

Data Quality Control

The quality of the metabolomic data was assessed based on the reproducibility of quality control samples (n QC = 5), which represented a pooled sample of patient plasma. Figure 1 shows the results of a principal component analysis (PCA) of the study groups.

The quality control sample group demonstrated high reproducibility, suggesting high data quality in the metabolomics experiment.

The technical quality of the data was assessed using the coefficient of variation (CV) for all detected metabolites. For RP and HILIC chromatography, the median CVs were 17.8% and 16.1%, respectively, with 54.8% and 57.9% of metabolites having CVs < 20%, and 69.9% and 70.5% having CVs < 30%. These values confirm good data quality and reproducibility of the measurements.

A sum-of-squares (SS) decomposition revealed that for most metabolites (>50%), the proportion of variance explained by group membership (SAE vs. control) was 6.4% and 10.2% for RP and HILIC HPLC-MS analysis, respectively. Despite the limited sample size, a source of variation analysis revealed that for 21.2% (RP) and 32.5% (HILIC) of metabolites, the group factor explained more than 30% of the total variance. For 6.4% and 10.2% of metabolites, the group factor's contribution exceeded 50%, indicating the presence of stable and significant differences between groups even with a limited number of observations.

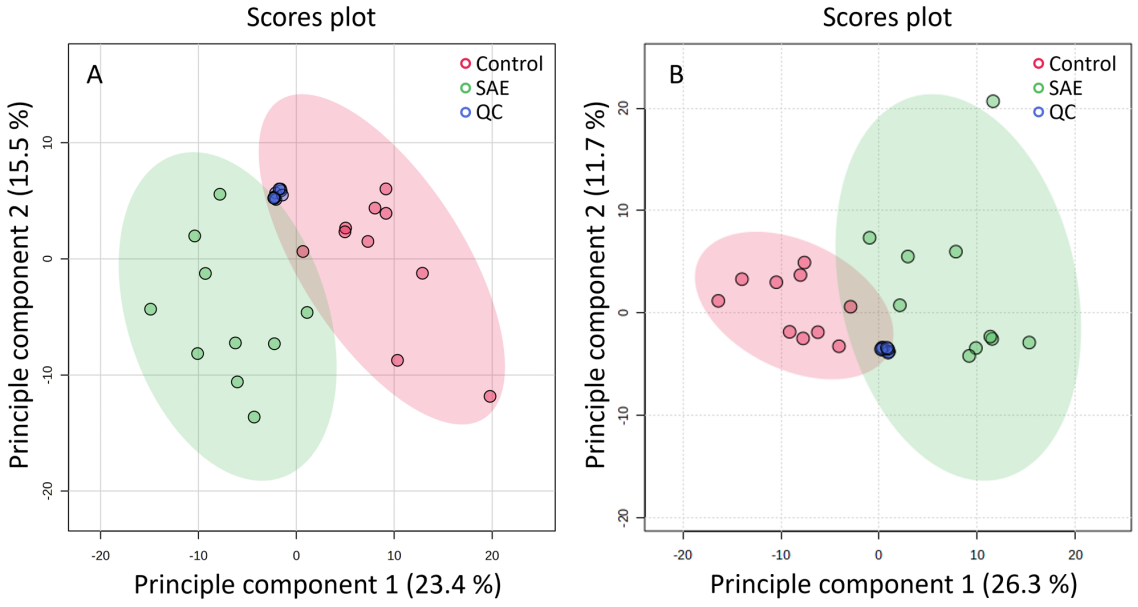

Figure 1. The results of the analysis of the obtained experimental data by the method of principal components (PCA) are the differentiation of control groups, diseases and QC for RP (**A**) and HILIC (**B**) chromatography.

RESULTS

In-hospital mortality in patients with sepsis was 60%. The average SOFA score on the first day of sepsis diagnosis was 9.5 points, and the SAPS II score was 44.5 points. The primary infectious sources were nosocomial pneumonia (50%) and secondary peritonitis (50%). The average procalcitonin level on the first day was 43.9 ng/ml.

All patients were diagnosed with septic shock (hypotension that was not relieved by fluid infusion therapy and required vasopressors to maintain mean arterial pressure above 65 mm Hg, and an increase in arterial lactate concentration above 2 mmol/l).

At the time of blood sampling for metabolomic analysis, there were no patients with the clinical variant of SAE of the delirium type in the study group. All patients experienced impaired consciousness ranging from obtundation to coma and autonomic instability on the first day of sepsis. They underwent multicomponent neurovegetative stabilization (the opioid analgesic fentanyl and propofol).

In this study, an untargeted metabolomics approach was used to examine the plasma metabolome of SAE patients. Despite the limited sample size, the group factor (SAE, control) explained more than 30% of the observed data variability for 21.2% and 32.5% of metabolites, and more than 50% for 6.4% and 10.2% for RP and HILIC HPLC-MS analysis, respectively. Only 40.4% and 31.3% of variables explained less than 10% of the variance, thus indicating a significant proportion of biologically systematic differences between the study groups. The most significant metabolites, in turn, showed high effect sizes ($\log_2$(FC) and Cohen's d), confirmed by the consistency of the nonparametric test.

After data normalization, chemometric analysis was performed in the two sample groups using partial least squares discriminant analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA). Figure 2 shows significant differences between the study groups. Since these analyses are supervised multivariate methods prone to data overestimation and describe the direction of maximum covariance between the data set and group membership, we cross-validated the resulting models and assessed their predictive ability. The resulting models demonstrated high explanatory and predictive ability (R^2Y = 0.933 and 0.718; Q^2 = 0.861 and 0.619). According to the results of permutation tests (n = 20, 100, and 1000 permutations), none of the random models exceeded the original model. This indicates the statistical significance of the OPLS-DA models ($p < 0.001$).

During the OPLS-DA analysis of the experimental data, the significance of the variable contributions to the prediction used in the model was assessed. A high VIP value can serve as a basis for selecting variables as potential disease biomarkers.

As part of the statistical analysis of the data, Volcano plots were constructed (FC difference range ≥ 2.0; false discovery rate q < 0.01), and the direction, magnitude, and significance of changes in the intensity of variables in the study groups were assessed (Fig. 3). These data allow us to visualize and identify groups of variables that significantly increased or decreased in the study groups, as well as to evaluate their correlation between the patient groups.

