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# Linking Immunological Parameters and Recovery of Patient's Motor and Cognitive Functions In The Acute Period of Ischemic Stroke

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## Summary

**Objective**. To evaluate the relationship between immunological parameters and functional outcome in patients with varying severity of ischemic stroke based on statistical methodology.

**Materials and methods.** The prospective study included 78 patients diagnosed with ischemic stroke, who were distributed into two groups: group 1 — 38 mild stroke patients, NIHSS score < 5, group 2 – 40 moderate stroke patients, NIHSS score 5–15. Signs of stroke severity, degree of disability, cognitive decline, and activities of daily living were chosen as criteria to estimate the functional outcome by calculating the difference between the NIHSS, mRS, MoCA, and BI scales at the time of admission and on Day 12 of hospital stay. Lab tests included assessment of plasma concentrations of CXC and CC subfamilies of cytokines, interleukins and TNF-a on Day 2 of hospital stay. Machine learning algorithms, the Python programming language, the Pandas and SciPy libraries, and discriminant analysis were used for statistical processing.

**Results.** The following parameters were found significant: concentrations of IL-1b and MPIF-1/CCL23 for group 1, and concentrations of IL-16, MPIF-1/CCL23, Eotaxin-2/CCL24, Gro-a/CXCL1 and IL-8/CXCL8 for group 2 patients. Positive correlation was established between NIHSS dynamics and concentrations of TNF-$\alpha$ ($R$=0.227, $P$=0.001), MPIF-1/CCL23 ($R$=0.380, $P$=0.00061) and Gro-a/CXCL1 ($R$=0.211, $P$=0.00001), and between changes in mRS and concentrations of MPIF-1/CCL23 ($R$=0.277, $P$=0.00006), Gro-a/CXCL1 ($R$=0.211, $P$=0.0075) and IL-16 ($R$=0, 211, $P$=0.00001). There was a significant negative correlation between cognitive dysfunction and concentrations of Eotaxin-2/CCL24 ($R$=–0.378, $P$=0.00075), Gro-a/CXCL1 ($R$=–0.313, $P$=0.0035), and IP-10/CXCL1 ($R$=–0.214, $P$=0.00023), and between limited activities of daily living (BI) and concentrations of MPIF-1/CCL23 ($R$=–0.345, $P$=0.0024) and Gro-a/CXCL1 ($R$=–0.210, $P$=0.00001).

**Conclusion.** Chemokines form the CC family — MPIF-1/CCL23 and Eotaxin-2/CCL24, and the CXC-Gro-a/CXCL1 and IL-16 clusters are the principal cytokines associated with the dynamics of patient's motor and cognitive functions recovery in the acute period of ischemic stroke. Although obtained results demonstrate negative effect of increased MPIF-1/CCL23, Gro-a/CXCL1, IL-16 and Eotaxin-2/CCL24 concentrations on the improvement of motor and cognitive impairments, further studies are needed to verify the CXC and CC subfamilies chemokines as prognostic markers of patient's functional outcome in the acute period of ischemic stroke.

***Keywords: ischemic stroke; functional outcome; severity of stroke; cytokine; biomarker***

**Conflict of interest.** The authors declare no conflict of interest.

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## Introduction

Ischemic stroke (IS) dominates the structure of cerebrovascular disease, causing disability and death worldwide [1]. In addition to abnormal blood flow and coagulation, inflammatory and neuroimmune processes mediated and regulated by proinflammatory cytokines play important roles in the pathogenesis of ischemic circulatory disorders [2].

Increased levels of cytokines, including chemokines and cell adhesion molecules, which are directly related to the severity and extent of cerebral infarction, exacerbate ischemic brain damage and have an impact on the functional outcome of stroke [3–5]. Currently, there is a large body of evidence describing the role of interleukins in the development of cerebral ischemia [6, 7].

Another promising direction is to investigate the relationship between stroke severity and chemokine production, which influences immune cell activation, differentiation, and migration [8–10]. More than 60 chemokines with different structures and biological properties have been identified in

humans. According to the current classification, they have four subfamilies, two of which are CXC and CC [11].

Results of immunologic studies in patients with IS show a direct correlation between the expression of CC subfamily chemokines, CCL3 (MIP-1$\alpha$), CCL5 (RANTES), CCL7 (MCP-3), such as CCL13 (MCP-4), CCL14 (HCC-1), CCL15 (LKN-1) and CCL23 (MPIF-1), and stroke severity [12–14]. The CXC subfamily chemokines most associated with ischemic mechanisms are CXCL1 (Gro-a), CXCL-2 (Gro-b), CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC), CXCL12 (SDF-1a+b), CXCL16 (SCYB16), and CXCL8 (IL-8) [15]. Understanding the processes of differential expression of cytokines of different subfamilies in patients with acute stroke will help to expand the understanding of the role of the immune response in the pathogenesis of IS, as well as to identify individual cytokines or their combinations as potential biomarkers for assessing the severity of ischemia and risk stratification of adverse outcomes after stroke.

The use of multivariate discriminant analysis based on machine learning (ML) techniques is currently a promising area in basic medicine [16]. Machine learning has been used primarily in clinical settings for diagnosis and prognosis, greatly improving the ability to predict the risk of developing various diseases, their progression, and functional outcome [17].

The aim of the study was to evaluate the influence of immunological parameters on the functional outcome of patients with different severity of ischemic stroke using statistical methods.

## Materials and Methods

The prospective cohort study was approved by the Independent Ethical Committee of the Clinical Research Center of the Immanuel Kant Baltic Federal University (Protocol No. 34, dated September 29, 2022) and conducted from October 2022 to February 2023.

The study included 78 primary vascular center patients diagnosed with ischemic stroke. The study sample size was not pre-specified.

To verify the subtype of MI according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [18], routine clinical and diagnostic examinations were performed.

On admission, patients underwent neurological examination and routine diagnostic tests, including brain CT/MRI, transcranial Doppler study of extra- and intracranial vessels, 12-lead ECG, complete blood count and biochemistry, and pulse oximetry.

Additional tests included MR/CT angiography, echocardiography, ECG Holter monitoring, detailed coagulation studies, evaluation for systemic diseases, blood homocysteine measurement, and lumbar puncture, if indicated. Thrombolytic therapy was not administered due to contraindications or hospitalization beyond the therapeutic window.

Neuroimaging parameters were obtained by computed tomography (CT) and magnetic resonance imaging (MRI). Initial ischemic changes in the middle cerebral artery were assessed using the ASPECTS (Alberta stroke program early CT score) scale.

Functional status of all patients was assessed on admission and at discharge using standard scales [19]. Stroke severity was assessed by the National Institutes of Health Stroke Scale (NIHSS), disability severity by the modified Rankin Scale (mRS), cognitive decline by the Montreal Cognitive Assessment (MoCA) scale, and activities of daily living by the Barthel Index (BI).

