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Amino acid dysregulation in cerebrospinal fluid of stroke patients serving as diagnostic biomarkers in post-neurosurgical bacterial meningitis

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Post-neurosurgical bacterial meningitis (PNBM) is a severe complication following neurosurgical operations. However, clinical diagnosis of PNBM is difficult because of the complicated pathological conditions. This study aims to investigate alterations in amino acid metabolism in hemorrhagic stroke patients with PNBM through targeted metabolomics analysis. Cerebrospinal fluid (CSF) samples were collected from 66 hemorrhagic stroke patients who underwent neurosurgical operation in our department. Baseline data were retrospectively analyzed for two patient groups: the post-neurosurgical bacterial meningitis group (PNBM, $n = 40$) and the non-post-neurosurgical bacterial meningitis (non-infected control group, $n = 26$), classified based on established diagnostic criteria for intracranial infection. A targeted analysis of 36 amino acids and their derivatives in CSF was performed using liquid chromatography-mass spectrometry (LC-MS). Candidate biomarkers were identified through Student's t-test, fold change (FC) analysis, Variable Importance in Projection (VIP), and logistic Least Absolute Shrinkage and Selection Operator (LASSO) regression. The diagnostic performance was evaluated using Receiver Operating Characteristic (ROC) curve analysis. Twenty-one amino acids and their derivatives were found to be significantly downregulated in the PNBM group. Logistic LASSO regression identified Glycine ($p^{orginal} = 2.24 \times 10^{-20}$, fold change = 0.34, AUC = 0.91), L-Threonine ($p^{orginal} = 5.05 \times 10^{-28}$, fold change = 0.39, AUC = 0.92), and L-Homoserine ($p^{orginal} = 2.18 \times 10^{-17}$, fold change = 0.28, AUC = 0.92) as potential biomarkers for diagnosing PNBM in the context of hemorrhagic stroke. Metabolic pathway analysis, corrected for false discovery rate, revealed three CSF-based amino acid metabolic pathways potentially associated with PNBM. In conclusion, these altered amino acids offer new insights into the pathophysiology of PNBM and providing helpful information on potential therapeutic targets in PNBM in the context of hemorrhagic stroke.

Keywords Amino acids, Hemorrhagic stroke, Bacterial meningitis, Metabolomics, Operation

Despite the widespread use of aseptic techniques and preventive measures, post-neurosurgical bacterial meningitis (PNBM) remains an unavoidable complication. PNBM not only exacerbates primary brain injury and neurological deficits but also leads to prolonged hospital stays and, in some instances, increased mortality, thus imposing a substantial burden on healthcare systems[1]. The incidence of post-surgical meningitis is influenced by a variety of factors, including patient age, surgery duration, and the use of external ventricular drainage (EVD) or lumbar drainage (LD)[2]. Recent basic and clinical research, including high-throughput profiling and the application of various biochemical reagent kits, has yielded invaluable insights into the early diagnosis and pathophysiology of the disease over the past several decades.

Currently, the diagnosis of PNBM relies on a combination of clinical signs, such as fever and neurological deterioration, alongside hematological markers, CSF analysis, and microbiological cultures[3,4]. While these

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diagnostic methods are widely employed, they exhibit significant limitations. For example, the presence of a large number of erythrocytes in the subarachnoid space can interfere with the interpretation of CSF findings. Erythrocyte lysis generates cell-free hemoglobin, which can induce aseptic inflammation[5,6]. Moreover, CSF culture is limited by prolonged incubation times, low microbial load, and the influence of prior antibiotic therapy, all of which can result in false-negative results, potentially leading to diagnostic inaccuracies and delayed intervention[4]. These challenges highlight the urgent need for more sensitive and specific molecular biomarkers that can enable earlier and more accurate diagnosis of PNBM, thereby facilitating timely treatment and improving patient outcomes.

In recent years, metabolomics has provided a comprehensive view of the metabolic state of an organism through the profiling of small-molecule metabolites in biological fluids, offering valuable insights into the biochemical alterations associated with various disease states. This approach has been successfully employed for the identification of biomarkers for the early diagnosis and mechanistic understanding of diverse diseases, including cardiovascular, infectious, and neurological disorders[7]. Among the broad spectrum of metabolites, amino acids play a pivotal role in protein synthesis. They serve as essential structural components and energy substrates, crucial for normal cell growth, differentiation, and homeostasis. Moreover, alterations in amino acid levels are frequently observed in a variety of pathological conditions[8]. Previous studies have shown that disturbances in L-arginine metabolism are linked to cerebral vasospasm and poor outcomes in patients with intracranial aneurysms[9,10]. Ardiansyah et al. demonstrated that elevated baseline CSF tryptophan levels and plasma kynurenine concentrations in patients with tuberculous meningitis are associated with an increased risk of mortality[11]. During infection, amino acids not only support protein synthesis but also play a critical role in regulating metabolic reprogramming and signal transduction, thereby enhancing the effector functions of immune cells[12]. However, research specifically focusing on amino acid metabolic disturbances in PNBM patients following neurosurgical operation remains limited. Therefore, a deeper understanding of these metabolic alterations could provide critical insights into the pathophysiology of PNBM, and targeting immune cell amino acid metabolism may offer new therapeutic approaches for its treatment.