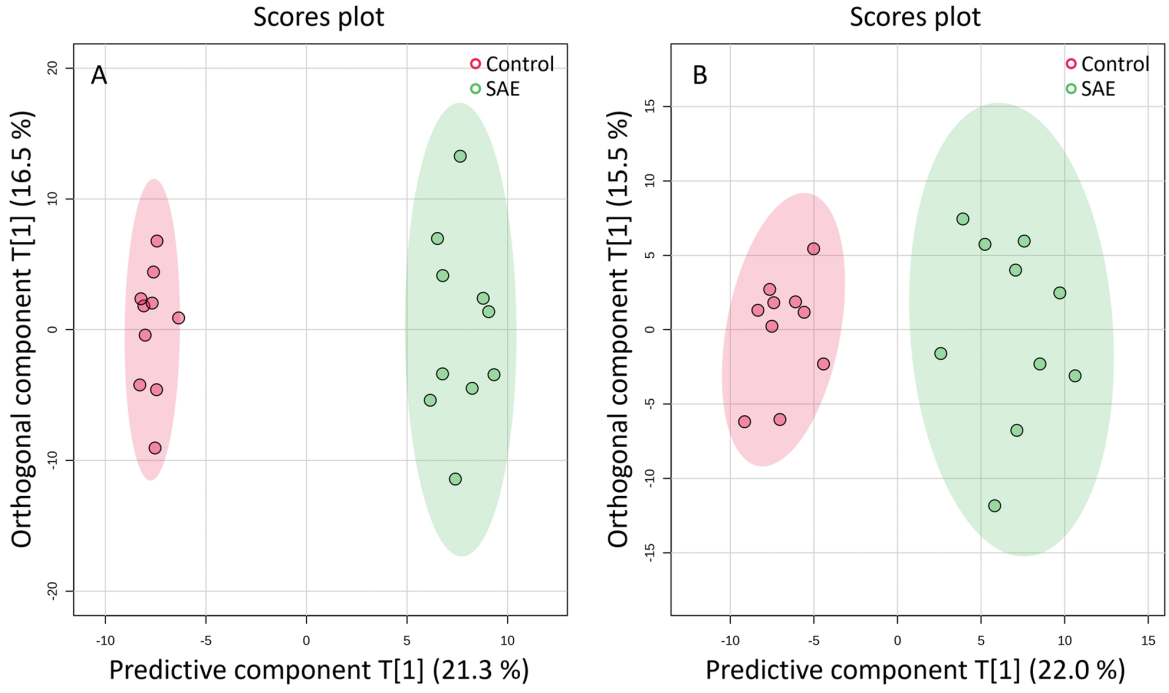

Figure 2. OPLS-DA analysis of the normalized experimental data obtained for blood plasma samples from patients with sepsis (disease group) and without the disease (control group) for RP (**A**) and HILIC (**B**) chromatography.

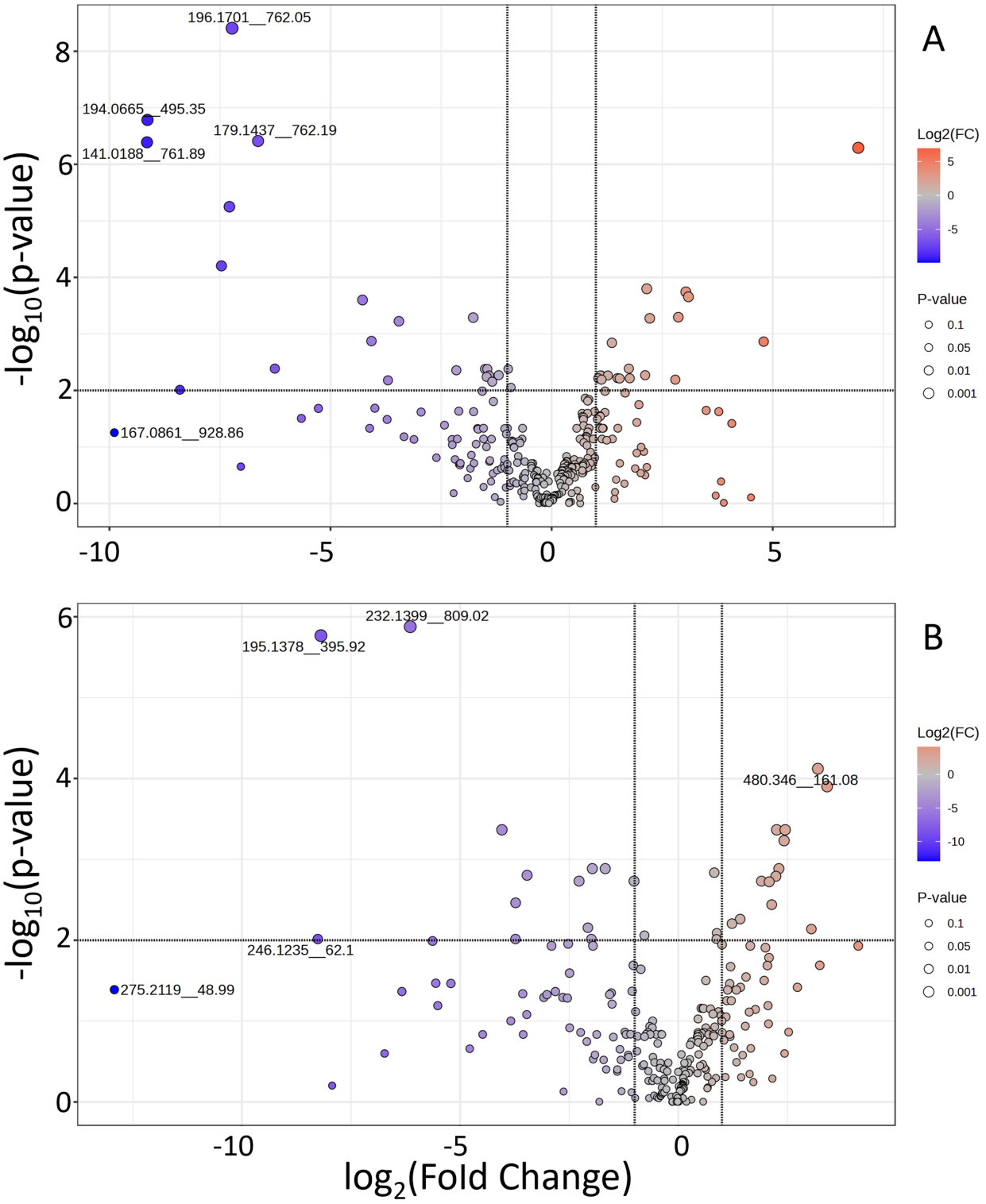

Figure 3. Volcano slot diagrams for plasma samples of patients with sepsis (disease group) and without the disease (control group) for RP (A) and HILIC (B) chromatography. The blue color indicates the variables, the intensity of which increases in the disease group; red color indicates the variables with decreased intensity in the disease group. The color version of this figure is available in the electronic version of the journal.