Patients were divided into two groups based on the NIHSS score. Group 1 included 38 patients with mild neurological deficit (NIHSS $<5$ points), while group 2 included 40 patients with moderate neurological impairment (NIHSS 5–15 points). The baseline NIHSS score was 4 [3; 4] points in group 1 and 10 [7; 13] points in group 2. Patients in groups 1 and 2 were comparable in terms of demographic and clinical characteristics (Table 1).

Inclusion criteria were clinical signs and symptoms consistent with a diagnosis of ischemic stroke; age 60 to 80 years; NIHSS score $\leqslant 15$; full consciousness at the time of the study.

Exclusion criteria were pre-existing neurological and psychiatric conditions, hemorrhagic stroke and transient ischemic attack, vertebrobasilar IS, gross motor and/or sensory aphasia.

The criteria for the functional outcome of acute ischemic stroke were the change in the patient's condition, expressed in absolute values by calculating the difference between the NIHSS, mRS, BI, and MoCA parameters at the time of admission and at day 12 of hospitalization (gain/decrease index, delta, $\triangle$) ($\triangle$MoCA, $\triangle$NIHSS, $\triangle$BI, $\triangle$mRS).

The laboratory study included measurement of levels of biologically active molecules (cytokines) in blood plasma. Blood samples were taken on the 2$^{nd}$ day of hospitalization. Interleukins (IL-1b, IL-2, IL-4, IL-6, IL-16), chemokines of CC subfamily (MCP-1/CCL2, MIP-1a/CCL3, MCP-3/CCL7, MCP-4/CCL13, MIP-1d/CCL15, MPIF-1/CCL23, Eotaxin-2/CCL24, Eotaxin/CCL11) and CXC subfamily (Gro-a/CXCL1, Gro-b/CXCL-2, IP-10/CXCL10, SCYB16/CXCL16, IL-8/CXCL8), and TNF-$\alpha$ were measured.

The analysis was performed by flow fluorimetry on an automated dual-beam laser analyzer (Bio-Plex® 200 Systems, Bio-Rad, USA) using a commercial test system (Bio-Plex Human Panel, 40-Plex Assay, Bio-Rad, USA). Results are expressed in pg/mL.

Statistical analysis was performed using the standard SPSS Statistics V23.0 for Windows package, the Python programming language, the Pandas and SciPy libraries, and methods of multivariate analysis using machine learning (ML) algorithms.

The distribution of quantitative variables was assessed using the Shapiro–Wilk test. Variables with normal

distribution were reported as arithmetic mean *(M)* and standard deviation *(SD)*. Data with normal distribution were compared using ANOVA variance test for dependent and independent samples.

For non-normal distribution, quantitative variables were reported as median *(Me)* and lower and upper quartiles *[Q1–Q3]*. For non-normal distribution, the non-parametric Wilcoxon test was used. The Mann–Whitney *U*-criterion was used to compare two groups for a non-normal variable. Differences in frequencies between two independent groups were analyzed using the two-tailed Fisher's exact test, $\chi^2$ test with Yates' correction. The level of statistical significance was set at $P<0.05$. Bonferroni correction ($P<0.0125$) was used for multiple comparisons of variables to eliminate false positives.

The Z-score was not used to calculate the MoCA test and to prevent the occurrence of type II error (false-negative conclusion) due to the lack of control group reference values.

The correlation coefficient *(r)* was calculated to assess the association of functional outcome parameters on the NIHSS, mRS, BI, and MoCA scales with serum cytokine levels on day 2 of hospitalization.

The *r*-value was between –1 and 1, where 1 is a complete inverse correlation, 0 is no correlation, and 1 is a complete direct correlation. The Fechner method was chosen to evaluate the correlation of continuous variables, including those measured in points. The standard *P* value of 0.05 was chosen as the significance level. Correlation coefficients with a *P* value greater than 0.05 were discarded.

Correlation analysis was performed separately for each group, considering four groups of predictors. Grouping according to functional outcome parameters was not performed because the statistical results did not significantly affect the general pattern of correlations.

Discriminant analysis using ML algorithms was performed in two steps. First, the gradient boosting method [20] with interpretable results was used to process continuous variables of serum cytokine levels. Second, the Boruta thresholding and significance identification method [21] was used to determine the significance of a parameter and eliminate spurious correlations.

The significance of a parameter was defined as the total information gain due to its selection. Missing and incomplete values were not present in the original data set.

## Results

At the start of the study, all patients were stable after initial treatment. No deaths were observed during hospitalization.

Based on the tests performed, the following clinical and neuroimaging manifestations of ischemic stroke were identified (Table 1).

Group 2 patients had a significant reduction in cognitive function according to the MoCA scale ($P<0.001$), daily activity according to BI ($P<0.001$), and disability severity according to mRS ($P<0.001$) compared to group 1. No significant differences in other parameters were found between the groups ($P>0.05$).

Discriminant analysis using ML showed that the most significant parameters (based on information gain, IG) in group 1 patients were IL-1b and MPIF-1/CCL23 levels. In group 2 patients, the levels of MPIF-1/CCL23, Eotaxin-2/CCL24, Gro-a/CXCL1, IL-8/CXCL8 and IL-16 were found to be relevant (Fig. 1).

In summary, the immunologic parameters directly related to stroke severity were CD4 (IL-16), CXCR2 (Gro-a/CXCL1), CXCR1-2 (IL-8/CXCL8), CCR3 (Eotaxin-2/CCL24), and CCR1 (MPIF-1/CCL23) receptor chemoattractants.

The values of NIHSS, mRS, MoCA, and BI scores at admission and day 12 of hospitalization are shown in Table 2.

Analysis of the changes in the main clinical scores revealed a significant increase in MoCA and BI scores and a decrease in NIHSS and mRS scores after initial therapy and early rehabilitation in patients in both groups (Fig. 2).

