

"KARAGANDA MEDICAL UNIVERSITY" NC JSC

ABSTRACT

for the dissertation submitted for the degree of Doctor of Philosophy (PhD)

«Diagnostic Significance of Changes in Blood Plasma Metabolites in Acute Cerebrovascular Diseases»

Educational Program: 8D10100 - Medicine

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Relevance of the Study.

Acute cerebrovascular diseases (ACVD) remain among the leading causes of mortality and disability worldwide. According to contemporary epidemiological studies, approximately 12 million new cases of stroke occur annually, while the total number of stroke survivors reaches 94 million. More than 7 million people die from stroke each year, and the overall disease burden exceeds 160 million disability-adjusted life years (DALYs) [1, 2].

Despite advances in modern medicine, the global burden of stroke has increased over recent decades: between 1990 and 2021, stroke incidence increased by 70%, mortality by 44%, and disability-adjusted life years (DALYs) by 32%. The particular significance of this problem is underscored by the high lifetime risk of stroke, reaching 25%, as well as its occurrence among individuals of working age.

Despite the widespread implementation of neuroimaging techniques, early diagnosis of ischemic stroke during the first hours after symptom onset remains challenging, particularly in cases without visible computed tomography changes and in the presence of stroke mimics [3, 4]. Currently, there are no widely available laboratory biomarkers capable of improving the accuracy of early and differential diagnosis of acute cerebrovascular diseases [5].

In this context, the investigation of metabolic alterations, particularly the amino acid profile reflecting early pathobiochemical processes during cerebral ischemia, represents a promising direction for the development of novel diagnostic approaches.

The greatest clinical interest lies in studying metabolic alterations specifically during the hyperacute phase of stroke, when decisions regarding reperfusion therapy are made and the capabilities of routine diagnostic methods remain limited.

Amino acids are of particular interest as potential early biomarkers of ACVD because they are directly involved in key mechanisms of ischemic brain injury, including excitotoxicity, neuroinflammation, endothelial dysfunction, impaired energy metabolism, and oxidative stress. It has been shown that alterations in the amino acid profile occur at early stages of ischemia and may reflect pathobiochemical processes before pronounced structural changes become detectable by neuroimaging [6].

The selection of amino acids for the targeted panel was based on their pathophysiological roles in cerebral ischemia. Glutamic acid is one of the key mediators of excitotoxicity and neuronal injury in ischemic stroke [7]. Arginine is considered an important substrate for nitric oxide (NO) synthesis and a marker of endothelial dysfunction, while disturbances in its metabolism are associated with vascular and inflammatory changes in stroke [8]. Tryptophan is involved in the kynurenine pathway, which is associated with neuroinflammation, immune response, and the severity of ischemic brain injury [9]. Phenylalanine and branched-chain amino acids, including valine, reflect disturbances in energy and catabolic metabolism during acute cerebral injury and are considered promising metabolomic biomarkers of stroke [8]. Additional interest in lysine stems from its involvement in carnitine synthesis and its indirect association with mitochondrial energy metabolism [10].

To assess the amino acid profile, high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) was used, offering high sensitivity, specificity, and the ability to simultaneously quantify low-molecular-weight metabolites in biological samples. HPLC-MS is widely used in contemporary metabolomic studies of stroke and enables the identification of early metabolic alterations with potential diagnostic significance [11].

Despite growing interest in metabolomic studies of stroke, data on the diagnostic significance of the amino acid profile during the hyperacute phase of ACVD remain limited and inconsistent. Most studies were conducted at later stages of the disease, were based on small samples, or did not account for the dynamic nature of metabolic changes.

Thus, the search for early metabolic biomarkers reflecting the pathobiochemical mechanisms of cerebral ischemia, as well as the development of clinical and laboratory models for the early diagnosis of ischemic stroke incorporating metabolic biomarkers, represents a relevant direction in modern vascular neurology and underscores the need for the present study.