The resulting experimental data set was analyzed using the Random Forest machine learning algorithm using an ensemble of decision trees (number of trees: 500, number of predictors: 7). This method, unlike PLS-DA, is unsupervised. The final classification prediction for the samples is the average of the predictions from individual trees. To calculate the significance of variables, the Mean Decrease Accuracy (MDA) method was used across all cross-validated predictions. Classifier performance was assessed using the OOB (out-of-bag) error.

An untargeted metabolomics approach requires annotation and preliminary metabolite identification, followed by metabolic pathway analysis. Metabolic pathways are chains of enzymatic chemical reactions that occur within cells during substrate conversion into end products. Enrichment analysis is a bioinformatics approach used to identify biological pathways most affected by metabolites of interest identified in experimental data. Using this approach it is possible to identify relevant pathways associated with a pathological condition.

We have performed Pathway Analysis using the Mummichog algorithm; with this algorithm it is possible to map all metabolite matches on a metabolic map and find local enrichment that reflects true activity (Fig. 4).

Functional analysis resulted in identification of the metabolic pathways most affected by pathological processes in patients with sepsis (Fig. 4). These included taurine and acylcarnitine metabolism, folate biosynthesis, and xenobiotic metabolism (cytochrome P450 substrates). All significant variables annotated using the Mummichog algorithm and involved in the described metabolic processes were also used in constructing the OPLS-DA model and had a VIP value > 1.0, thus indicating statistically significant change between experimental groups.

Generic supplemental enrichment analysis (GSEA) of the experimental data also yielded a pre-annotated list of metabolites and metabolic pathways of interest in untargeted metabolomics research (Fig. 5).

Preliminary annotation of the obtained variables was performed based on the obtained analytical characteristics (exact mass value, retention time, MS/MS spectrum) using the KEGG (Kyoto Gene and Kyoto Encyclopedia), Lipidmaps, HMDB (Human Metabolome Database), NIST20 libraries, as well as the MZmine and GNPS services with built-in mass spectrometric libraries GNPS, HMDB, Massbank, Fiehn, MetaboBASE, and others.

Based on the results obtained during chemometric processing of the experimental data using the Volcano plot, OPLS-DA, and Random Forest methods, as well as taking into account the functional analysis of metabolic pathways, the variables used to build models by all the described algorithms were selected. The following limitations were used: fold change in variable intensities between experimental groups FC > 2.0 and false discovery rate (FDR) q < 0.01 (Volcano plot); the significance of the contribution of variables to the forecast was VIP > 1.0 (OPLS-DA) and Mean Decrease Accuracy MDA > 0.0 (Random Forest). As a result, 20 significant variables were selected for subsequent annotation for RP chromatography and 44 for HILIC. Visualization of significantly changed metabolites between the study groups using heatmap construction is presented in Figure S1 of the Supplementary Materials.

Table 2 shows the results of the statistical power calculation for significant annotated metabolites; it demonstrates the probability of detecting the observed effect using the current sample size. Power values greater than 0.8 indicate a sufficient sample size, while the standardized Cohen's d effect size (d > 0.8 for large effects and d > 1.0 for very large effects) for individual metabolites suggests that we detected statistically significant results given the limited sample size of the pilot study.

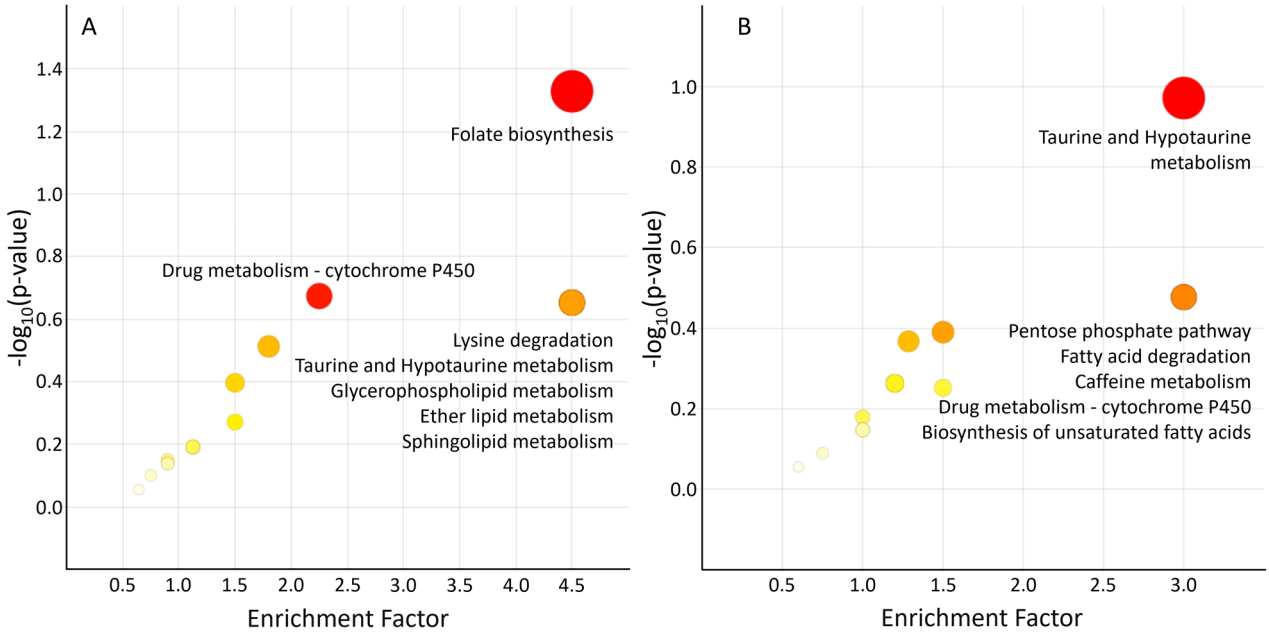

Figure 4. Analysis of metabolic pathways using the Mummichog algorithm and the KEGG library ($p < 0.05$) for RP (**A**) and HILIC (**B**) chromatography. The graphs depict the impact of the pathological condition on metabolic pathways based on the experimental data.