In this study, we established a clinical cohort and classified patients into PNBM and non-infected control groups based on stringent clinical diagnostic criteria. Utilizing targeted metabolomics analysis, we aimed to identify and quantify distinct amino acid profiles in CSF from both groups, to identify potential molecular biomarkers for the early diagnosis of PNBM. Additionally, we conducted a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis to investigate the potential amino acid metabolic pathways associated with the condition[13–15].

Methods

Participants and sample collection

A total of 66 patients with hemorrhagic stroke were recruited in this study. The diagnosis was confirmed by two senior neurosurgeons with supporting evidence from neuro-imaging tests. The inclusion criteria were: (1) patients with neurosurgical treatments; (2) patients with complete demographic and clinicopathological data; and (3) adequate amount and quality of CSF samples available for metabolomics analysis. The exclusion criteria were: (1) patients concomitant or complicated with other types of CNS disorders (e.g., brain tumors, neurodegenerative disorders, seizure, or psychiatric disorders); (2) inadequate amount or quality of CSF samples available for the study (e.g., severely hemolyzed or contaminated sample during transportation or storage; 3) patients who had systemic inflammatory diseases or malignant tumors. This study involving human participants was conducted according to the Declaration of Helsinki and was approved by the Institutional Ethics Board of the First Affiliated Hospital of Anhui Medical University (Approval code: 20190146, March 2019).

CSF samples were collected aseptically via cisternal drainage or lumbar puncture in patients with suspected CNS infection 2–7 days post-craniotomy. All samples were stored at −80 °C. All patients received laboratory tests of CSF characteristics because of the potential infectious signs, such as hyperpyrexia, meningeal irritation signs, or altered consciousness and mental states. The diagnostic criteria of PNBM were based on the guidelines issued by the Infectious Diseases Society of America (IDSA) Clinical Practice Guidelines for Healthcare-Associated Ventriculitis and Meningitis[16] and a Chinese Expert Consensus of Diagnostic and Therapy for the Neurosurgical Central Nervous System Infections in 2021. According to the guidelines, PNBM diagnosis briefly relies on either positive results of Gram's staining/bacterial culture or the CSF indications (simultaneously satisfying CSF white blood cells > 100×10^6/L. CSF glucose < 2.2 mmol/L, and CSF-to-blood glucose ratio < 0.4). All enrolled patients underwent craniotomy did not have prophylactic antibiotics. For suspected postoperative infections, CSF was obtained via lumbar puncture or cistern drainage prior to empirical antibiotic administration. Antimicrobial therapy was subsequently tailored according to CSF culture results and biochemical markers. Furthermore, although stroke-induced immunosuppression is common in stroke patients, the rate of intracranial infection after the onset of the disease is relatively low in most ischemic stroke patients who do not undergo craniotomy. This type of patient was not included in this study.

Amino acids quantitative analysis

The content of 36 amino acids and their derivatives in CSF samples were analyzed by the LC-MS method. The analysis was formed in Sinotech Genomics Biotechnology Co. Ltd. and referenced with an established protocol. Briefly, After thawing the cerebrospinal fluid samples at 4 °C, 300 µL of supernatant was freeze-dried in a 2 mL centrifuge tube. The samples were then subjected to shock, centrifugation, filtration, and dilution. The amino acid contents were measured using the UPLC-QQQ-MS method (AB SCIEX 5500 QQQ Mass Spectrometer) in multiple reaction monitoring (MRM) mode. Sample analysis was carried out with an Acquity UPLC system (Waters Corp., Milford, MA, USA). The LC separations were carried out on an Acquity UPLC Amide BEH (1.7 µm, 100 × 2.1 mm), with a flow rate of 0.3 mL/min and an injection volume of 4 µL, column temperature

is 40 °C. Mobile phase A consisted of 0.05% aqueous formic acid in water. Mobile phase B consisted of 0.05% aqueous formic acid inacetonitrile. Mass spectrometry was performed as follows: curtain gas, 35 arb; collision gas, 9 arb; ion source gas, 45 arb; ion spray voltage, 4500 V; ion source temperature, 450 °C. We used MultiQuant software to extract and preprocess the data. The standard solution was added to the sample vial to quantify and identify peaks of Amino acids.

Statistical analysis

SPSS software (23.0 version, IBM, USA) was used for statistical analysis. All continuous data were depicted as the mean ± standard deviation (mean ± SD) and then analyzed with Student's t-test or as medians with interquartile range (IQR) and then analyzed using the Mann–Whitney U test. Binary data were analyzed using the chi-squared test. Unsupervised principal component analysis (PCA), partial least squares discriminate analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed using the R package *ropls* to distinguish between the PNBM and non-infected control groups[17,18].

To identify the key features influencing PNBM, we utilized fold change (FC) thresholds, variable importance in projection (VIP) values, and p-values from Student's t-tests to analyze differences in all 36 amino acids and their derivatives between the two groups. Statistically significant differences were defined by FC thresholds of < 0.5 (downregulation) or > 2 (upregulation), VIP values > 1, and p-values < 0.05[19,20].