Aim of the study: to investigate the targeted plasma amino acid profile in patients during the early period of ACVD in order to identify early metabolic markers of stroke, determine their diagnostic significance for the timely verification and differential diagnosis of clinical forms of the disease, and develop a clinical and laboratory model integrating metabolomic parameters.

Objectives of the Study:

1. To perform a comprehensive clinical, laboratory, and metabolomic analysis of patients with ACVD and the control group (CG), including assessment of the diagnostic value of standard laboratory parameters, determination of plasma concentrations of the targeted amino acid panel by HPLC-MS at two time points during the early period of the disease (3-4.5 hours and 12 hours after the onset of neurological symptoms), as well as analysis of intergroup differences and associations among the studied parameters.
2. To assess the clinical significance of changes in the amino acid profile in patients with different forms of ACVD based on the analysis of metabolite dynamics and their associations with the severity of neurological deficit according to the NIHSS and with routine laboratory diagnostic parameters during the hyperacute period of the disease.
3. To evaluate the diagnostic value and comparative performance of routine diagnostic and metabolomic parameters in the early diagnosis of ACVD using ROC analysis, with determination of cut-off values, sensitivity, specificity, and AUC for differentiating between different types of ACVD and the CG.
4. To develop and validate a clinical and laboratory model for the early diagnosis of patients with ACVD based on the integration of clinical, standard laboratory, and metabolomic parameters.

Object of the Study: patients with ACVD with confirmed diagnoses of ischemic stroke (IS), hemorrhagic stroke (HS), or transient ischemic attack (TIA), enrolled at the stroke centers of Akzhan Multidisciplinary Medical Center and the Professor Kh. Zh. Makazhanov Multidisciplinary Hospital of the Health Department of the Karaganda Region, as well as control group participants without signs of ACVD who underwent outpatient assessment at medical organizations in the Karaganda Region from January 2024 to June 2025.

Scientific novelty:

1. For the first time, it was established that the plasma amino acid profile during the early period of ACVD is characterized by features specific to individual forms of the disease and by temporal dynamics: specific patterns of metabolic alterations in IS, HS, and TIA were identified; the most informative amino acid markers (phenylalanine, tryptophan, and arginine) were determined; and the influence of biomaterial sampling time on their diagnostic performance was demonstrated.

2. For the first time, a clinical and laboratory model for the early diagnosis of IS was developed and validated based on the integration of clinical, standard laboratory, and metabolomic parameters, demonstrating high discriminative performance (AUC = 0.940).

3. A method for the simultaneous targeted determination of glutamic acid, valine, lysine, phenylalanine, arginine, and tryptophan in blood plasma using HPLC-MS was developed and validated, and a certificate confirming the inclusion of relevant information in the State Register of Rights to Copyright-Protected Objects was obtained for this method.

Theoretical Significance

The study results expand current understanding of ACVD pathophysiology by demonstrating that alterations in the amino acid profile during the hyperacute phase reflect distinct features of the metabolic response across different forms of the disease. The established phase-dependent nature of metabolic changes within the first 12 hours after stroke onset highlights the importance of the temporal factor in interpreting biochemical markers of acute cerebrovascular injury.

Practical Significance

1. The identified form-specific alterations in the plasma amino acid profile of patients with ACVD during the hyperacute phase may be used as additional biochemical markers for the early differential diagnosis of various forms of ACVD within the limited therapeutic window.

2. The established dependence of the diagnostic performance of amino acids on biomaterial sampling time highlights the need for dynamic assessment of metabolic parameters during the early period of the disease and may be considered when planning laboratory evaluation of patients with ACVD.

3. The developed clinical and laboratory model incorporating metabolomic parameters for the differential diagnosis of IS (NIHSS, ESR, valine, and arginine) demonstrates high diagnostic accuracy (AUC = 0.94; sensitivity, 89.3%; specificity,

89.7%) and enables quantitative estimation of disease probability. The model may be used as a clinical decision-support tool and requires further external validation.