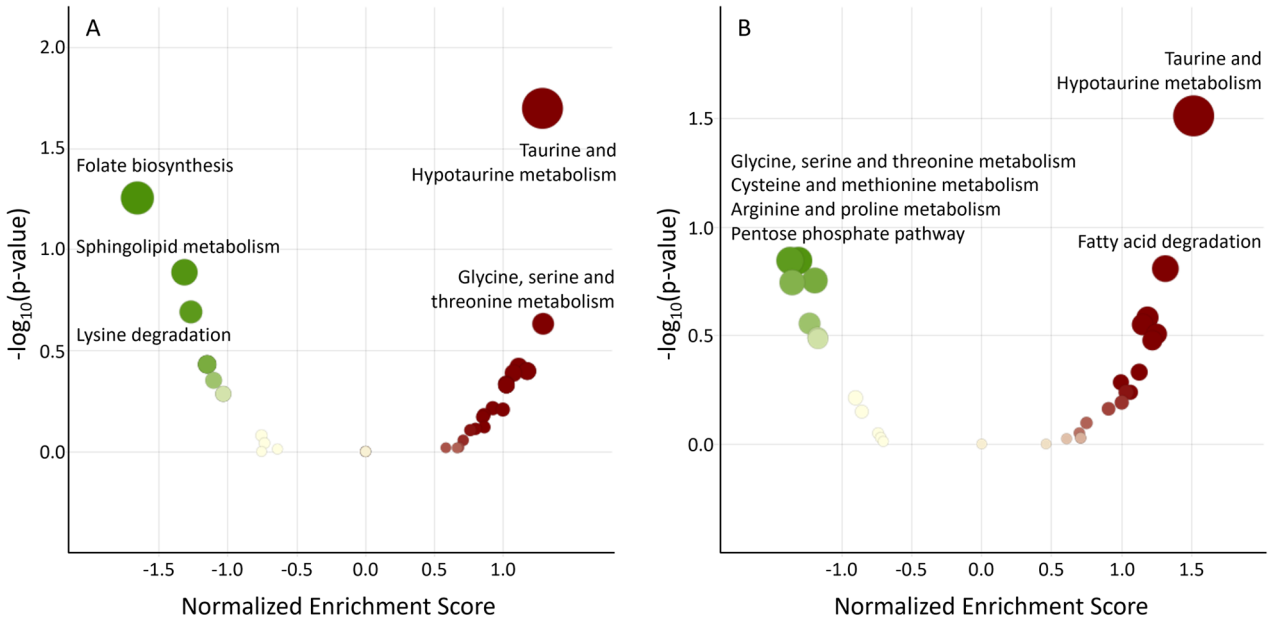

Figure 5. Analysis of metabolic pathways using the RSA algorithm and the KEGG library ($p < 0.05$) for RP (**A**) and HILIC (**B**) chromatography. The graphs depict the impact of the pathological condition on metabolic pathways based on the experimental data.

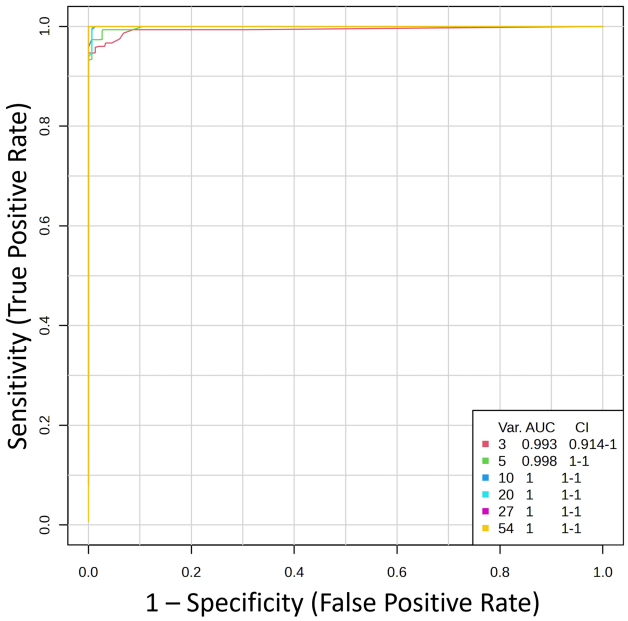

Figure 6. Averaged ROC curves of all models based on the performance of permutation tests and AUC for models obtained using a different number of variables. The ordinate axis shows sensitivity (the frequency of true positive results); the abscissa axis shows specificity (the frequency of false positive results). The color version of this figure is available in the electronic version of the journal.

The test power values at $\alpha = 0.05$ for most metabolites are close to 1, thus reflecting fairly large effects with Cohen's d values > 1.5–5.0. The use of FDR correction by means of the Benjamini-Hochberg method allowed maintaining high test power (> 0.7 for significant metabolites) with acceptable control of false-positive discoveries and a balance between sensitivity and method rigor for the obtained metabolomic data.

To assess the diagnostic significance of the identified pre-annotated metabolites based on a data set containing significant candidates for potential SAE biomarkers, we constructed ROC curves and calculated the areas under the curves (AUC). The ROC (Receiver Operating Characteristic) curve allows evaluating the quality of the classification model by separating the two experimental groups for each variable used. The AUC (Area Under ROC) characteristic is also used to assess the quality of the model: AUC values in the range of 0.9 to 1.0 indicate high accuracy; from 0.8 to 0.9 indicate very good accuracy; from 0.7 to 0.8 indicate good accuracy; 0.6 to 0.7 indicates moderate accuracy; and less than 0.6 indicates low diagnostic accuracy of the variable. Figure 6 shows the results of the multivariate analysis based on the ROC curve using the Random Forest algorithm.

Based on the ROC curve analysis, we were able to identify metabolites with high diagnostic significance in the plasma samples used for analysis (Supplementary Materials, Fig. S2).