Furthermore, logistic least absolute shrinkage and selection operator (LASSO) regression was employed to facilitate variable selection by applying an L1-penalty to the regression coefficients. This approach enhances model interpretability, reduces complexity, and mitigates the risk of overfitting. Binary data were analyzed using the chi-squared test or Fisher's exact test. Pearson's correlation coefficients were assessed between the amino acids and CSF characters. To evaluate the diagnostic performance of the identified amino acids in the context of PNBM, receiver operating characteristic (ROC) curve analysis was performed, with diagnostic accuracy assessed using the area under the curve (AUC).

Metabolic pathway analysis

To investigate potential amino acid groups or pathways associated with PNBM, metabolic pathway enrichment analysis was performed using MetaboAnalyst 6.0. The KEGG database was utilized for this analysis, with the significance threshold for pathway analysis set at 0.05[21]. Additionally, the study focused exclusively on amino acids that met the recommended criteria of VIP values > 1.0 and p-values < 0.05.

Results

Patient characteristics

In this study, the levels of glucose, protein, white blood cells(WBC), chloride in CSF, and CSF-to-blood glucose ratio were recorded for all patients. Among all participants, 40 patients were categorized into the PNBM group, while 26 patients were included in the PNBM group, based on the established diagnostic criteria. There were no differences in age, sex, and blood glucose between the two groups. Student's t-test or Mann–Whitney U test revealed significant differences in the levels of glucose ($p < 0.001$), protein ($p < 0.001$), and WBC ($p < 0.001$) in the CSF, as well as the CSF-to-blood glucose ratio ($p < 0.001$) between patients in the PNBM and non-infected control groups. Furthermore, there were no significant differences in the severity of the primary disease and other comorbidities between the two groups (p value for Glasgow coma scale score was 0.493; p value for hypertension was 0.859; p value for hyperlipidemia was 0.512; p value for comobidity with lung infection was 0.401). The demographic and CSF clinicopathological features are summarised in Table 1.

Multivariate statistical analyses

A total of 35 out of 36 amino acids and their derivatives were detected in CSF samples, except for L-homocysteine. Unsupervised PCA revealed that the scores for PNBM and non-infected control groups were $R^2X = 0.681$; The majority of samples fell within the 95% confidence interval (Hotelling's T-squared ellipse) (Fig. 1A). PLS-DA demonstrated significant separation between the two groups, with $R^2X = 0.808$, $R^2Y = 0.928$, and $Q^2 = 0.793$ (Fig. 1B). OPLS-DA also demonstrated significant separation between the two groups, with $R^2X = 0.464$, $R^2Y = 0.809$, and $Q^2 = 0.798$ (Fig. 1C). The Q^2 value, being closer to 1.0, indicates strong model stability and predictive reliability. Collectively, these PCA, PLS-DA, and OPLS-DA results suggest that the original model was both stable and effective.

Subsequently, a Student's t-test was performed for all amino acids, identifying 33 amino acids with p-values less than 0.05. By combining these results with FC and VIP values, we finally identified 21 amino acids as having statistically significant differences, all of which were down-regulated in the PNBM group. The specific name and results of the analysis are provided in Table 2. The volcano plot, correlation heatmap, and hierarchical clustering heatmap are shown in Fig. 2(A, B, C).

Pathway analysis

As shown in Fig. 2D, eight potential amino acid metabolic pathways were identified as potentially associated with PNBM. After applying FDR correction, three metabolic pathways were ultimately recognized: Glycine, Serine, and Threonine metabolism ($p = 4.4 \times 10^{-4}$, FDR = 0.018); Arginine biosynthesis ($p = 4.5 \times 10^{-4}$, FDR = 0.018); and Phenylalanine, Tyrosine, and Tryptophan biosynthesis ($p = 7.7 \times 10^{-4}$, FDR = 0.02). These pathways involve four amino acids in Glycine, Serine, and Threonine metabolism (Dimethylglycine, Glycine, Sarcosine, and L-Threonine), three amino acids in Arginine biosynthesis (L-Aspartic acid, Citrulline, and N-Acetyl-L-glutamic acid), and two amino acids in Phenylalanine, Tyrosine, and Tryptophan biosynthesis (Phenylalanine and L-Tyrosine), respectively.

	PNBM ($n=40$)	non-PNBM ($n=26$)	*P* value
Age (years, mean ± SD)	50.03 ± 15.46	53.58 ± 15.94	0.371
Gender			0.760
Male	20	12	
Female	20	14	
Hypertension	13	9	0.859
Hyperlipidemia	8	7	0.512
Glasgow coma scale score	12.5 [11.0, 15.0]	13 [11.0, 15.0]	0.493
Comorbid with pulmonary infection	5	1	0.401
CSF characters			
Glucose(mmol/L, IQR)	2.01 ± 0.93	4.42 ± 0.42	< 0.001
Protein (g/L, IQR)	2.12 [1.10, 3.50]	0.63 [0.40, 5.75]	< 0.001
White blood cells ($x10^6$/L, IQR)	1054 [224, 2372]	34 [6, 293]	< 0.001
Chlorine (mmol/L, mean ± SD)	119.9 ± 7.9	127.2 ± 6.4	< 0.001
Blood glucose (mmol/L, IQR)	6.59 ± 3.33	6.89 ± 2.43	0.689
cGlu/bGlu ratio (IQR)	0.34 ± 0.17	0.69 ± 0.17	< 0.001

Table 1. Clinicopathological characteristics of patients recruited in this study. PNBM: post-neurosurgical bacterial meningitis, CSF: cerebrospinal fluid, cGlu/bGlu ratio: CSF-to-blood glucose ratio, IQR: interquartile range.