**Table 1. Demographic and clinical characteristics of patients with carotid ischemic stroke in groups 1 and 2.**

| Parameter | Values in groups | | *P* value |
|---|---|---|---|
| | Group 1 (*N*=38) | Group 2 (*N*=40) | |
| **Demographic feature** | | | |
| Men, *N* (%) | 23 (60.5 ) | 24 (60.0 ) | 0.964 |
| Women, *N* (%) | 15 (39.5 ) | 16 (40.0 ) | 0.964 |
| Mean age | 68.32±5.62 | 66.81±4.92 | 0.212 |
| **IS subtype (TOAST), *N* (%)** | | | |
| IS due to atherosclerosis of large arteries (atherothrombotic) | 8 (21.1) | 13 (32.5) | 0.256 |
| IS due to cardiogenic embolism (cardioembolic) | 20 (52.6) | 14 (35.0) | 0.117 |
| IS due to occlusion of small arteries (lacunar) | 7(18.4) | 11 (27.5) | 0.340 |
| IS of undetermined etiology | 3 (7.9) | 2 (5.0) | 0.601 |
| **Clinical stroke scores, *Me [Q1; Q3]*** | | | |
| Barthel Index, BI | 83 [70; 100] | 76 [55; 80] * | 0.001 |
| Modified Rankin scale, mRS | 1.6 [0; 3] | 3.5 [1; 5] * | 0.008 |
| MoCA | 23 [16; 29] | 21 [18; 25] * | <0.001 |
| NIHSS | 4 [3; 4] | 10 [7; 13]* | <0.001 |
| ASPECTS | 8 [8; 9] | 8 [7; 9] | 1.000 |

**Note.** * — significant differences between groups.

Analysis of the changes in the parameters of the main clinical scales showed a significant increase in MoCA and BI, and a decrease in NIHSS and mRS after standard therapy and early rehabilitation.

When studying the correlations between the initial cytokine levels and the functional outcome parameters based on MoCA, NIHSS, BI, mRS scores (△MoCA, △NIHSS, △BI, △mRS) in both groups, correlations of different strength and direction were revealed.

In group 1 patients, a significant correlation was found between MPIF-1/CCL23 levels and △mRS ($r$=0.217, $P$=0.0004), △BI ($r$=–0.225, $P$<0.0001) and △NIHSS ($r$=0.214, $P$<0.0001). There was a negative correlation between Gro-a/CXCL1 levels ($r$=–0.213, $P$=0.005) and changes in cognitive function (△MoCA). The mRS outcome scores (△mRS) were positively correlated with IL-16 levels ($r$=0.244, $P$=0.0007, Fig. 3).

Correlations between functional outcome parameters and levels of CXC subfamily chemokines in group 2 patients are shown in Figure 3.

In group 2 patients, there was a significant negative correlation between the MoCA cognitive deficit score (△MoCA) and the levels of eotaxin-2/CCL24 ($r$=–0.388, $P$=0.00075), Gro-a/CXCL1 ($r$=–0.319, $P$=0.0035), and IP-10/CXCL1 ($r$=–0.274, $P$=0.00023). A significant inverse correlation of △BI values with the levels of MPIF-1/CCL23 ($r$=–0.345, $P$=0.0024) and Gro-a/CXCL1 ($r$=–0.210, $P$=0.00001) was also observed. We found a significant direct correlation of △mRS with MPIF-1/CCL23 ($r$=0.294, $P$=0.00006), Gro-a/CXCL1 ($r$=0.230, $P$=0.0075) and IL-16 ($r$=0.200, $P$=0.00001) levels. Furthermore, a positive correlation of △NIHSS functional outcome scores with levels of TNF-$\alpha$ ($r$=0.227, $P$=0.001), MPIF-1/CCL23 ($r$=0.288, $P$=0.00061) and Gro-a/CXCL1 ($r$=0.214, $P$=0.00001) was observed.

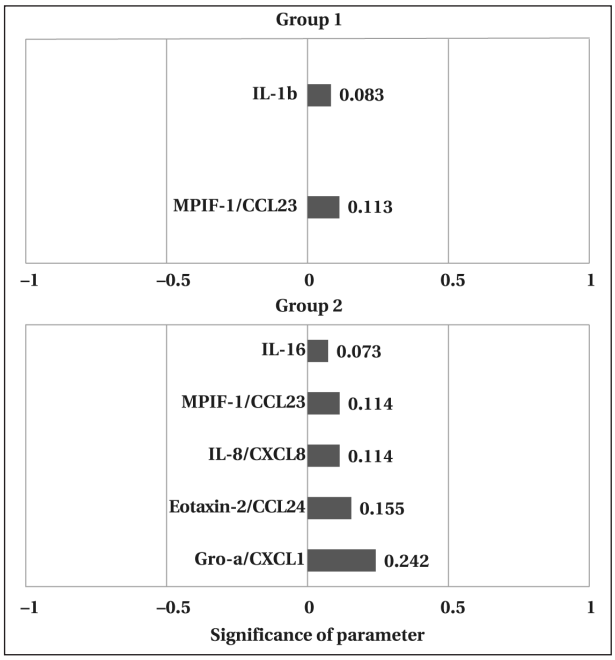


**Fig. 1. Relevant cytokine levels in patients with mild and moderate IS on day 2 of hospitalization.**

## Discussion

Predicting the functional outcome of patients with ischemic stroke is a challenging task for most clinicians due to a poor understanding of the pathogenetic mechanisms of ischemia development and the lack of clear prognostic algorithms [22–24]. The multifaceted nature of factors influencing the functional outcome of the disease necessitates the use of structured and combined techniques for personalized assessment of patient status in early IS [25].

The study of patient status using tools such as mRS, Barthel Index and MoCA showed that in patients with moderate stroke severity, the neurological and cognitive status is characterized by a predomi-

**Table 2. Comparative characteristics of clinical parameters at admission and on day 12 of hospital stay**

| Parameters (points) | Values in groups | | | | *P* value |
|---|---|---|---|---|---|
| | Group 1 (*N*=38) | | Group 2 (*N*=40) | | |
| | Day 1 | Day 12 | Day 1 | Day 12 | |
| BI | 83 [70; 100] | 93 [80; 100] | 76 [55; 80] | 87 [75; 100] | $P_1$=0.0004*<br>$P_2$⩽0.0001*<br>$P_3$=0.163 |
| mRS | 1.6 [0; 3] | 0.5 [0; 1] | 3.5 [1; 5] | 2 [0; 4] | $P_1$⩽0.0001*<br>$P_2$⩽0.0001*<br>$P_3$⩽0.001* |
| MoCA | 23 [16; 29] | 24 [15; 29] | 21 [18; 25] | 22.5 [11; 27] | $P_1$=0.0057*<br>$P_2$=0.0016*<br>$P_3$=0.034 |
| NIHSS | 4 [3; 4] | 2 [0; 4] | 10 [7; 13] | 5 [0; 9] | $P$⩽0.0001*<br>$P_2$⩽0.0001*<br>$P_3$⩽0.0001* |

**Note.** $P_1$ – significant difference between the parameters on the 1st and 10th days of of hospital stay in group 1; $P_2$ — significant difference between the parameters on the 1st and 10th days of hospital stay in group 2; $P_3$ — significant difference of parameters between groups on the 12th day of hospitalization; * — statistically significant differences between the groups.