4. The obtained results may be used to develop rapid diagnostic approaches intended for use in emergency medical care settings, which requires further research and technological validation.

Main Provisions Submitted for Defense

1. It was established that, as early as 3-4.5 hours after ACVD onset, the concentrations of valine ($p = 0.007$), glutamic acid ($p < 0.001$), phenylalanine ($p = 0.0009$), arginine ($p = 0.016$), and tryptophan ($p = 0.002$) differed significantly among patients with IS, HS, and TIA and the CG, indicating a form-specific pattern of the early amino acid response. At 12 hours, intergroup differences persisted for valine ($p = 0.003$), glutamic acid ($p = 0.0006$), phenylalanine ($p = 0.043$), and tryptophan ($p = 0.005$).

2. It was demonstrated that the dynamics of the amino acid profile during the first 12 hours after ACVD onset exhibit a form-specific pattern: in IS, a decrease in valine concentration ($p = 0.008$) and increases in arginine ($p = 0.00015$) and tryptophan ($p = 0.007$) levels were observed; in TIA, increases in valine ($p = 0.019$), phenylalanine ($p = 0.001$), and tryptophan ($p = 0.0003$) concentrations were observed; in HS, a statistically significant change was identified only for tryptophan ($p = 0.010$), whereas glutamic acid concentration remained stable across all study groups ($p > 0.05$).

3. It was established that the inclusion of metabolomic parameters in the clinical and laboratory model significantly improves the diagnostic performance for verification of IS relative to the CG, increasing the area under the ROC curve from 0.734 to 0.803 ($\Delta AUC = 0.072$; 95% CI, 0.019-0.125; $p = 0.007$) and sensitivity from 63.1% to 77.0%, while maintaining comparable specificity.

4. The developed and validated clinical and laboratory model for the early diagnosis of IS, based on the integration of clinical, standard laboratory, and metabolomic parameters, demonstrates high diagnostic accuracy in the differential diagnosis of IS (AUC = 0.940; sensitivity, 89.3%; specificity, 89.7%) and enables quantitative estimation of disease probability.

This dissertation was carried out within the framework of grant funding from the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan under the research project “Studying the effectiveness of using serum biomarkers and transcranial doppler ultrasound in the early diagnosis of acute cerebrovascular diseases” (IRN AP23490807, State Registration No. 0124PK00941, 2024-2026).

The ethical aspects of the study were approved by the Local Bioethics Committee of Karaganda Medical University (Protocol No. 19 dated November 11, 2024).

The dissertation topic was approved at the meeting of the Senate of Karaganda Medical University (Protocol No. 3 dated October 31, 2024).

The results of the dissertation research were presented at:

1. International Scientific and Practical Conference “Genetics and Physiology:

Past, Present and Future” (IGPH2025), Almaty, Republic of Kazakhstan (May 28, 2025).

2. 10th International Scientific and Practical Conference of the Caspian States “Current Issues of Modern Medicine,” Astrakhan, Russian Federation (May 29, 2025).

3. International Scientific and Practical Conference “From Science to Practice: Modern Perspectives and New Approaches,” Izhevsk, Russian Federation (November 25, 2025).

4. Scientific and Practical Conference of Young Scientists “Innovative Approaches in Medical Science and Education” within the framework of the International Congress “Multidisciplinary Approaches in Medicine: Integration into Clinical Practice, Science and Education,” Karaganda, Republic of Kazakhstan (April 17, 2026).

5. Extended meeting of the Council of the Institute of Life Sciences, Karaganda Medical University (Minutes No. 6, May 14, 2026).

Author’s Contribution

The author was directly involved in all stages of the dissertation research, from the analysis of national and international scientific literature to the interpretation of the obtained results. The author formulated the study aim, hypothesis, and objectives, developed the study design, and independently analyzed patients’ medical documentation.