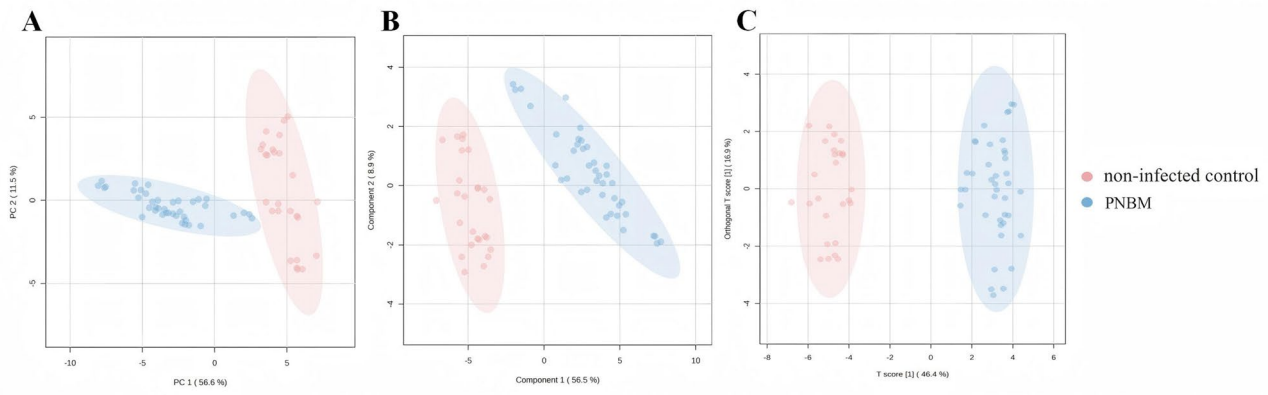

Fig. 1. Multivariate statistical analysis comparing the PNBM and non-infected control groups. (**A**) Principal component analysis (PCA) scores plot; (**B**) partial least squares discriminant analysis (PLS-DA) scores plot; (C) Orthogonal partial least squares discriminant analysis (OPLS-DA) scores plot.

Identification of four amino acids as PNBM potential biomarkers

Based on the above 21 amino acids, we performed logistic LASSO regression to further screen the significant amino acids that could distinguish PNBM from controls. Before training the LASSO model, the tuning parameter λ needed to be determined. To provide a comprehensive overview, Fig. 3A presents the coefficient path plot. Each curve in the figure represents the trajectory of coefficient estimates for each feature as λ changes. As λ increases (i.e., as regularization strength increases), fewer parameters are retained in the model. Ultimately, only the features with the strongest correlation to the dependent variable remain. The horizontal axis represents the logarithm of λ, while the vertical axis depicts the values of the feature coefficients.

When modeling the 21 amino acid metabolite data using the logistic LASSO model, we obtained the following results: (1) Among the 21 amino acids in the feature set, only four (Glycine, L-Threonine, L-Homoserine, and N-Acetyl-L-alanine) were retained after dimensionality reduction, while features with lower correlation were excluded. This dimensionality reduction enhanced the interpretability of the model. (2) Predictive analysis using these four amino acids showed high diagnostic accuracy. Specifically, diagnostic results based on Glycine, L-Threonine, and L-Homoserine individually achieved an AUC > 0.9, as illustrated in Fig. 3B. These findings align with significant enrichment in two metabolic pathways: *Glycine, Serine, and Threonine metabolism* and *Phenylalanine, Tyrosine, and Tryptophan biosynthesis*. This suggests that the amino acid metabolites identified by the logistic LASSO model are biologically meaningful and hold potential as biomarkers for the diagnosis of PNBM.