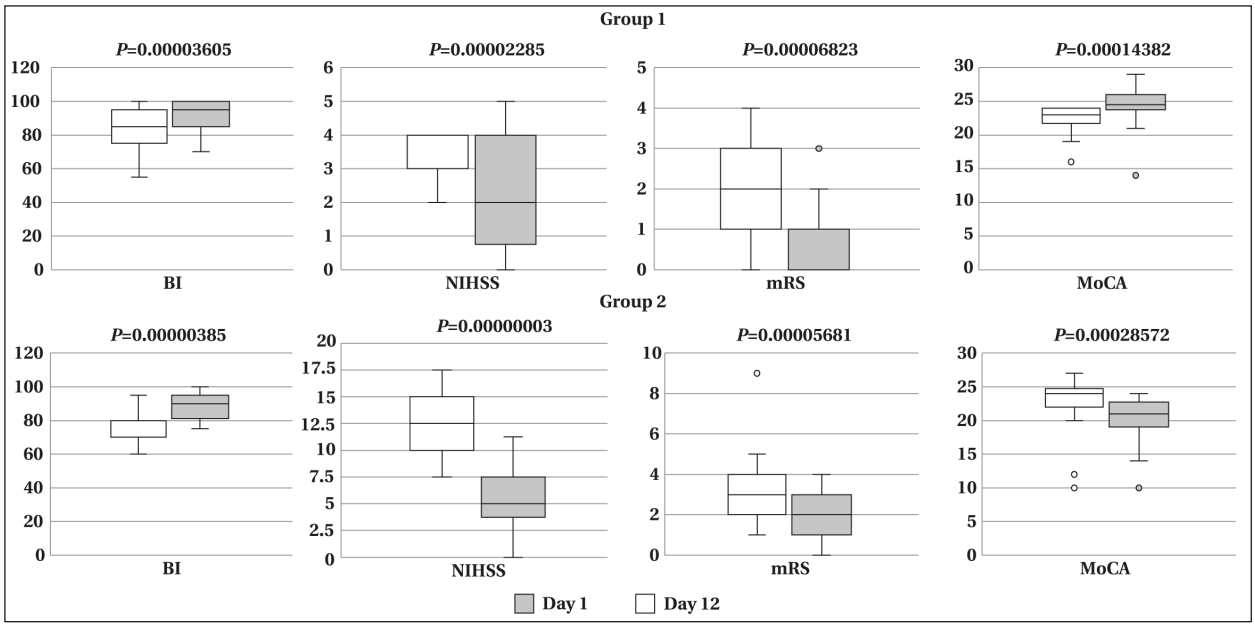


**Fig. 2. Changes in the values of the main clinical scales before and after treatment.**

nant decrease in daily activity, cognitive function and degree of independence, confirming the association of these parameters with the NIHSS level.

The results obtained are consistent with other studies showing that currently the main predictors of patient recovery are motor and cognitive impairment, age, severe aphasia, and baseline daily activity [26–28]. However, the use of rating scales is not sufficient to develop a rational prognostic model of stroke recovery.

Thus, the NIHSS scale, which is a universal tool for monitoring the effectiveness of therapy, is

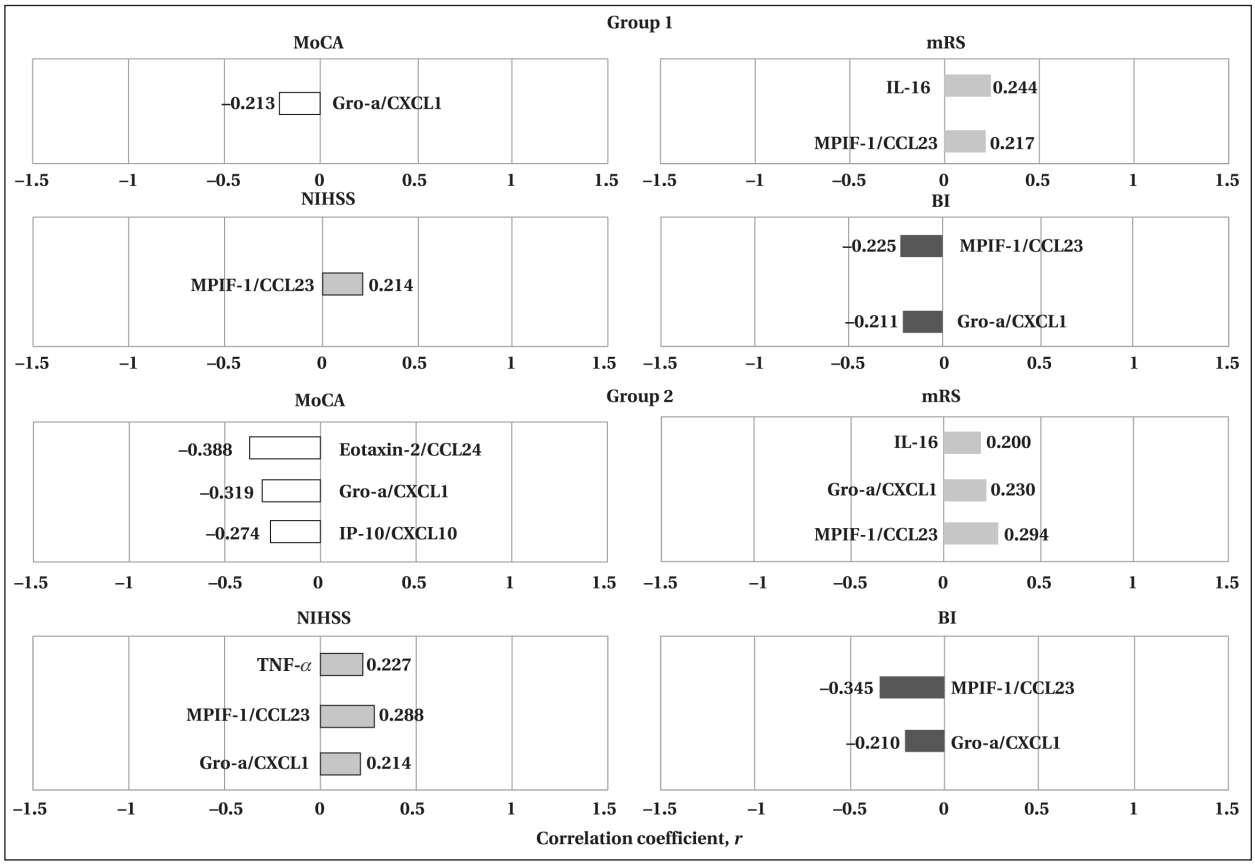


**Fig. 3. Correlation between cytokine levels (pg/mL) and main clinical scales (points) in patients with mild *(a)* and moderate *(b)* IS.**

of limited value in assessing symptoms of lesions in the anterior and posterior arterial supply territories and the nondominant brain hemisphere [29]. Scales that have demonstrated reliability and validity for various activities of daily living and stroke outcomes (BI and mRS) are not sensitive enough to assess cognitive profile, speech and visual function [30]. The MoCA scale has insufficient specificity for the advanced diagnosis of cognitive dysfunction and is generally used as a screening tool for moderate cognitive impairment.