The author personally created the electronic databases, performed data preparation for statistical analysis, conducted statistical analyses, and interpreted the obtained results. The author also participated in the writing, formatting, and submission of scientific publications related to the dissertation topic.

Implementation of Research Results into Practice

The results of the dissertation research have been implemented in the educational process of Karaganda Medical University and in the clinical practice of City Center for Primary Health Care LLP and Saran City Hospital. The findings are used in the training of undergraduate students, interns, and residents, as well as in the assessment of clinical, laboratory, and metabolic parameters in patients with ACVD during the hyperacute phase of the disease.

The implementation of the research results is confirmed by official implementation acts approved by the heads of the respective medical organizations.

Publications Related to the Dissertation

Based on the dissertation research, two scientific articles have been published, including one in a scientific journal recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, and one in an international peer-reviewed scientific journal indexed in the Scopus database (Q2, 91st percentile). As a result of the dissertation research, three certificates of entry of information into the State Register of Rights to Copyright-Protected Objects have been obtained.

Study Design, Materials and Methods

Participant recruitment was conducted at the stroke center of the Professor Kh.

Zh. Makazhanov Multidisciplinary Hospital and at the stroke center based at the Akzhan Multidisciplinary Medical Center in Karaganda, Kazakhstan, from January 2024 through June 2025 inclusive.

The study was conducted as a prospective clinical observational non-interventional study with elements of a case-control design. The study included patients with ischemic stroke (IS), hemorrhagic stroke (HS), transient ischemic attack (TIA), as well as individuals in the control group (individuals without signs of ACVD). Comprehensive clinical-neurological, laboratory, and instrumental assessments were performed, including assessment of the plasma amino acid profile during the hyperacute phase of the disease (3-4.5 and 12 hours after symptom onset). Blood samples were collected once from participants in the control group. ROC analysis, logistic regression, and internal model validation methods were used to assess the diagnostic performance of biomarkers.

The study was conducted in accordance with the principles of the Declaration of Helsinki and the current regulatory documents of the Republic of Kazakhstan.

Inclusion criteria for the IS group (n = 122):

- age over 18 years;
- NIHSS score ranging from 3 to 22 points;
- absence of CT and/or MRI signs of intracerebral hemorrhage or brain tumor at admission; neuroimaging signs of IS identified during follow-up within 24 hours of admission;
- time from the onset of neurological symptoms to study inclusion not exceeding 4.5 hours;
- written informed consent to participate in the study provided by the patient or the patient's relatives.

Inclusion criteria for the HS group (n = 50):

- age over 18 years;
- NIHSS score ranging from 3 to 22 points;
- absence of CT and/or MRI signs of IS or brain tumor at admission; neuroimaging signs of HS identified during follow-up within 24 hours of admission;
- time from the onset of neurological symptoms to study inclusion not exceeding 4.5 hours;
- written informed consent to participate in the study provided by the patient or the patient's relatives.

Inclusion criteria for the TIA group (n = 50):

- age over 18 years;
- NIHSS score ranging from 3 to 22 points;
- absence of CT and/or MRI signs of intracerebral hemorrhage, IS, or brain tumor at admission and within 24 hours of admission;
- time from the onset of neurological symptoms to study inclusion not exceeding 4.5 hours;
- written informed consent to participate in the study provided by the patient or the patient's relatives.

Inclusion criteria for the CG (individuals without signs of ACVD) (n = 75):

- age over 18 years;

- grade 3 arterial hypertension (AH);
- absence of focal neurological deficits on neurological examination;
- no history of ACVD, myocardial infarction, or other acute vascular diseases;
- absence of acute neurological or acute vascular diseases at the time of inclusion;
- written informed consent to participate in the study.

Exclusion criteria for all groups: presence of oncological or cardiovascular diseases (subacute bacterial endocarditis, acute pericarditis), gastrointestinal bleeding, significant liver dysfunction, end-stage renal failure, or other diseases that may affect the laboratory parameters under investigation.