Amino acid	HMDB	Mean ± SD (μg/ml)		VIP	FC	*P* value
		PNBM	Non-PNBM			
N-Acetyl-L-alanine	HMDB0000766	114.1 ± 69.8	407.7 ± 85.3	1.22	0.280	4.82E-23
Alanine	HMDB0000161	235.0 ± 115.5	575.1 ± 43.0	1.16	0.410	3.95E-23
N, N-Dimethylglycine	HMDB0000092	4.3 ± 2.5	13.8 ± 5.5	1.15	0.311	1.71E-9
Valine	HMDB0000883	200.4 ± 72.7	511.8 ± 170.2	1.18	0.392	5.88E-10
Threonine	HMDB0000167	282.6 ± 122.6	730.6 ± 57.7	1.18	0.387	5.05E-28
Hydroxyproline	HMDB0000725	10.8 ± 8.5	32.7 ± 3.4	1.01	0.332	1.63E-20
Aspartic acid	HMDB0000191	68.3 ± 62.5	136.7 ± 31.1	1.09	0.499	1.80E-7
Lysine	HMDB0000182	2400.5 ± 1376.7	6364.9 ± 932.6	1.13	0.377	4.79E-21
Phenylalanine	HMDB0000159	523.6 ± 350.2	1404.9 ± 390.7	1.15	0.373	6.44E-14
citrulline	HMDB0000904	58.4 ± 28.4	138.4 ± 10.7	1.12	0.42	2.58E-22
Tyrosine	HMDB0000158	186.9 ± 105.8	455.3 ± 100.9	1.17	0.411	3.96E-15
N-Acetyl-L-glutamic	HMDB0001138	8.4 ± 5.1	22.9 ± 4.1	1.16	0.366	3.62E-18
Tryptophan	HMDB0000929	317.4 ± 356.9	1314.4 ± 1322.4	1.04	0.240	8.26E-18
Kynurenine	HMDB0000684	17.7 ± 10.4	102.7 ± 63.8	1.02	0.170	3.87E-7
Cystine	HMDB0000192	80.4 ± 230.8	229.3 ± 261.1	1.00	0.350	0.018
Sarcosine	HMDB0000271	230.1 ± 114.4	566.5 ± 43.7	1.15	0.410	3.77E-23
L-Leucic Acid	HMDB0000746	4.4 ± 2.2	16.7 ± 8.8	1.01	0.260	1.65E-7
S-(5'-Adenosyl)-L-homocysteine	HMDB0000939	1.9 ± 1.7	5.8 ± 1.3	1.14	0.338	1.27E-14
Glycine	HMDB0000123	193.0 ± 121.9	568.6 ± 89.9	1.07	0.340	2.24E-20
Homoserine	HMDB0000719	2.4 ± 1.2	8.5 ± 1.9	1.26	0.280	2.18E-17
S-Adenosyl-L-methionine	HMDB0001185	3.4 ± 2.9	35.4 ± 17.6	1.36	0.096	1.15E-9

Table 2. List of statistically significant differences in amino acid concentrations between PNBM and non-PNBM group. VIP: variable influence on projection, FC: fold change.

Correlation analysis between amino acids with CSF characters

We performed a correlation analysis between the 21 amino acids with CSF characters, including CSF glucose, CSF WBC, CSF proteins and CSF-to-blood glucose ratio. The results indicated that the 4 animo acids which were retained to be significant after dimensionality reduction (LASSO regression model) had minor correlations with the CSF characters (Fig. 4).

Discussions

PNBM is a common complication following neurosurgical operations. The concomitant injury from first and secondary brain injury following intracranial hemorrhage further exacerbates the poor prognosis in these patients, presenting significant challenges for clinical diagnosis and management. Amino acid metabolism plays a crucial role in the pathophysiology of inflammation and the regulation of immune function. In this study, we investigated alterations of amino acids in CSF with PNBM patients in the context of hemorrhagic stroke. This study focused on amino acid profile changes in PNBM patients following hemorrhagic stroke, given that craniotomy is generally not required in the treatment of most ischemic stroke patients. By analyzing the amino acid metabolism profile, we identified 21 amino acids that were significantly down-regulated in PNBM patients. Among these, three amino acids were found to have potential as diagnostic biomarkers for PNBM. Additionally, KEGG pathway analysis revealed that three metabolic pathways were significantly associated with PNBM, suggesting that amino acid metabolism is disrupted in these patients.

Currently, targeted analysis of amino acid profiles in CSF using LC-MS platforms demonstrates significant clinical feasibility. The technique is mature and reliable, offering high sensitivity even for low-abundance analytes. Clinical samples are relatively easy to obtain and require only small volumes, as patients often undergo repeated lumbar punctures or continuous CSF drainage, allowing for dynamic monitoring of amino acid fluctuations. The analytical workflow is relatively straightforward and rapid, and can be implemented using existing LC-MS infrastructure in clinical laboratories. However, compared to conventional diagnostic assays, specialized training for laboratory personnel and the establishment of standardized operating procedures are required. Moreover, further studies are needed to validate the consistency of amino acid profiling with final clinical diagnoses, particularly in comparison to traditional diagnostic biomarkers.

Due to the low abundance of microbes in CSF, the application of antibiotics, and the interference of clinical symptoms such as central fever and positive meningeal irritation after hemorrhagic stroke, the diagnosis of PNBM becomes particularly challenging in patients undergoing neurosurgical operations. Previous studies have suggested several inflammatory cytokines in CSF, including interleukin (IL)−6, IL-8, IL-10, interferon-gamma, YKL-40, and procalcitonin, as potential auxiliary diagnostic markers for intracranial infection[22–24]. However, the levels of these cytokines are also elevated during the secondary brain injury phase following hemorrhagic stroke[25]. Additionally, recent research has proposed lactate, β−2 transferrin, transferrin, and glycans in CSF as valuable biomarkers for the diagnosis of PNBM[26–28]. Nevertheless, these markers can be influenced by various

