Concentration of immunoglobulin free light chains in cerebrospinal fluid in the diagnosis of multiple sclerosis

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Objective: *to determine the sensitivity and specificity of method of determining the concentration of immunoglobulin free light chains (FLCs) in cerebrospinal fluid (CSF) in the diagnosis and differential diagnosis of multiple sclerosis (MS).*

Material and methods. *80 patients participated in the study. The main group consisted of 54 patients diagnosed with MS according to the 2017 McDonald criteria. The comparison group (n=26) comprised patients with other diseases of the nervous system. An enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of FLCs (kappa- and lambda-chains) in the CSF.*

Results. *In the group of patients with MS, an increase in the concentration of free kappa-chains (κ-FLCs) in the CSF was found compared to the comparison group ($p<0.001$). With an increase in the concentration of κ-FLCs, a decrease in the sensitivity and an increase in the specificity of the method for the diagnosis of MS was observed. The κ-FLCs cut-off value of 0.17 μg/ml had a sensitivity of 68.5 % and a specificity of 92.3 %. The cut-off value of 0.22 μg/ml had a sensitivity of 59.3 % and a specificity of 100 %. The concentrations of lambda-FLCs in the CSF in the MS group and in the comparison, group did not differ significantly ($p=0.1$).*

Conclusion. *The results obtained indicate an increase in the concentration of κ-FLCs in the CSF of MS patients. This biomarker showed a high specificity for this pathology. However, further development of optimal thresholds is required to clarify the diagnostic value of CSF κ-FLCs concentration in MS patients.*

Keywords: *multiple sclerosis; immunoglobulin free light chains; kappa-chain; lambda-chain; MacDonald criteria.*

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Introduction. Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) [1]. The prevalence of MS in Russia exceeds 70 cases per 100.000 population [2]. MS is the most common non-traumatic cause of persistent disability in young adults among CNS diseases [3].

The pathogenesis of MS involves inflammation, demyelination, and axonal degeneration of neurons, which underlie its clinical manifestations [4]. Currently, the diagnosis of MS is based on the 2017 McDonald criteria, which include the presence of clinical relapses, CNS lesions identified by magnetic resonance imaging (MRI), optic nerve damage verified by optical coherence tomography (OCT), and visual evoked potentials (VEP) [5]. The only laboratory test widely used in the diagnosis of MS is the detection of oligoclonal bands (OCB) of immunoglobulin G (IgG) in the cerebrospinal fluid (CSF) [5, 6]. However, the presence of OCB is highly sensitive but low in specificity for this disease. In addition, this assay is based on subjective qualitative analysis. Intrathecal synthesis of immunoglobulins can also be detected in several other inflammatory and autoimmune CNS diseases [6, 7, 8].

Timely diagnosis of MS is crucial, as early diagnosis, initiation of therapy, and prevention of disability progression are priority tasks for neurologists. Differential diagnosis remains a significant issue, as many diseases with similar clinical manifestations can mimic MS and lead to multifocal CNS lesions [9, 10]. Therefore, identifying new biomarkers for MS could improve the quality of MS diagnosis and facilitate timely treatment initiation. Immunoglobulins free light chains could serve as such markers.

Immunoglobulins are proteins produced by activated B-cells which perform various functions in the immune response. All immunoglobulin molecules consist of four polypeptide chains: two heavy and two light chains. There are five types of heavy chains in immunoglobulins: alpha, delta, gamma, mu, epsilon, which divide them into five classes: IgA, IgD, IgG, IgM, IgE. The molecule also contains one of the two types of free light chains: kappa or lambda [11].

In recent years, the content of free light chains of immunoglobulins in the CSF has been considered as a potential marker for MS, as well as a possible predictor of the disease's inflammatory activity [12].

Study Objective. The aim of the study is to determine the sensitivity and specificity of immunoglobulins free light chains in the CSF for the diagnosis of MS.