All patients in the study groups underwent a comprehensive clinical evaluation, including collection of subjective complaints, medical and life history obtained from the patients and their relatives, as well as a detailed assessment of neurological status. Neurological examination was performed by a neurologist during the first hours after admission to the stroke center using a standard clinical protocol. The severity of neurological deficit was assessed at admission using the NIHSS.

Additionally, the medical records of the study patients were analyzed, including anamnestic data and the results of instrumental examinations (brain CT/MRI). As part of the study, complete blood count (CBC), biochemical blood test, and coagulation profile parameters were assessed at admission.

To evaluate the diagnostic performance of plasma biomarkers during the early period of ACVD, venous blood was collected twice from patients in the study groups: the first sample was collected no later than 4.5 hours after the onset of acute neurological symptoms, and the second sample was collected 12 hours after symptom onset.

Statistical analysis was performed using Statistica and jamovi software packages, as well as the R environment. Due to the non-normal distribution of most variables, nonparametric methods were applied, including the Kruskal-Wallis, Mann-Whitney, and Wilcoxon tests. Correlations were assessed using Spearman's correlation coefficient. The diagnostic significance of the studied parameters was evaluated using ROC analysis with calculation of AUC, sensitivity, and specificity. Independent predictors were identified using multivariable logistic regression with calculation of odds ratios (ORs) and 95% confidence intervals (95% CIs). Diagnostic models were compared using the DeLong test. Internal model validation was performed using the bootstrap method. Statistical significance was set at $p < 0.05$.

The study was approved by the Local Bioethics Committee, conducted in accordance with the principles of the Declaration of Helsinki, and all data were anonymized.

Results.

A total of 297 participants were included in the present study, including 122 patients with IS, 50 with HS, 50 with TIA, and 75 control group participants. The groups were comparable in terms of sex and age; a weak positive correlation was identified between age and the severity of neurological deficit ($r = 0.22$; $p < 0.05$). Women demonstrated greater severity of neurological deficit compared with men ($p = 0.007$).

The severity of neurological deficit differed significantly among the groups ($p < 0.001$): the most pronounced impairments were observed in HS (NIHSS 10.0 [7.0-15.0]), whereas the lowest scores were observed in TIA (3.0 [3.0-6.0]). The median time from symptom onset to hospitalization was 120 [80-160] minutes and did not differ among the groups.

According to neuroimaging findings at admission, signs of stroke were detected in 41.8% of patients. In HS, focal changes were visualized in nearly all cases; in IS, they were detected in less than half of patients (36.9%); and in TIA, they were absent. These findings reflect the limited capability of early neuroimaging to verify IS during the hyperacute phase. At 24 hours, neuroimaging signs of stroke were confirmed in the majority of patients with IS and HS.

Routine laboratory parameters were characterized predominantly by moderate nonspecific alterations. Statistically significant intergroup differences were identified in indicators of the systemic inflammatory response, including leukocyte count and erythrocyte sedimentation rate (ESR) ($p < 0.001$), which limits the independent diagnostic significance of standard laboratory parameters during the hyperacute phase.

During the hyperacute phase (no later than 3-4.5 hours after the onset of acute neurological symptoms), disease-form-specific alterations in the amino acid profile were identified:

- patients with IS demonstrated elevated phenylalanine levels (16.19 [12.80-19.17] vs. 13.64 [10.95-16.01] $\mu\text{mol/L}$ in the control group; $p < 0.01$);
- patients with HS demonstrated decreased glutamic acid levels (0.66 [0.57-0.88] vs. 0.98 [0.78-1.31] $\mu\text{mol/L}$ in the control group; $p < 0.05$);
- patients with TIA demonstrated decreased tryptophan levels ($p < 0.01$) and elevated arginine levels (0.31 [0.17-0.41] $\mu\text{mol/L}$; $p < 0.05$).

At 12 hours, intergroup differences in the concentrations of key amino acids persisted ($p < 0.05$), with HS showing a tendency toward lower values for most of the studied parameters.