Therefore, in order to optimize the prediction of functional outcome of IS, it is necessary to expand the range of predictive markers and, in addition to rating scale scores, to consider laboratory parameters as informative criteria of patient recovery in acute IS [31]. To improve the reliability of prognosis, it is reasonable to rely on mathematical modeling algorithms and discrete function construction based on clinical and other data.

Modern trends in the study of immunological and biological mechanisms of ischemia development point to new directions in the identification of biomarkers of functional outcome in patients with various severity of stroke and the role of cytokines in the regulation of pathogenetic mechanisms of ischemia [32, 33].

In the current study, the results of the assessment of interleukin levels using machine learning methods revealed the importance of IL-16 levels in patients with moderate stroke severity. The association of elevated IL-16 levels with neurological deficits has been attributed to its direct effect on the expression of inflammatory cytokines TNF-$\alpha$, IL-1β, and IL-6, which exacerbate ischemia and brain damage [34, 35].

A study of the expression of CCL cluster chemokines showed that the main members of this group associated with stroke severity were MPIF-1/CCL23 and Eotaxin-2/CCL24. The chemokine ligand CCL23, which has chemotactic activity against T lymphocytes, monocytes and neutrophils and stimulates the production of proinflammatory cytokines and adhesion molecules, is currently considered a new promising biomarker for early diagnosis of brain lesions [36, 37]. The results of the present study demonstrate a direct correlation between the increase in MPIF-1/CCL23 levels and reduced daily activity and independence levels, as measured by mRS and BI scales, in patients with moderate severity of IS. The results obtained are consistent with previous studies showing a positive correlation of MPIF-1/CCL23 expression with NIHSS scores and a negative correlation with BI scores, suggesting the use of this chemokine as a tool for predicting functional outcome in patients with ischemic stroke [38, 39].

Eotaxin-2/CCL24 is a potent chemoattractant for eosinophils, basophils, and lymphocytes in many tissues, including the brain [40]. Despite the lack of convincing data on changes in eotaxin-2 levels during acute IS in the current literature, our results show a clear increase in serum eotaxin-2/CCL24 in patients with moderate ischemia, as well as a correlation between its level and cognitive dysfunction as measured by the MoCA scale.

The most likely explanation for these results is the experimentally demonstrated effect of eotaxin-2 on the mechanisms of atherogenesis by inducing the expression of toll-like receptor 4 (TLR4) with subsequent endothelial dysfunction and atherosclerosis progression [41].

Levels of CXC subfamily chemokines (Gro-a/CXCL1 and IL-8/CXCL8) were important parameters associated with stroke severity. A clear inverse correlation was found between Gro-a/CXCL1 levels and cognitive function score as measured by the MoCA scale and level of daytime activity as measured by the Barthel scale.

Gro-a/CXCL1, acting through CXCR2 receptors, is a potent chemoattractant and activator of neutrophils. Along with macrophages, neutrophils are the predominant immune cells in the ischemic zone and are directly involved in the mechanisms of atherogenesis, aseptic inflammation and thrombosis in acute ischemia [42, 43].

Previously, a number of researchers have identified the role of CXCL1 in the production of reactive oxygen species, which in turn induce and modulate neuroinflammation [44]. Recent studies demonstrate the correlation between CXCL1 expression levels in acute IS and the volume of hypodense brain areas according to neuroimaging data [45]. The increased serum Gro-a/CXCL1 in patients with acute IS reflects early systemic production of CXCL1. No less significant are the results of studies on the involvement of the CXCR1 ligand in the mechanisms of neurogenesis [46, 47].

In experimental animal models, Gro-a/CXCL1 expression has been demonstrated in the hippocampal dentate gyrus and microglia following induced brain injury, including hypoxic-ischemic injury [48]. Several clinical studies have produced similar results, demonstrating an increase in CXCL1 in human hippocampal neural progenitor cells and detecting CXCL1 expression in the cerebrospinal fluid of Alzheimer's patients [49].

Thus, previous experimental and clinical data are consistent with our findings and may explain the association of Gro-a/CXCL1 expression with cognitive dysfunction and lower BI scores in patients with moderate severity of IS.

IL-8 is a chemotactic cytokine similar to Gro-a/CXCL1 that attracts neutrophils. Elevated levels of IL-8/CXCL8 promote chemotaxis of inflammatory

cells, resulting in neutrophil infiltration into the ischemic area, which exacerbates the local inflammatory response and stroke [50]. There is experimental and clinical evidence showing a positive correlation between the severity of clinical impairment and disability and serum IL-8 levels [51]. We found that CXCL8 was associated with stroke severity, but without correlation with other clinical scales.

**Limitations.** The main limitation was the small number of patients due to limited laboratory diagnostic capacity and the exclusion of patients with severe stroke from the study.

## Conclusion

The main cytokines associated with changes in functional and cognitive parameters in patients with acute IS are CC family chemokines such as MPIF-1/CCL23 and Eotaxin-2/CCL24 and CXC cluster chemokines such as Gro-a/CXCL1 and IL-16. The initial increase in MPIF-1/CCL23 and Gro-a/CXCL1 levels negatively affects the recovery of neurological deficits, daily activity and independence of patients regardless of the severity of IS. IL-16 expression is predominantly associated with Modified Rankin Scale disability scores. Elevated eotaxin-2/CCL24 levels were more strongly associated with cognitive performance in patients with moderate IS.

Despite the results demonstrating the negative effect of MPIF-1/CCL23, Gro-a/CXCL1, IL-16 and Eotaxin-2/CCL24 elevation on the improvement of motor and cognitive impairment, further studies are needed to identify chemokines of the CXC and CC subfamilies as prognostic markers for patient functional outcome in acute IS.

The implementation of machine learning methods in neurological practice may result in accurate and accessible predictions of stroke patients' functional outcomes, which is a key goal of contemporary clinical medicine and healthcare. Discriminant analysis of a wide range of disease-related variables will enable clinicians to more accurately predict a stroke patient's potential for recovery without the need for time-consuming diagnostic techniques.