Patients and Methods. The study included 80 patients, of whom 45 were women and 35 were men. The main group consisted of 54 patients diagnosed with MS based on the 2017 McDonald criteria. The average age in the main group was

35.7±9.6 years (95% CI 33.1–38.3). The comparison group included 26 patients with other CNS diseases: autoimmune encephalitis (n=4), idiopathic transverse myelitis (n=4), idiopathic optic neuritis (n=4), neuromyelitis optica spectrum disorder (n=3), diffuse glioma (n=2), cerebral small vessel disease (n=2), acute disseminated encephalomyelitis (n=1), progressive solitary sclerosis (n=1), Alzheimer's disease (n=1), progressive multifocal leukoencephalopathy (n=1), metachromatic leukodystrophy (n=1), ischemic myelopathy (n=1), and migraine (n=1). The average age in the comparison group was 46.3±11.6 years (95% CI 41.6–50.9).

All study participants underwent a lumbar puncture with 3–5 ml of CSF collected. An enzyme-linked immunosorbent assay (ELISA) was used to determine the concentrations of immunoglobulins free light chains (kappa and lambda chains) in the CSF.

For comparison of the two groups in the analysis of quantitative data, the Mann–Whitney U test was used. Differences were considered statistically significant at $p<0.05$. Predictive models were developed using binary logistic regression to determine sensitivity and specificity, and ROC analysis was used to identify threshold values with optimal performance indicators. Predictive models were considered statistically significant at $p<0.05$. Statistical analysis was conducted using IBM SPSS Statistics software, version 26.

Results. The comparison of immunoglobulins free light chains levels depending on the presence of MS yielded the following data (Table 1)."

According to the data obtained, there were no statistically significant differences in the concentration of LFLC between the MS group and the comparison group ($p=0.1$). The concentration of KFLC in the CSF of patients with MS was significantly higher than in the comparison group ($p<0.001$).

The relationship between the diagnosis of MS and the level of kappa chains in the CSF is described by Equation (1):

$$P = 1 / (1 + e^{-z}) \cdot 100\%;$$
$$z = -0{,}92 + 10{,}7 \cdot X_{KC}, \quad (1)$$

where P represents the probability of MS diagnosis (%), and X_{KC} is the concentration of kappa chains in the CSF (μg/ml).

The derived regression model is statistically significant ($p<0.001$). According to Nagelkerke's coefficient of determination, 44.9% of the variance in the probability of diagnosing MS is determined by the factor included in Model (1). The sensitivity of Model (1) was 74.1%, and the specificity was 73.1%.

Comparison of immunoglobulin free light chain concentrations depending on the presence or absence of MS, Me [25th; 75th percentile]

Indicator	Main group (n=54)	Comparison group (n=26)	p
Kappa free light chains (KFLC), μg/ml	0,3 [0,08; 0,66]	0,05 [0,04; 0,1]	<0,001*
Lambda free light chains (LFLC), μg/ml	0,06 [0,03; 0,23]	0,05 [0,03; 0,23]	0,1

Note. * – differences are statistically significant ($p<0.05$)

Based on the regression coefficient value, the level of KFLC in the CSF had a direct correlation with the probability of diagnosing MS.

When evaluating the dependency of the probability of diagnosing MS on the concentration of KFLC in the CSF, the following ROC curve was obtained (Figure 1).

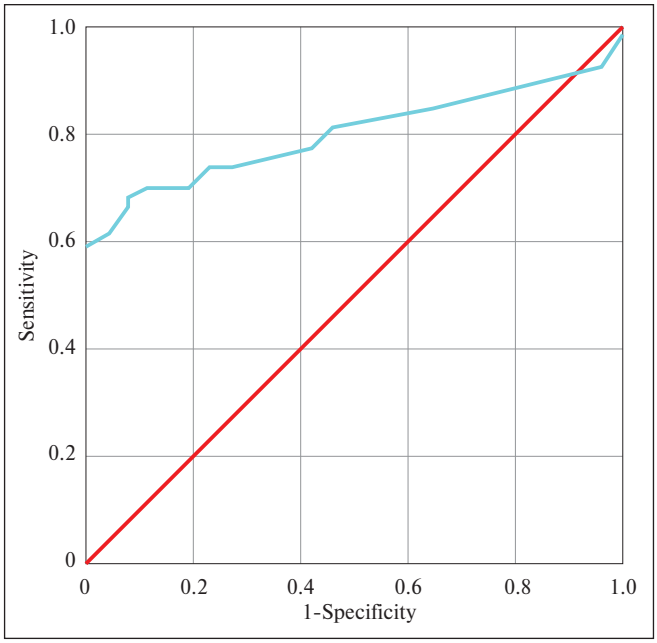
The ROC curve obtained was characterized by an AUC value of 0.8±0.05 (95% CI: 0.71–0.9). The model was statistically significant ($p<0.001$).