Analysis of temporal dynamics revealed multidirectional changes in metabolites: in IS, valine levels decreased, and arginine levels increased; in TIA, valine and phenylalanine levels increased ($p < 0.05$), whereas tryptophan levels increased across all clinical groups.

Individual amino acids demonstrated limited diagnostic value (AUC 0.61-0.65); however, analysis of temporal dynamics showed that an increase in Δ arginine was associated with a higher probability of IS (OR = 2.29; $p = 0.017$), whereas an increase in Δ valine was associated with a lower probability (OR = 0.91; $p = 0.037$).

Clinical outcomes were characterized by pronounced intergroup heterogeneity: mortality was 22.0% in HS, 9.8% in IS, and was absent in TIA ($p = 0.0018$). Cerebral edema was the leading cause of death (73.9%).

Associations were identified between the amino acid profile and markers of systemic inflammation: valine, phenylalanine, and Δ arginine levels correlated with NLR at admission (r up to 0.57), indicating an association between metabolic alterations and the systemic inflammatory response.

Based on the identified factors, a clinical-laboratory model for the early diagnosis

of IS was developed. The baseline model demonstrated satisfactory discriminative ability (AUC = 0.734; sensitivity 63.1%; specificity 74.7%), whereas the inclusion of metabolic markers (phenylalanine and tryptophan) significantly improved the discriminative ability of the model (AUC = 0.803; sensitivity 77.0%; specificity 73.2%; $p = 0.007$).

Independent predictors of IS were leukocyte count (OR = 1.24), phenylalanine (OR = 1.34), and tryptophan (OR = 0.78), reflecting their multidirectional contributions to the probability of developing the disease.

In the differential diagnosis of IS and HS, the combined clinical-metabolic model (systolic blood pressure, prothrombin time, and valine) demonstrated moderate diagnostic performance (AUC = 0.732; sensitivity 72.9%; specificity 71.4%).

At the final stage of the study, a combined clinical-laboratory model integrating metabolic parameters for the differential diagnosis of IS was developed. The model included neurological deficit severity according to the NIHSS, ESR, and plasma concentrations of valine and arginine.

Independent predictors of IS were NIHSS score (OR = 1.67; $p < 0.001$), ESR (OR = 1.11; $p = 0.006$), valine (OR = 1.13; $p < 0.001$), and arginine (OR = 0.78; $p = 0.012$), reflecting the combined contribution of clinical, inflammatory, and metabolic factors to the probability of IS.

The developed model demonstrated high diagnostic performance (AUC = 0.94; 95% CI 0.91-0.97), with a sensitivity of 89.3% and a specificity of 89.7% at a cut-off value of 0.451.

Internal validation using the bootstrap method confirmed the stability of the model (AUC = 0.94; 95% CI 0.90-0.97) and the reproducibility of its diagnostic characteristics.

The resulting model formed the basis of a mathematical algorithm for the early diagnosis of IS.

Conclusions:

1. At the first time point (3-4.5 hours), patients with IS demonstrated an increase in phenylalanine levels to 16.19 [12.80-19.17] $\mu\text{mol/L}$ compared with 13.64 [10.95-16.01] $\mu\text{mol/L}$ in the control group ($p < 0.01$); patients with HS demonstrated a decrease in glutamic acid levels to 0.66 $\mu\text{mol/L}$ compared with 0.98 $\mu\text{mol/L}$ in the control group ($p < 0.05$); and patients with TIA demonstrated decreased tryptophan and increased arginine levels ($p < 0.05$). Intergroup differences in individual metabolites persisted at 12 hours after symptom onset, confirming the dynamic nature of metabolic alterations in ACVD.