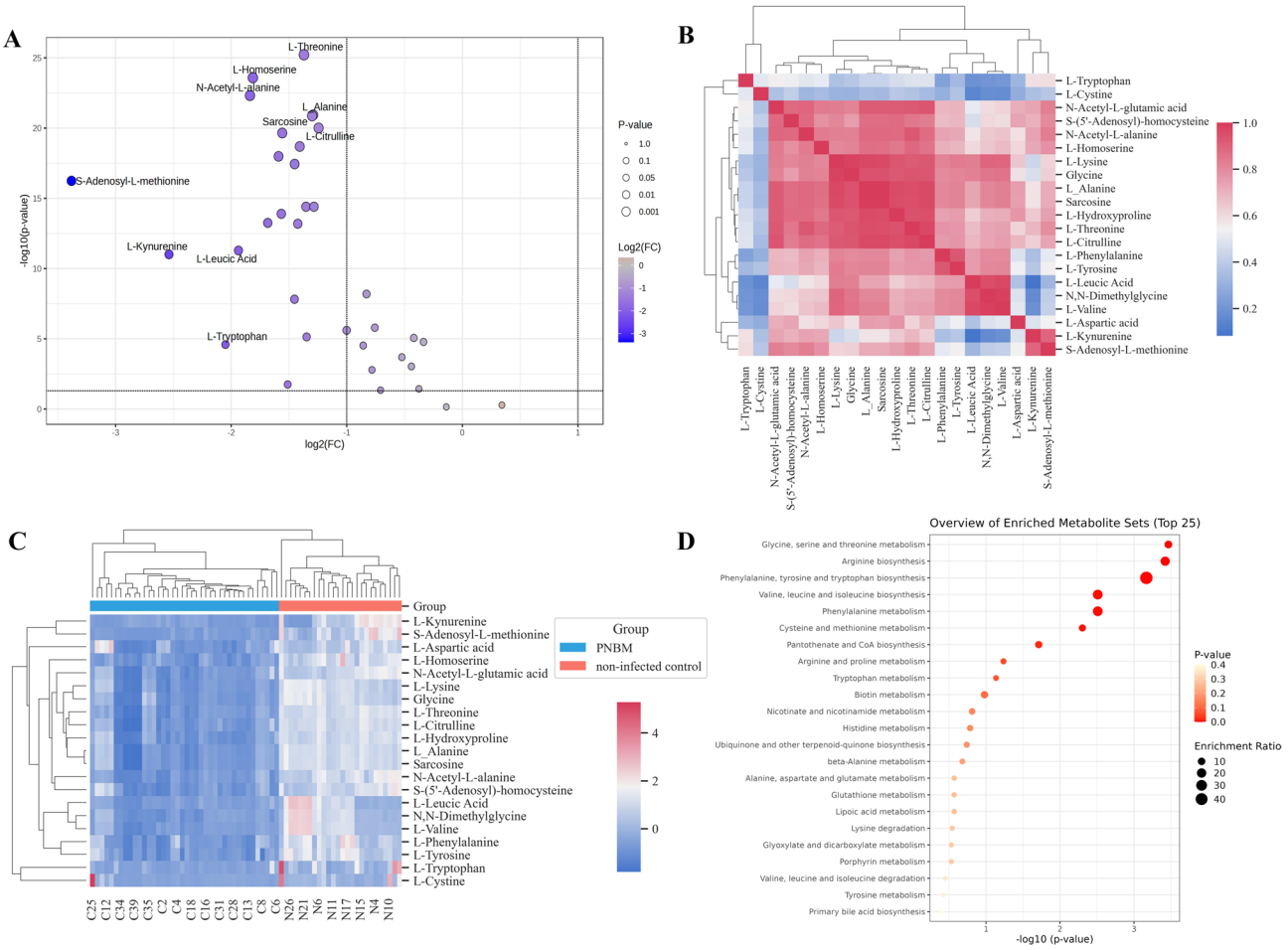

Fig. 2. Statistical information and enrichment analysis of 21 amino acids. (**A**) Volcano plot of differential amino acids. Each point represents an amino acid. The features down-regulated are plotted in purple; The dotted lines show the threshold as FC = 0.5 and p-value = 0.05; (**B**) Heatmap of correlation analysis between differential amino acids. C Heatmap of hierarchical clustering analysis. D KEEG Pathway analysis for amino acids.

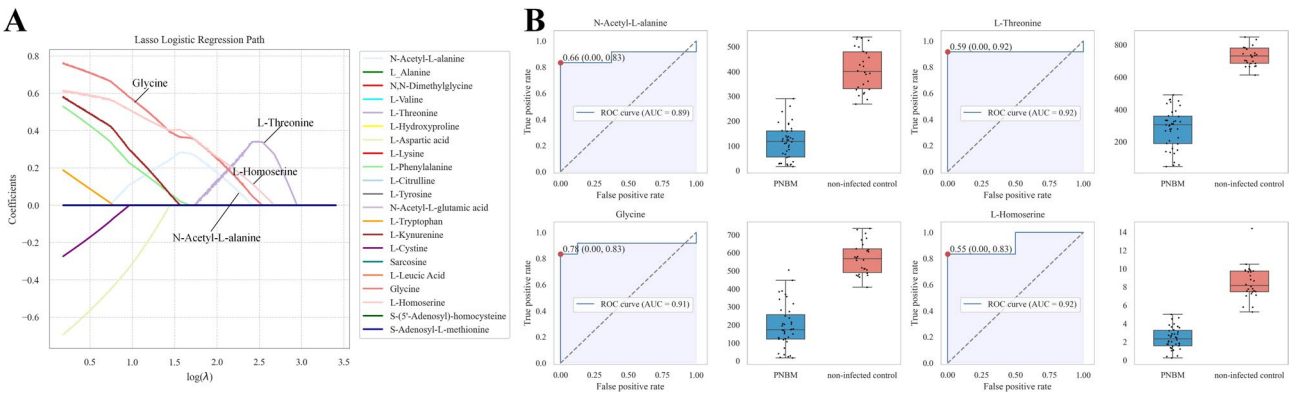

Fig. 3. Four amino acids were identified as potential biomarkers for PNBM through logistic LASSO regression and ROC curve analysis. (**A**) The coefficient path plot of the logistic LASSO model; (**B**) ROC curves for each amino acid (N-Acetyl-L-alanine, Glycine, L-Threonine, and L-Homoserine) and their distribution across the PNBM and non-infected control groups.