**References**


1. *Клочихина О. А., Шпрах В. В., Стаховская Л. В., Полунина О. С., Полунина Е. А.* Динамика показателей заболеваемости инсультом и смертности от него за восьмилетний период на территориях, вошедших в федеральную программу реорганизации помощи пациентам с инсультом. *Acta Biomedica Scientifica*. 2021; 6 (1): 75–80. [*Klochikhina O. A., Shprach V. V., Stakhovskaya L. V., Polunina O. S., Polunina E. A.* Dynamics of stroke morbidity and mortality over an eight-year period in the territories included in the Federal program for the reorganization of care for stroke patients. *Acta Biomedica Scientifica*. 2021; 6 (1): 75–80. (in Russ.)]. DOI: 10.29413/ABS.2021–6.1.10.
2. *Song S. H., Kim J. H., Lee J. H., Yun Y. M., Choi D. H., Kim H. Y.* Elevated blood viscosity is associated with cerebral small vessel disease in patients with acute ischemic stroke. *BMC Neurol*. 2017; 17 (1): 20. DOI: 10.1186/s12883-017-0808-3. PMID: 28143595.
3. *Голубев А. М., Гречко А. В., Захарченко В. Е., Канарский М. М., Петрова М. В., Борисов И. В.* Характеристика кандидатных молекулярных маркеров при ишемическом и геморрагическом инсульте. Obshchaya Reanimatologiya = General Reanimatology. 2021; 17 (5): 23–34. [*Golubev A. M., Grechko A. V., Zakharchenko V. E., Kanarsky M. M., Petrova M. V., Borisov I. V.* Comparative characterization of candidate molecular markers in ischemic and hemorrhagic stroke. *General Reanimatilogy/ Obshchaya Reanimatologya*. 2021; 17 (5): 23–34. (in Rus. & Engl.)]. DOI: 10.15360/1813-9779-2021-5-23-34.
4. *Maida C. D., Norrito R. L., Daidone M., Tuttolomondo A., Pinto A.* Neuroinflammatory mechanisms in ischemic stroke: focus on cardioembolic stroke, background, and therapeutic approaches. *Int J Mol Sci*. 2020; 21 (18): 6454. DOI: 10.3390/ijms21186454. PMID: 32899616.
5. *Прилуцкая И. А., Крюк Ю. Я.* Уровни фактора некроза опухолей $\alpha$ у больных ишемическим инсультом. Медицинская иммунология. 2019; 21 (4): 755–764. [*Prilutskaya I. A., Kryuk Yu. Ya.* Levels of tumor necrosis factor alpha in patients with ischemic stroke. *Medical immunology/Meditsinskaya Immunologiya*. 2019; 21 (4): 755–764. (in Russ.]. DOI: 10.15789/1563-0625-2019-4-755-764.
6. *Быкова А. Ю., Кулеш А. А., Кайлева Н. А., Куклина Е. М., Шестаков В. В.* Взаимосвязь динамики сывороточных концентраций интерлейкина-1β, интерлейкина-6 и интерлейкина-10 с клиническими данными в остром периоде ишемического инсульта в зависимости от стратегии реперфузионной терапии. *Бюллетень сибирской медицины*. 2020; 18 (4): 16–25. [*Bykova A. Yu., Kulesh A. A., Kayleva N. A., Kuklina E. M., Shestakov V. V.* Interrelation of the dynamics of serum concentrations of interleukin-1β, interleukin-6 and interleukin-10 with clinical data in acute period of ischemic stroke and the strategy of reperfusion therapy. *Bulletin of Siberian Medicine/ Bulleten Sibirskoy Meditsiny*. 2020; 18 (4): 16–25. (in Russ.)]. DOI: 10.20538/1682-0363-2019-4-16-25.
7. *Вознюк И. А., Ильина О. М., Коломенцев С. В.* Ишемический инсульт как клиническая форма и патогенетическая модель в структуре поражения центральной нервной системы при COVID-19. *Вестник восстановительной медицины*. 2020; 4 (98): 90–98. [*Voznyuk I. A., Ilyina O. M., Kolomentsev S. V.* Ischemic stroke as a clinical form and pathogenetic model in the structure of central nervous system lesions in COVID-19. *Bulletin of Restorative Medicine/ Vestnik Vosstanovitelnoy Meditsiny*. 2020; 4 (98): 90–98. (in Russ.)]. DOI: 10.38025/2078-1962-2020-98-4-90-98.
8. *Ransohoff R. M., Trettel F.* Editorial Research Topic «Chemokines and chemokine receptors in brain homeostasis». *Front Cell Neurosci*. 2015; 9: 132. DOI: 10.3389/fncel.2015. 00132. PMID: 25904848.
9. *Голубев А. М., Петрова М. В., Гречко А. В., Захарченко В. Е., Кузовлев А. Н., Ершов А. В.* Молекулярные маркеры ишемического инсульта. *Obshchaya Reanimatologiya = General Reanimatology*. 2019; 15 (5): 11–22. [*Golubev A. M., Petrova M. V., Grechko A. V., Zakharchenko V. E., Kuzovlev A. N., Ershov A. V.* Molecular markers of ischemic stroke. *General Reanimatology/Obshchaya Reanimatologya*. 2019; 15 (5): 11–22. (in Rus.& Engl.)]. DOI: 10.15360/1813-9779-2019-5-11-22.
10. *Jayaraj R. L., Azimullah S., Beiram R., Jalal F. Y., Rosenberg G. A.* Neuroinflammation: friend and foe for ischemic stroke. *J Neuroinflammation*. 2019; 16 (1): 142. DOI: 10.1186/s12974-019-1516-2. PMID: 31291966.
11. *Hughes C. E., Nibbs, R. J. B.* A guide to chemokines and their receptors. *FEBS J*. 2018; 285 (16): 2944–2971. DOI: 10.1111/febs. 14466. PMID: 29637711.
12. *Feng Y. Q., Xu Z. Z., Wang Y. T., Xiong Y., Xie W., He Y. Y., Chen L., et al.* Targeting C-C chemokine receptor 5: key to opening the neurorehabilitation window after ischemic stroke. *Front Cell Neurosci*. 2022; 16: 876342. DOI: 10.3389/fncel.2022. 876342. PMID: 35573839.
13. *Chen C., Chu S. F., Liu D. D., Zhang Z., Kong L. L., Zhou X., Chen N. H.* Chemokines play complex roles in cerebral ischemia. *Neurochem Int*. 2018;