DISCUSSION

According to our data, long-chain acylcarnitines (CAR(6:1); CAR(16:0); CAR(16:1); CAR(18:1); CAR(18:2); CAR(20:4)) as intermediates of intracellular

Table 2. Estimated statistical power for biomarker candidates

Metabolite	$\log_2$(FC)	Cohen's d	*p*-value (*t*-test)	p_{adj} (FDR)	Power ($\alpha = 0.05$)	Power (α_{corr})
RF-HPLC-MS analysis						
Acylcarnitines						
CAR(22:5)	4.311	3.169	1.32×10^{-6}	4.51×10^{-4}	1.000	0.981
CAR(20:4)	2.376	2.844	5.45×10^{-6}	6.44×10^{-4}	1.000	0.956
CAR(18:2)	1.619	2.655	1.28×10^{-5}	7.84×10^{-4}	1.000	0.941
CAR(22:6)	3.420	2.209	1.06×10^{-4}	0.002	0.997	0.903
CAR(18:1)	1.649	2.134	1.53×10^{-4}	0.003	0.995	0.881
CAR(9:0)	2.034	1.767	9.37×10^{-4}	0.009	0.962	0.760
CAR(18:0)	1.519	1.698	0.001	0.012	0.948	0.727
CAR(16:0)	1.396	1.647	0.002	0.014	0.936	0.708
CAR(6:1)	-5.125	-1.406	0.006	0.035	0.844	0.573
CAR(16:1)	1.838	1.304	0.009	0.048	0.787	0.522
Lysophospholipids						
LPC(20:5)	3.639	2.293	7.04×10^{-5}	0.002	0.998	0.917
LPC(16:0)	2.105	2.211	1.05×10^{-4}	0.002	0.997	0.901
LPC(14:0)	2.431	2.077	2.02×10^{-4}	0.003	0.992	0.866
LPC(16:1)	1.860	1.965	3.50×10^{-4}	0.005	0.986	0.832
LPC(15:0)	2.565	1.864	5.78×10^{-4}	0.007	0.976	0.794
LPC(18:2)	1.744	1.462	0.004	0.029	0.871	0.604
LPC(20:4)	1.844	1.456	0.004	0.029	0.868	0.601
LPS(O-18:0)	6.341	2.068	2.11×10^{-4}	0.004	0.992	0.869
Other metabolites						
Pentadienic acid	-6.971	-1.927	4.24×10^{-4}	0.005	0.983	0.820
N,N,N-Trimethyllysine	-1.115	-1.708	0.001	0.012	0.950	0.797
HILIC-HPLC-MS analysis						
Lysophospholipids						
LPS(O-18:0)	6.607	4.372	1.26×10^{-8}	1.62×10^{-6}	1.000	1.000
LPC(P-16:0)	3.977	3.438	4.30×10^{-7}	3.05×10^{-5}	1.000	0.999
LPC(17:0)	3.283	2.951	3.38×10^{-6}	1.53×10^{-4}	1.000	0.990
LPC(20:4)	2.784	2.617	1.53×10^{-5}	3.70×10^{-4}	1.000	0.975
LPC(14:0)	3.374	2.550	2.08×10^{-5}	4.70×10^{-4}	0.988	0.970
LPC(P-18:0)	2.860	2.424	3.77×10^{-5}	6.28×10^{-4}	0.999	0.960
LPC(20:3)	2.789	2.357	5.20×10^{-5}	7.18×10^{-4}	0.999	0.957
LPC(22:6)	2.369	1.965	3.50×10^{-4}	0.003	0.986	0.870
LPC(20:5)	3.071	1.876	5.43×10^{-4}	0.005	0.977	0.840
LPC(18:0)	3.440	1.853	6.10×10^{-4}	0.005	0.975	0.834
LPC(18:1)	2.518	1.791	8.31×10^{-4}	0.006	0.966	0.814
LPC(18:3)	0.816	1.775	9.01×10^{-4}	0.006	0.963	0.811
LPC(18:2)	1.723	1.664	0.002	0.009	0.940	0.766

Table 2. Estimated statistical power for biomarker candidates (continued)

Metabolite	$\log_2$(FC)	Cohen's d	*p*-value (*t*-test)	p_{adj} (FDR)	Power ($\alpha = 0.05$)	Power (α_{corr})
LPC(16:0)	2.312	1.633	0.002	0.010	0.932	0.757
LPG(12:0)	-5.201	-3.421	4.61×10^{-7}	3.09×10^{-5}	1.000	1.000
LPE(18:0)	3.423	2.775	7.43×10^{-6}	2.31×10^{-4}	1.000	0.984
LPE(20:5)	1.859	1.595	0.002	0.011	0.921	0.744
LPE(16:0)	1.811	1.496	0.004	0.016	0.885	0.691
Phospholipids						
PC(O-38:6)	1.784	2.680	1.15×10^{-5}	3.18×10^{-4}	1.000	0.979
PC(O-36:5)	1.701	2.629	1.44×10^{-5}	3.70×10^{-4}	1.000	0.975
PC(38:5)	1.176	1.700	1.31×10^{-5}	0.008	0.949	0.779
PC(38:6)	1.170	1.631	0.002	0.010	0.931	0.757
PC(38:3)	1.050	1.370	0.007	0.025	0.826	0.626
PG(33:1)	1.374	1.442	0.005	0.020	0.862	0.665
PA(42:1)	1.084	1.360	0.007	0.026	0.820	0.621
Sphingolipids						
SM(d18:2/15:0)	5.612	2.558	2.01×10^{-5}	4.50×10^{-4}	1.000	0.986
SM(d18:1/16:0)	1.778	2.246	8.84×10^{-5}	1.07×10^{-3}	0.997	0.941
SM(d18:1/16:1)	1.224	1.747	1.03×10^{-3}	0.007	0.958	0.798
Acylcarnitines						
CAR(18:0)	2.321	2.472	3.01×10^{-5}	5.78×10^{-4}	0.999	0.963
CAR(16:0)	1.746	1.920	4.37×10^{-4}	0.004	0.982	0.855
CAR(18:2)	1.602	1.805	7.73×10^{-4}	0.006	0.968	0.818
CAR(18:1)	1.711	1.711	1.24×10^{-3}	0.008	0.951	0.784
CAR(14:0)	1.791	1.675	1.48×10^{-3}	0.036	0.943	0.836
CAR(14:1)	1.699	1.661	0.002	0.009	0.939	0.765
CAR(10:1;O2)	-3.823	-1.452	0.004	0.019	0.866	0.669
CAR(18:2;O2)	-2.256	-1.425	0.005	0.021	0.854	0.657
Other metabolites						
Arg-Gly	-8.182	-4.889	2.23×10^{-9}	1.11×10^{-6}	1.000	1.000
Asn-hydroxyPro	-4.861	-2.487	2.80×10^{-5}	5.78×10^{-4}	0.999	0.963
Asp-Leu	-6.782	-1.647	0.002	0.009	0.936	0.763
MG(17:0)	-7.421	-4.571	6.37×10^{-9}	1.36×10^{-6}	1.000	1.000
Docosahexaenoic acid	3.172	1.733	1.11×10^{-3}	0.007	0.956	0.795
Taurine	2.676	3.112	1.68×10^{-6}	9.28×10^{-5}	1.000	0.994
p-Aminobenzoic acid	5.866	2.015	2.73×10^{-4}	0.003	0.989	0.883
Quinoline	-2.264	-1.785	8.58×10^{-4}	0.006	0.965	0.813