2. An increase in Δ arginine was associated with an increased probability of IS (OR = 2.29; $p = 0.017$), whereas an increase in Δ valine was associated with a decreased probability of IS (OR = 0.91; $p = 0.037$). Statistically significant associations were identified between the amino acid profile and the severity of neurological deficit according to the NIHSS, with the strongest association observed for tryptophan ($r = -0.31$; $p < 0.05$). In patients with an unfavorable outcome, tryptophan concentrations were statistically significantly lower at both study time points ($p < 0.01$).

3. It was established that the inclusion of metabolic markers in the diagnostic model improves the effectiveness of early ACVD diagnosis compared with the use of routine laboratory parameters alone, increasing the AUC from 0.734 to 0.803 ($p = 0.007$), with an increase in sensitivity from 63.1% to 77.0% while maintaining comparable specificity (74.7% and 73.2%, respectively).

4. The developed combined clinical-laboratory model for the diagnosis of IS with integrated metabolic parameters, incorporating NIHSS, ESR, valine, and arginine, demonstrates high discriminative ability (AUC = 0.94; 95% CI, 0.91-0.97; sensitivity, 89.3%; specificity, 89.7%). Internal validation using the bootstrap method confirmed its robustness and reproducibility (AUC = 0.94; 95% CI, 0.90-0.97), while maintaining its diagnostic characteristics (sensitivity, 89.3%; specificity, 89.6%).

REFERENCES

1. GBD 2021 Demographics Collaborators. Global age-sex-specific mortality, life expectancy, and population estimates in 204 countries and territories and 811 subnational locations, 1950-2021, and the impact of the COVID-19 pandemic: a comprehensive demographic analysis for the Global Burden of Disease Study 2021 // *Lancet*. - 2024. - Vol. 403, № 10440. - P. 19892056.

2. GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019 // *Lancet Neurology*. - 2021. - Vol. 20, № 10. - P. 795-820.

3. Powers W.J., Rabinstein A.A., Ackerson T., Adeoye O.M., Bambakidis N.C., Becker K. et al. Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association // *Stroke*. - 2019. - Vol. 50, № 12. - P. e344-e418..

4. Demeestere J., Wouters A., Christensen S., Lemmens R., Lansberg M.G. Review of Perfusion Imaging in Acute Ischemic Stroke: From Time to Tissue // *Stroke*. - 2020. - Vol. 51, № 3. - P. 1017-1024.

5. Campbell B.C.V., Khatri P. Stroke // *Lancet*. - 2020. - Vol. 396, № 10244. - P. 129-142.

6. Ding L., Zhang M., Fan B., Deng F., Li Z., Han Y. et al. Metabolomics reveals key biomarkers for ischemic stroke: a systematic review of emerging evidence // *Frontiers in Neurology*. - 2025. - Vol. 16. - 1630390.

7. Neves D., Salazar I.L., Almeida R.D., Silva R.M. Molecular mechanisms of ischemia and glutamate excitotoxicity // *Life Sciences*. - 2023. - Vol. 328. - Art. 121814.

8. Wang X., Liu T., Song H., Cui S., Liu G., Christoforou A. et al. Targeted Metabolomic Profiling Reveals Association Between Altered Amino Acids and Poor Functional Recovery After Stroke // *Frontiers in Neurology*. - 2019. - Vol. 10. - 1425.

9. Grigolashvili M., Seryogina A., Beisembayeva M., Muratbekova S., Turkevich I., Shayakhmetova Y. The Role of Activation of the Tryptophan-Kynurenine Axis in the Pathogenesis of Acute Cerebrovascular Diseases: A

Literature Review // Journal of Clinical Medicine of Kazakhstan. - 2025. - Vol. 22, № 1. - P. 21-27.

10. Nelson D.L., Cox M.M. Lehninger Principles of Biochemistry. - 8th ed. - New York: W.H. Freeman and Company, 2021. - 1190 p.

11. Alseekh S., Aharoni A., Brotman Y., Contrepois K., D'Auria J., Ewald J. et al. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices // Nature Methods. - 2021. - Vol. 18, № 7. - P. 747-756.