112: 146–158. DOI: 10.1016/ j.neuint.2017.06.008. PMID: 28629771.
14. *Воробьев С. В., Янишевский С. Н., Кудрявцев И. В., Шубина К. М., Антушева М. С., Кузнецова Р. Н., Серебрякова М. К., с соавт.* Участие иммунного ответа в патогенезе ишемического инсульта. *Медицинский Совет*. 2023; (3): 8–16. [*Vorobyov S. V., Yanishevsky S. N., Kudryavtsev I. V., Shubina K. M., Antusheva M. S., Kuznetsova R. N., Serebryakova M. K., et al.* Involvement immune response in the pathogenesis of ischemic stroke. *Medical Council/ Meditsinskiy Sovet*. 2023; (3): 8–16. (in Russ.)]. DOI: 10.21518/ms2023-024.
15. *Amin M., Vakilian A., Mahmoodi M. H., Hassanshahi G., Falahati-Pour S. K., Dolatabadi M. R., Nadimi A. E.* Circulatory levels of C-X-C motif chemokine ligands 1, 9, and 10 are elevated in patients with ischemic stroke. *Eurasian J Med*. 2017; 49 (2): 92–96. DOI: 10.5152/eurasianjmed. 2017. PMID: 28638249.
16. *Sidey-Gibbons J. A. M., Sidey-Gibbons C. J.* Machine learning in medicine: a practical introduction. *BMC Med Res Methodol*. 2019; 19 (1): 64. DOI: 10.1186/s12874-019-0681-4. PMID: 30890124.
17. *Deo R. C.* Machine learning in medicine. *Circulation*. 2015; 132 (20): 1920–30. DOI: 10.1161/CIRCULATIONAHA.115.001593. PMID: 26572668.
18. *Adams H. P., Jr Bendixen B. H., Kappelle L. J., Biller J., Love B. B., Gordon D. L., Marsh E. E. 3rd*. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*. 1993; 24 (1): 35–41. DOI: 10.1161/01.str.24.1.35. PMID: 7678184.
19. *Kasner S. E.* Clinical interpretation and use of stroke scales. *Lancet Neurol*. 2006; 5 (7): 603–612. DOI: 10.1016/S1474-4422 (06)70495–1. PMID: 16781990.
20. *Chen T., Guestrin* C. XGBoost: a scalable tree boosting system. *Proceedings of the 22nd ACM SIGKDD International Conference on knowledge discovery and data mining (KDD '16)*. Association for computing machinery, New York, NY, USA. 2016: 785–794. DOI: 10.1145/2939672.2939785.
21. *Kursa M. B., Jankowski A., Rudnicki W. R.* Boruta — a system for feature selection. *Fundamenta Informaticae*. 2010; 101 (4): 271–285. DOI: 10.3233/FI-2010-288.
22. *Shin S., Lee Y., Chang W. H., Sohn M. K., Lee J., Kim D. Y., Shin Y. I., et al.* Multifaceted assessment of functional outcomes in survivors of first-time stroke. *JAMA Netw Open*. 2022; 5 (9): e2233094. DOI: 10.1001/jamanetworkopen.2022. 33094. PMID: 36149652.
23. *Yeh H. J., Huang N., Chou Y. J., Cheng S. P., Lee W. K., Lai C. C., Cheng C. C.* Older age, low socioeconomic status, and multiple comorbidities lower the probability of receiving inpatient rehabilitation half a year after stroke. *Arch Phys Med Rehabil*. 2017; 98 (4): 707–715. DOI: 10.1016/j.apmr. 2016.08.468. PMID: 27633939.
24. *Alonso A., Ebert A. D., Kern R., Rapp S., Hennerici M. G., Fatar M.* Outcome predictors of acute stroke patients in need of intensive care treatment. *Cerebrovasc Dis*. 2015; 40 (1–2): 10–17. DOI: 10.1159/000430871. PMID: 26022716.
25. *Глущенкова Н. В., Саркисян О. Г., Гончарова З. А.* Злокачественный ишемический инсульт: клинические и биохимические особенности диагностики. *Южно-Российский журнал терапевтической практики*. 2023; 4 (2): 35–45. [*Glushenkova N. V., Sarkisian O. G., Goncharova Z. A.* Malignant ischemic stroke: clinical and biochemical features of diagnosis. *South Russian Journal of Therapeutic Practice/Yuzhno-Rossiyskiy Zhurnal Terapevticheskoy Praktiki*. 2023; 4 (2): 35–45. (in Russ.)]. DOI: 10.21886/2712-8156-2023-4-2-35-45.
26. *Kongsawasdi S., Klaphajone J., Wivatvongvana P., Watcharasaksilp K.* Prognostic factors of functional outcome assessed by using the modified rankin scale in subacute ischemic stroke. *J Clin Med Res*. 2019; 11 (5): 375–382. DOI: 10.14740/jocmr3799. PMID: 31019633.
27. *Harvey R. L.* Predictors of functional outcome following stroke. *Phys Med Rehabil Clin N Am*. 2015; 26 (4): 583–98. DOI: 10.1016/j.pmr. 2015.07.002. PMID: 31019633.
28. *Chen W. C., Hsiao M. Y., Wang T. G.* Prognostic factors of functional outcome in post-acute stroke in the rehabilitation unit. *J Formos Med Assoc*. 2022; 121 (3): 670–678. DOI: 10.1016/ j.jfma.2021.07.009. PMID: 34303583.
29. *Abzhandadze T., Reinholdsson M., Stibrant Sunnerhagen K.* NIHSS is not enough for cognitive screening in acute stroke: a cross-sectional, retrospective study. *Sci Rep*. 2020; 10 (1): 534. DOI: 10.1038/s41598-019-57316-8. PMID: 31953508.
30. *Siniscalchi A.* Use of stroke scales in clinical practice: current concepts. *Turk J Emerg Med*. 2022; 22 (3): 119–124. DOI: 10.4103/2452-2473.348440. PMID: 35936953.
31. *Тынтерова А. М., Моисеева Е. М., Голубев А. М., Шушарина Н. Н.* Роль эндотелинергических и нитроксидергических реакций в прогнозировании функционального исхода пациентов с различной степенью тяжести ишемического инсульта. *Obshchaya Reanimatologiya = General Reanimatology*. 2023; 19 (5). [*Tynterova A. M., Moiseeva E. M., Golubev A. M., Shusharina N. N.* The role of endothelinergic and nitroxydergic reactions in predicting the functional outcome in patients with ischemic stroke of different severity. *General Reanima-*