$\log_2$(FC) – logarithm to the base 2 of the fold change in the metabolite level; Cohen's d – standardized effect size according to Cohen; p_{adj} (FDR) – *p*-value, with Benjamini-Hochberg correction; Power ($\alpha = 0.05$) – power of the test with standard $\alpha = 0.05$; Power (α_{corr}) – power of the test with Benjamini-Hochberg correction (FDR); $\alpha_{corr} = \alpha \times i/m$, where *i* is the rank of the variable, *m* is the number of variables (497 and 590 for RP- and HILIC-HPLC-MS analysis, respectively); LPS – lysophosphatidylserines; LPC – lysophosphatidylcholines; LPG – lysophosphatidylglycerols; LPE – lysophosphatidylethanolamines; PC – phosphatidylcholines; PG – phosphatidylglycerols; PA – phosphatidic acids; SM – sphingomyelins; CAR – carnitines; MG – monoglycerides.

fatty acid metabolism were statistically significantly decreased in the SAE patient group. Acylcarnitine is an important biomolecule involved in many metabolic processes, including fatty acid metabolism. Acylcarnitine is essential for the transport of long-chain fatty acids into mitochondria, which produce energy within cells. It serves as an intermediate in the carnitine shuttle system, transporting acyl groups of fatty acids from the cytoplasm into the mitochondrial matrix. In the mitochondria, the acyl groups undergo beta-oxidation, ultimately facilitating ATP synthesis.

Acylcarnitines play a key role in the transport of fatty acids into the brain, thus facilitating their breakdown and use for energy production [10]. The importance of acylcarnitine for brain function and development has been demonstrated in studies on energy metabolism, neurotransmitter synthesis, and neuroprotection [11]. Acylcarnitine is also involved in the maintenance of myelin within the myelin neural sheath, which reflects the functional maturity of neurons. Dysregulation of acylcarnitine metabolism has been associated with cognitive deficits and neurodegenerative diseases [11].

Decreased acylcarnitine levels detected in a group of SAE patients with the altered level of consciousness, progressing to delirium and coma, confirm the importance of metabolomic screening in acute brain injury to further identification of ways to correct neurological and cognitive deficits in patients with sepsis.

Advances in lipid research [12] have led to the consideration of lysophosphatidylcholines as central mediators of lipotoxicity by initiating stress-mediated cell apoptosis and disrupting oxidative phosphorylation of liver mitochondria, leading to mitochondrial dysfunction [13]. In the brain, neuronal membrane phospholipids serve as a reservoir for second messengers and are involved in apoptosis, modulating the activity of transporters and membrane-bound enzymes [13]. Neurological disorders and acute traumatic brain injury (TBI) have been shown to be associated with significant changes in the glycerophospholipid composition of neuronal membranes. The phospholipid composition of myelin is similar to that of white matter and differs significantly from that of gray matter [14]. White matter damage in the brain during sepsis is confirmed by periventricular and semi-oval massive lesions based on neuroimaging data, such as brain MRI [15], which clinically manifests as delirium. Of the 10 SAE patients examined, delirium developed early in the sepsis diagnosis in 50% of patients.

Changes in lysophosphatidylcholine levels lead to increased permeability of cell membranes, and along with the accumulation of lipid peroxides and disruption of energy metabolism, these metabolites may be responsible for neurodegeneration, which underlies long-term cognitive impairment in SAE patients [14]. Glycerophospholipids of neuronal membranes are also destroyed during the onset of neuroinflammation, a universal pathophysiological mechanism in response to cerebral injury [14]. In our study, the level of lysophosphatidylcholines (LysoPC(14:0); LysoPC(16:0); LysoPC(20:4n6), as well as LysoPC(P-16:0); LysoPC(17:0); α-GPC; glycerophospholipids LysoPE(18:0) significantly decreased in SAE patients, along with a significant decrease in plasma docosahexaenoic acid, an essential polyunsaturated fatty acid of the omega-3 class, a component of lipids in most mammalian tissues. Increased concentrations of the precursor of carnitine and the coenzyme of fatty acid oxidation N6-trimethyl-L-lysine in SAE patients are also consistent with data on the disruption of long-chain fatty acid transport across the mitochondrial membrane.

Taurine metabolism was also affected in patients with sepsis and SAE. Sulfur-containing conditionally essential amino acid taurine (aminosulfonic acid) is an inhibitory neurotransmitter in the central nervous system, one of the main antioxidants, an anti-inflammatory and anti-apoptotic factor, and a physiological stabilizer of cell membranes [16]. Its level was significantly reduced in our study conducted in SAE patients.

Our study also revealed impairments in folate biosynthesis processes — a significant decrease in the concentration of *p*-aminobenzoic acid, an intermediate metabolite in the folic acid synthesis.

It should be especially emphasized that the above-mentioned changes in metabolite concentrations were detected in blood plasma collected within 24 h of sepsis diagnosis in patients with SAE (i.e., in the early stages of the disease).

Advances in systems biology theory and the development of metabolomics technologies have led to recognition of the importance of metabolomics for clinical work in the field of intensive care medicine [17]. Many previous metabolomic studies in patients with sepsis have shown that metabolite profiles change significantly in patients with systemic inflammatory response syndrome (SIRS) compared to healthy controls and that low-molecular-weight metabolites can be used to detect infections and predict disease prognosis [4, 18]. Clinical metabolomics studies in SAE are still limited. Based on bioinformatics analysis, we have identified 64 metabolites as potential biomarkers of sepsis and disease severity.