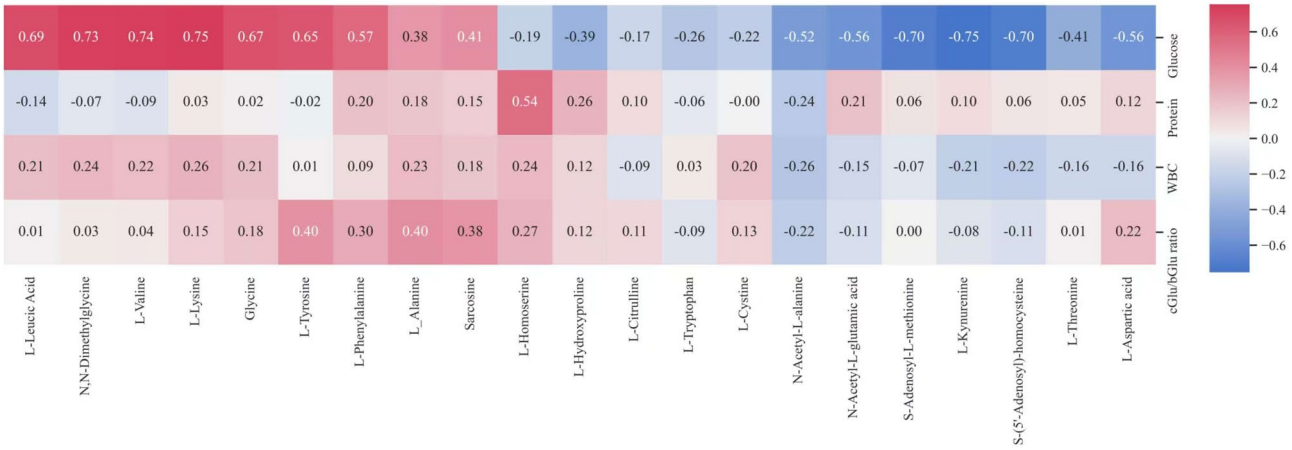

Fig. 4. Correlation analysis were performed between amino acids and the CSF characters.

physical and chemical alterations in the CSF, such as the cell-free hemoglobin and cytokines released from Lysed erythrocytes[6,29]. In our study, we employ LASSO regression to select characteristic variables, a method that effectively mitigates overfitting and addresses issues related to multicollinearity. Finally, L-Threonine, Glycine, and L-Homoserine were identified as potential biomarkers for diagnosis of PNBM in the context of hemorrhagic stroke, all exhibiting an AUC greater than 0.9. However, the specific quantification value and the diagnostic efficiency should also be validated in an augmented population. Furthermore, the use of microdialysis to collect CSF samples for real-time monitoring of amino acid fluctuations could offer more precise sampling and quantification and may be very helpful in clarifying the amino acid metabolism in the pathophysiological processes of PNBM.

Interestingly, we observed that not only the three characteristic amino acids identified in the final screening were down-regulated in the PNBM group, but 21 amino acids that exhibited statistical significance were also down-regulated. Bartosz et al. reported an increase in the levels of 31 amino acids and their derivatives following hemorrhagic stroke, with 18 of these showing statistical significance. Notably, hydroxyproline and 3-amino-isobutyric acid were the only two compounds that did not exhibit significant changes[30]. The potential underlying mechanisms may include alterations in blood-brain barrier (BBB) permeability, the release of amino acids from erythrocytes, and the mobilization of amino acids for cellular repair[31]. However, our results suggest that, during infection in hemorrhagic stroke patients, the levels of these amino acids significantly decrease to varying extents, except for L-Glutamic acid, which increases. Glutamic acid, known as an excitatory amino acid, has been shown to exacerbate neuronal injury and cerebral vasospasm following hemorrhagic stroke and is regarded as a risk factor for poor prognosis[32]. Pneumolysin, produced by Streptococcus pneumoniae following invasion of the central nervous system, triggers glutamate release from astrocytes and plays a critical synapto- and dendritotoxic role in mouse pneumococcal meningitis[33]. Although no statistically significant difference in glutamate levels was observed between the two groups in our study, the potential role of glutamic acid in the dual nerve damage associated with bacterial meningitis in the context of hemorrhagic stroke warrants further investigation through larger studies and foundational experiments.