*tology/Obshchaya Reanimatologya*. 2023; 19 (5). (in Russ.&Engl.)]. DOI: 10.15360/1813-9779-2023-5-2354.
32. *Pawluk H., Woźniak A., Grześk G., Kołodziejska R., Kozakiewicz M., Kopkowska E., Grzechowiak E., et al.* The role of selected pro-inflammatory cytokines in pathogenesis of ischemic stroke. *Clin Interv Aging*. 2020; 15: 469–484. DOI: 10.2147/CIA.S233909. PMID: 32273689.
33. *Mizum A., Yenari M. A.* Anti-inflammatory targets for the treatment of reperfusion injury in stroke. *Front Neurol*. 2017; 8: 467–487. DOI: 10.3389/fneur.2017.00467. PMID: 28936196.
34. *Ганковская Л. В., Стаховская Л. В., Греченко В. В., Кольцова Е. А., Уварова О. С., Демина М. Д., Громова Т. В., с соавт.* Гиперэкспрессия TLR2 и TLR4 у больных с ишемическим инсультом в остром периоде заболевания. *Медицинская иммунология*. 2020; 22 (4): 665–674. [*Gankovskaya L. V., Stakhovskaya L. V., Grechenko V. V., Koltsova E. A., Uvarova O. S., Demina M. D., Gromova T. V., et al.* Hyperexpression of TLR2 and TLR4 in patients with ischemic stroke in the acute period of the disease. *Medical immunology/Meditsinskaya Immunologiya*. 2020; 22 (4): 665–674. (in Russ.)]. DOI: 10.15789/1563-0625-HOT-1971.
35. *Kleinschnitz C., Kraft P., Dreykluft A., Hagedorn I., Göbel K., Schuhmann M. K., Langhauser F., et al.* Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature. *Blood*. 2013; 121: 679–91. DOI: 10.1182/blood-2012-04-426734. PMID: 23160472.
36. *Faura J., Bustamante A., Penalba A., Giralt D., Simats A., Martínez-Sáez E., Alcolea D., et al.* CCL23: a chemokine associated with progression from mild cognitive impairment to Alzheimer's disease. *J Alzheimers Dis*. 2020; 73 (4): 1585–1595. DOI: 10.3233/JAD-190753. PMID: 31958084.
37. *Черных Е. Р., Шевела Е. Я., Морозов С. А., Останин А. А.* Иммунопатогенетические аспекты ишемического инсульта. *Медицинская иммунология*. 2018; 20 (1): 19–34. [*Chernykh E. R., Shevela E. Ya., Morozov S. A., Ostanin A. A.* Immunopathogenetic aspects of ischemic stroke. *Medical immunology/Meditsinskaya Immunologiya*. 2018; 20 (1): 19–34. (in Russ.)]. DOI: 10.15789/1563-0625-2018-1-19-34.
38. *Wang X., Yang Y., Zhao Z., Li P., Ma C., Zhu B.* Diagnostic value of serum MIF and CCL23 in the patients with acute cerebral infarction. *Clin Lab*. 2020; 66 (11). DOI: 10.7754/Clin.Lab.2020.200239. PMID: 33180438.
39. *Li Y. S., Chen W., Liu S., Zhang Y. Y., Li X. H.* Serum macrophage migration inhibitory factor levels are associated with infarct volumes and long-term outcomes in patients with acute ischemic stroke. *Int J Neurosci*. 2017; 127 (6): 539–546. DOI: 10.1080/00207454.2016.1211648. PMID: 27402018.
40. *Furer V., Hazan E., Mor A., Segal M., Katav A., Aloush V., Elkayam O., et al.* Elevated levels of eotaxin-2 in serum of fibromyalgia patients. *Pain Res Manag*. 2018; 2018: 7257681. DOI: 10.1155/2018/7257681. PMID: 29861805.
41. *Tsai C. S., Huang C. Y., Chen C. H., Lin Y. W., Shih C. M., Tsao N. W., Chiang K. H., et al.* Eotaxin-2 increased toll-like receptor 4 expression in endothelial cells in vitro and exacerbates high-cholesterol diet-induced atherogenesis *in vivo*. *Am J Transl Res*. 2016; 8 (12): 5338–5353 PMID: 28078007.
42. *Sreeramkumar V., Adrover J. M., Ballesteros I., Cuartero M. I., Rossaint J., Bilbao I., Nácher M., et al.* Neutrophils scan for activated platelets to initiate inflammation. Science. 2014; 346 (6214): 1234–1238. DOI: 10.1126/science.1256478. PMID: 25477463.
43. *Gill D., Sivakumaran P., Wilding P., Love M., Veltkamp R., Kar A.* Trends in C-reactive protein levels are associated with neurological change twenty-four hours after thrombolysis for acute ischemic stroke. *J Stroke Cerebrovasc Dis*. 2016; 25 (8): 1966–1999. DOI: 10.1016/J.JSTROKECEREBROVASDIS. 2016.05.003. PMID: 27212272.
44. *Boro M., Balaji K. N.* CXCL1 and CXCL2 regulate NLRP3 inflammasome activation via G-Protein-coupled receptor CXCR2. *J Immunol*. 2017; 199 (5): 1660–1671. DOI: 10.4049/jimmunol.1700129. PMID: 28739876.
45. *Zhu W., Nan Y., Wang S., Liu W.* Bioinformatics analysis of gene expression profiles of sex differences in ischemic stroke. *Biomed Res Int*. 2019; 2019: 2478453. DOI: 10.1155/2019/ 2478453. PMID: 31183363.
46. *Huang F., Lan Y., Qin L., Dong H., Shi H., Wu H., Zou Q., et al.* Astragaloside IV promotes adult neurogenesis in hippocampal dentate gyrus of mouse through CXCL1/CXCR2 signaling. *Molecules*. 2018; 23 (9): 2178. DOI: 10.3390/molecules23092178. PMID: 30158469.
47. *Zhang X. F., Zhao Y. F., Zhu S. W., Huang W. J., Luo Y., Chen Q. Y., Ge L. J., et al.* CXCL1 Triggers caspase-3 dependent tau cleavage in long-term neuronal cultures and in the hippocampus of aged mice: implications in Alzheimer's Disease. *J Alzheimers Dis*. 2015; 48 (1): 89–104. DOI: 10.3233/JAD-150041. PMID: 26401931.
48. *Shang Y., Tian L., Chen T., Liu X., Zhang J., Liu D., Wei J., et al.* CXCL1 promotes the proliferation of neural stem cells by stimulating the generation of reactive oxygen species in APP/PS1 mice. *Biochem Biophys Res Commun*. 2019; 515 (1): 201–206. DOI: 10.1016/j.bbrc.2019.05.130. PMID: 31146911.
49. *Korbecki J., Gąssowska-Dobrowolska M., Wójcik J., Szatkowska I., Barczak K., Chlubek M.,*

*Baranowska-Bosiacka I.* The importance of CXCL1 in physiology and noncancerous diseases of bone, bone marrow, muscle and the nervous system. *Int J Mol Sci*. 2022; 23 (8): 4205. DOI: 10.3390/ijms23084205. PMID: 35457023.

50. *Shaheen H. A., Daker L. I., Abbass M. M., Abd E. l., Fattah A. A.* The relationship between the severity of disability and serum IL-8 in acute ischemic stroke patients. *Egypt J Neurol Psychiatr Neurosurg*. 2018; 54 (1): 26. DOI: 10.1186/s41983-018-0025-z. PMID: 30294205.

51. *Zhang L., Xu D., Zhang T., Hou W., Yixi L.* Correlation between interleukin-6, interleukin-8, and modified early warning score of patients with acute ischemic stroke and their condition and prognosis. *Ann Palliat Med*. 2021; 10 (1): 148–155. DOI: 10.21037/apm-20-2200. PMID: 33440979.

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