Two studies have identified metabolites that can monitor treatment response in patients with sepsis [19, 20]. The first study aimed to monitor metabolome changes in patients with septic shock who received L-carnitine therapy, which would reduce the use of vasopressors [19]. A good response was found in patients with a low hydroxybutyrate level (153 μM): an increase in methionine, lysine, phenylalanine, and tyrosine was found after L-carnitine administration. In addition, low levels of carnitine and

acetylcarnitine were also observed in patients with good response [19]. A second study followed patients with septic shock; their multiple organ failure score (SOFA) was below 8 or decreased by more than 5 within 48 h of treatment initiation [20]. The untargeted metabolomics study comparing the extent of metabolite changes in responders and non-responders over time (from the initial level to 48 h) showed that myristic and oleic acid levels were more reduced in good responders [20]. In targeted metabolomics, changes in several metabolites showed different results. When comparing the extent of metabolite changes over time, kynurenine levels were elevated in responders but lower than in non-responders. Interestingly, some phospholipids had predictive ability to determine treatment response. Indeed, most SM, SM(OH), and PC were increased in responders, whereas they were decreased [20]. These results suggest that some metabolites may be potential markers for monitoring treatment response in patients with sepsis. Consistent with the different disease outcomes, we found that the concentrations of sulfur-containing amino acids in the non-survivor group were significantly lower than in the survivor patients.

This pilot study aimed to validate the methodology for untargeted metabolomic analysis of plasma from patients with SAE, optimize protocols for sample preparation and data analysis, and generate hypotheses regarding potential biomarkers. The limited number of patients in both experimental groups, due to the clinical context of SAE, suggests the possibility of identifying preferentially manifested metabolic differences. However, metabolites that significantly changed between the control and SAE groups were characterized by large effect sizes and high statistical power, making them reliable candidates for subsequent studies. Even with a relatively small sample size, for metabolites with the largest effect (Cohen's $d > 2.0$), the study power exceeded 0.9 after FDR correction, thus indicating sufficient sensitivity to detect biologically significant changes. The obtained results should be considered preliminary and reflect a combination of metabolic changes associated with both the disease and the treatment. To confirm them and clarify the contribution of the pathological process, a study on an expanded and independent cohort of patients is necessary, which we plan to conduct in the future.

CONCLUSIONS

Metabolomic studies show promise for the diagnostics and treatment of critically ill patients. The obtained results demonstrate the feasibility of using HPLC-HR-MS methodology to analyze metabolomic changes in SAE and applicability for identification of a number of potentially significant metabolites that require validation in larger studies. Some of the potential metabolite molecules and their pathways identified in our study may provide valuable clues and a basis for further in-depth research in intensive care. We plan to expand metabolomic studies of biological fluids in patients with SAE to assess the specificity of biomarkers of central nervous system injury and implement new diagnostic technologies.

FUNDING

This study was supported by the State-funded research project “Investigation of the mechanisms of the development of sepsis-associated encephalopathy and study of approaches to its treatment based on omics technologies” (no. 122041900090-4).

COMPLIANCE WITH ETHICAL STANDARDS

The study protocol was approved by the local ethics committee of the Almazov National Medical Research Centre (no. 0204-22 from meeting minutes no. 04-22 dated April 18, 2022). All patients provided written informed consent prior to participating in the study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

REFERENCES

1. *Hu J., Xie S., Xia W., Huang F., Xu B., Zuo Z., Liao Y., Qian Z., Zhang L.* (2024) Meta-analysis of evaluating neuron specific enolase as a serum biomarker for sepsis-associated encephalopathy. Int. Immunopharmacol., **131**, 111857. DOI: 10.1016/j.intimp.2024.111857
2. *Havelund J., Heegaard N., Færgeman N., Gramsbergen J.* (2017) Biomarker research in Parkinson’s disease using metabolite profiling. Metabolites, **7**(3), 42. DOI: 10.3390/metabo7030042
3. *Trongtrakul K., Thonusin C., Pothirat C., Chattipakorn S.C., Chattipakorn N.* (2021) Past experiences for future applications of metabolomics in critically ill patients with sepsis and septic shocks. Metabolites, **12**(1), 1. DOI: 10.3390/metabo12010001
4. *Hussain H., Vutipongsatorn K., Jiménez B., Antcliffe D.B.* (2022) Patient stratification in sepsis: using metabolomics to detect clinical phenotypes, sub-phenotypes and therapeutic response. Metabolites, **12**(5), 376. DOI: 10.3390/metabo12050376
5. *Liu J., Zhou G., Wang X., Liu D.* (2022) Metabolic reprogramming consequences of sepsis: adaptations and contradictions. Cell. Mol. Life Sci., **79**(8), 456. DOI: 10.1007/s00018-022-04490-0