It is well established that the nine essential amino acids required for human metabolism must be acquired through dietary sources. Despite all patients receiving enteral nutrition during the treatment period, the downregulation of the nine essential amino acids in the PNBM group suggests that there may be insufficient enteral and parenteral nutritional support in this cohort. Furthermore, significant depletion of amino acids involved in protein synthesis and energy supply is observed under conditions of stress and infection[12]. These may contribute to the downregulation of 21 amino acid levels observed and highlights the importance of future personalized nutritional assessments and tailored programs, which should be carefully considered by surgeons.

However, it is inadequate to explain the downregulation of these amino acid levels solely by insufficient intake and excessive consumption. To our knowledge, few studies have directly investigated the detailed mechanisms of amino acids in PNBM patients to date. However, It is well established that the pathophysiological process of PNBM mainly includes bacterial invasion and proliferation in the CNS, the triggered host immune response, and the progression of inflammation[8,34]. During CNS infection, both innate (monocytes and neutrophils) and adaptive immune cells (CD8+, CD4 + T cells, and B cells) are recruited from peripheral circulation across the damaged BBB, where they undergo specific functional changes. Meanwhile, resident immune cells in the CNS, such as microglia and astrocytes, also become activated and release a variety of cytokines and reactive oxygen species, thereby contributing to the inflammatory response and facilitating pathogen clearance[35]. These pathophysiological processes lead to the selective depletion of amino acids, particularly those involved in immune cell regulation, which may be a more important explanation for our findings.

KEGG enrichment analysis identified several potential amino acid metabolic pathways implicated in PNBM, with the three most relevant pathways being glycine, serine, and threonine metabolism; arginine biosynthesis; and phenylalanine, tyrosine, and tryptophan biosynthesis. Furthermore, glycine and threonine, identified through LASSO regression analysis, appear to be key amino acids involved in the first pathway. Eric H Ma et al.

demonstrated that while glucose concentrations are sufficient to support T cell activation and effector function, serine, glycine, and one-carbon units are essential for de novo nucleotide biosynthesis in proliferating T cells[36]. Shan et al. found that serine and glycine metabolism regulate the balance between M1 and M2 macrophage polarization by modulating the IGF1-P38 axis[37]. Moreover, Yang et al. observed that cultured microglia in a medium deficient in serine and glycine exhibited negligible expression of iNOS and, consequently, were unable to produce NO[38]. Therefore, we hypothesize that different immune cells selectively acquire specific amino acids to reprogram their metabolism and function, particularly those involved in glycine, serine, and threonine metabolism, thereby continuously adapting to the pathophysiological changes with PNBM. Given the complexity of amino acid metabolism in the context of hemorrhagic stroke, an accurate evaluation of the role of each amino acid within its local environment, as well as its regulatory effects on metabolism in different immune cells, will be crucial in future studies.

Some limitations in the study should be carefully considered. First, the sample size is relatively small, and this study serves as a preliminary investigation. The accuracy of the three amino acids identified as potential biomarkers needs to be further validated through larger, more robust studies. Second, due to the low positive rate of bacterial cultures, the specific microbial types involved remain unknown. Different bacterial infections may induce distinct alterations in amino acid metabolism. Third, because there are potential medical risks associated with CSF collection from healthy individuals, Therefore, results concerning the diagnostic biomarkers for PNBM are limited to patients with hemorrhagic stroke. Finally, given the complexity of amino acid metabolism in the body and the fact that our samples were collected at a specific time point, continuous monitoring of amino acid level fluctuations would be more conducive to a comprehensive understanding of the pathophysiology of PNBM.

Conclusions

In summary, through targeted analysis of the CSF metabolome, we identified twenty-one characteristic amino acids that were significantly downregulated in PNBM patients compared to the control group, three of them (Glycine, L-Threonine, and L-Homoserine) which may serve as potential biomarkers for the clinical diagnosis of PNBM in the context of hemorrhagic stroke. Additionally, we also identified three amino acid metabolic pathways associated with PNBM: glycine, serine, and threonine metabolism; arginine biosynthesis; and Phenylalanine, Tyrosine, and Tryptophan biosynthesis. These altered amino acids and pathways enhance our understanding of the complex metabolic changes associated with infection-induced immune responses and inflammation, offering new insights into the pathophysiology of PNBM and providing helpful information on potential therapeutic targets in PNBM in the context of hemorrhagic stroke. However, we should also admitted that the pathway analysis is primarily descriptive, and functional studies are needed to further establish causality.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Lei Ye, Hongwei Cheng, and Wei Chen conceived and designed research. Wei Chen, Lu Ding, Bohang Liu, and Lei Ye conducted experiments. Lei Ye, Yuanyuan Chen, and Chaojie Wang contributed new reagents or analytical tools. Wei Chen, Chaojie Wang, Lu Ding, and Yuanyuan Chen analyzed data. Wei Chen, Lei Ye, Yuanyuan Chen, and Hongwei Cheng wrote the manuscript. All authors critically reviewed the manuscript and approved the final draft.

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Declarations

Competing interests

The authors declare no competing interests.

Human ethics

This study involving human participants was conducted according to the Declaration of Helsinki and was approved by the Institutional Ethics Board of the First Affiliated Hospital of Anhui Medical University (Approval code: 20190146, March 2019).

Consent for publication

Written informed consents were obtained from all individual participants or the primary relatives.

Additional information

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