6. *Dryagina N.V., Kondratyeva E.A., Dubrovskii Ya.A., Kondratyev A.N.* (2020) Metabolome of the brain. Russian Neurological Journal, **25**(1), 4–12. DOI: 10.30629/2658-7947-2020-25-1-4-12
7. *Khan S., Ince-Dunn G., Suomalainen A., Elo L.L.* (2020) Integrative omics approaches provide biological and clinical insights: examples from mitochondrial diseases. J. Clin. Invest., **130**(1), 20–28. DOI: 10.1172/JCI129202
8. *Smirnov K.S., Maier T.V., Walker A., Heinzmann S.S., Forcisi S., Martinez I., Walter J., Schmitt-Kopplin P.* (2016) Challenges of metabolomics in human gut microbiota research. Int. J. Med. Microbiol., **306**(5), 266–279. DOI: 10.1016/j.ijmm.2016.03.006
9. *Xi B., Gu H., Baniasadi H., Raftery D.* (2014) Statistical analysis and modeling of mass spectrometry-based metabolomics data. In: Mass Spectrometry in Metabolomics (Raftery D., ed.), Springer New York (Methods in Molecular Biology), New York, NY, pp. 333–353. DOI: 10.1007/978-1-4939-1258-2_22
10. *McCann M.R., George de la Rosa M.V., Rosania G.R., Stringer K.A.* (2021) L-Carnitine and acylcarnitines: mitochondrial biomarkers for precision medicine. Metabolites, **11**(1), 51. DOI: 10.3390/metabo11010051
11. *Qu Q., Zeng F., Liu X., Wang Q.J., Deng F.* (2016) Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. Cell Death Dis., **7**(5), e2226. DOI: 10.1038/cddis.2016.132
12. *Musso G., Saba F., Cassader M., Gambino R.* (2023) Lipidomics in pathogenesis, progression and treatment of nonalcoholic steatohepatitis (NASH): recent advances. Prog. Lipid Res., **91**, 101238. DOI: 10.1016/j.plipres.2023.101238
13. *Farooqui A.A., Horrocks L.A., Farooqui T.* (2000) Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. Chem. Phys. Lipids, **106**(1), 1–29. DOI: 10.1016/S0009-3084(00)00128-6
14. *Farooqui A.A., Horrocks L.A., Farooqui T.* (2007) Modulation of inflammation in brain: a matter of fat. J. Neurochem., **101**(3), 577–599. DOI: 10.1111/j.1471-4159.2006.04371.x
15. *Trofimova N.V.*, *Andropova T.N., Talybov R.S., Sebelev K.I., Potemkina E.G., Ryzhkov A.V.* (2024) Neuroradiology of sepsis-associated encephalopathy. In: Sepsis-Associated Encephalopathy: Clinical, Metabolomic, Neurophysiological and Neuroimaging Parallels (Savvina I.A., Beloborodova N.V., Trofimova T.N., eds.), Nova Science Publishers, New York, pp. 109–138. DOI: 10.52305/QQGR0434
16. *Kodentsova V.M., Risnik D.V., Ladodo O.B.* (2022) Functional ingredient taurine: adequate and clinically effective doses. Meditsinskiy Sovet, **16**(14), 88–95. DOI: 10.21518/2079-701X-2022-16-14-88-95
17. *Serkova N.J., Standiford T.J., Stringer K.A.* (2011) The emerging field of quantitative blood metabolomics for biomarker discovery in critical illnesses. Am. J. Respir. Crit. Care Med., **184**(6), 647–655. DOI: 10.1164/rccm.201103-0474CI
18. *Su L., Huang Y., Zhu Y., Xia L., Wang R., Xiao K., Wang H., Yan P., Wen B., Cao L., Meng N., Luan H., Liu C., Li X., Xie L.* (2014) Discrimination of sepsis stage metabolic profiles with an LC/MS-MS-based metabolomics approach. BMJ Open Respir. Res., **1**(1), e000056. DOI: 10.1136/bmjresp-2014-000056
19. *Puskarich M.A., Finkel M.A., Karnovsky A., Jones A.E., Trexel J., Harris B.N., Stringer K.A.* (2015) Pharmacometabolomics of L-carnitine treatment response phenotypes in patients with septic shock. Ann. Am. Thorac. Soc., **12**(1), 46–56. DOI: 10.1513/AnnalsATS.201409-415OC
20. *Cambiaghi A., Pinto B.B., Brunelli L., Falcetta F., Aletti F., Bendjelid K., Pastorelli R., Ferrario M.* (2017) Characterization of a metabolomic profile associated with responsiveness to therapy in the acute phase of septic shock. Sci. Rep., **7**, 9748. DOI: 10.1038/s41598-017-09619-x

Received: 05.07.2025.
Revised: 20.10.2025.
Accepted: 13.11.2025.

МЕТАБОЛОМНЫЙ ПРОФИЛЬ ПАЦИЕНТОВ С СЕПСИС-АССОЦИИРОВАННОЙ ЭНЦЕФАЛОПАТИЕЙ

Е.Д. Кессених[1], К.М. Быкова[1], Е.А. Мурашко[1]*, Я.А. Дубровский[1], В.В. Дорофейков[1,2], И.А. Саввина[1,3]

[1]Национальный медицинский исследовательский центр имени В.А. Алмазова,
197341, Санкт-Петербург, ул. Аккуратова, 2; *эл. почта: kate.murashko@xmail.ru
[2]Национальный государственный университет физической культуры, спорта и здоровья имени П.Ф. Лесгафта,
190121, Санкт-Петербург, ул. Декабристов, 35
[3]Северо-Западный государственный медицинский университет имени И.И. Мечникова,
191015, Санкт-Петербург, ул. Кирочная, 41

Сепсис-ассоциированная энцефалопатия (САЭ) представляет собой острую дисфункцию головного мозга, которая возникает при отсутствии первичного очага инфекции в центральной нервной системе. Целью нашей работы было проведение пилотного нецелевого метаболомного исследования плазмы крови пациентов с САЭ для выявления метаболических изменений, потенциально связанных с патологическим состоянием, и формирования гипотез для дальнейшего изучения патогенеза, поиска перспективных биомаркеров и оценки тяжести состояния септического пациента. Метаболомное профилирование осуществлялось методом ВЭЖХ-МС-ВР с последующим статистическим анализом полученных данных. В результате слепого рандомизированного контролируемого клинического исследования выявлено существенное различие в метаболических профилях основной и контрольной групп. Функциональный анализ позволил обнаружить метаболические пути, наиболее затронутые патологическими процессами у пациентов с САЭ: метаболизм ацилкарнитинов, лизофосфатидилхолинов, таурина, биосинтез фолата и метаболизм лекарственных препаратов — субстрата цитохрома Р450. У больных с САЭ с нарушением сознания в виде делирия и комы отмечено снижение уровня длинноцепочечных ацилкарнитинов и содержания лизофосфатидилхолинов. Метаболомные профили пациентов с САЭ значимо различались в группе умерших и выживших пациентов: концентрации серосодержащих аминокислот в группе умерших были значительно ниже, чем в группе выживших. В нашем исследовании установлены 64 кандидата в биомаркеры, которые потенциально могут быть использованы для прогнозирования исходов сепсиса, что требует дальнейшего изучения с использованием расширенной и независимой когорты пациентов.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: метаболомика; метаболиты; сепсис-ассоциированная энцефалопатия; сепсис

Финансирование. Исследование проведено в рамках выполнения государственного задания “Изучение механизмов развития и разработка подходов к терапии сепсис-ассоциированной энцефалопатии на основе омиксных технологий” № 122041900090-4 в Национальном медицинском исследовательском центре имени В.А. Алмазова (НМИЦ имени В.А. Алмазова).

Поступила в редакцию: 05.07.2025; после доработки: 20.10.2025; принята к печати: 13.11.2025.