A KFLC concentration of 0.15 μg/ml provided a sensitivity of 70.4% and a specificity of 88.5%. An increase in kappa chain concentration was characterized by a decrease in sensitivity and an increase in specificity. A kappa chain threshold of 0.17 μg/ml had a sensitivity of 68.5% and a specificity of 92.3%. A threshold of 0.22 μg/ml had a sensitivity of 59.3% and a specificity of 100%.

Discussion. Evaluating immunoglobulins free light chains in the CSF as a potential biomarker for diagnosing MS is of interest, as there is currently no single specific laboratory test for this disease. Our study was conducted to determine the diagnostic significance of quantitative assessment of immunoglobulins free light chains in the CSF using enzyme-linked immunosorbent assay (ELISA) in patients with and without MS, and to determine the sensitivity and specificity of these indicators.

CSF examination is conducted in most cases when MS is suspected. In addition to assessing protein-cell composition, the clonality of IgG in the CSF and serum is studied.

Immunoglobulins in CSF can have various origins. Typically, immunoglobulins penetrate the blood-brain barrier (BBB) through passive transport. In MS, intrathecal synthesis of immunoglobulins often occurs, resulting in CSF-specific oligoclonal bands of IgG [6]. Detection of at least two OCB and the presence of MRI criteria for dissemination in space allow for the diagnosis of MS in patients with clinically isolated syndrome (CIS) [5]. However, OCB determination in the CSF has two



ROC curve characterizing the dependence of the probability of an MS diagnosis from the free kappa-chains concentration in the CSF

major drawbacks. Firstly, this laboratory technique requires parallel examination of paired CSF and serum samples, and typically takes about 4 hours to process results. Secondly, interpretation of the results depends on the subsequent subjective visual evaluation by the researcher and is qualitative rather than quantitative. Thus, these drawbacks necessitate considering a more objective standardized quantitative laboratory test for diagnosing MS.

The evaluation of immunoglobulins free light chains in the CSF in clinical diagnosis of MS has been extensively researched over the last twenty years. In several described clinical studies, the evaluation of the concentration of immunoglobulins free light chains in the CSF had comparable sensitivity and specificity with OCB investigation [12, 13]. Several methods for assessing the concentration of LFLC and KFLC have been described: the first using ELISA, the second by nephelometry, and the third by turbidimetry. In our study, we used ELISA and assessed the concentration of LFLC in the CSF. In our study no statistically significant differences were found between the MS group and the comparison group. Similar results were obtained in several other studies [12, 14, 15]. Likely, this is due to the insufficient sensitivity of the ELISA method. Thus, it can be concluded that this indicator is not diagnostically significant for MS, and quantitative assessment of the concentration of LFLC in CSF by ELISA cannot be used for diagnosing or differential diagnosis of MS.

In a systematic review dedicated to evaluating the concentration of KFLC, it was found that their elevated CSF level potentially provides accuracy up to 95% for MS and CIS [12]. In the large multicenter OFSEP study, which included 1621 patients (675 MS, 90 CIS, 297 other inflammatory CNS diseases, and 559 other non-inflammatory CNS diseases), convincing data were obtained that testing kappa chains is simpler, faster to perform, more reliable for differential diagnosis, and substantially cheaper than determining the type of oligoclonal IgG synthesis [16].

In our study, the concentration of KFLC in the CSF in the MS patient group was higher than in the comparison group, which was demonstrated by other authors as well [12, 13, 14]. Also, from the results presented in this study, it is evident that higher concentration of KFLC in the CSF results in a decreased sensitivity of MS diagnostics, and increased specificity. The results indicate that detecting increased KFLC concentrations in the CSF reflects the inflammatory process characteristic of MS and can be used as an additional laboratory biomarker in diagnosing this disease. However, optimal threshold values need to be developed to achieve the best combination of sensitivity and specificity. In our study, the indicator of 0.17 μg/ml provided a balance between sensitivity (68.5%) and specificity (92.3%). The presented result differs from the reference value of Russian laboratories (0.50 μg/ml).

The limitations of this study include the relatively small sample size, examination of immunoglobulins free light chains only in the CSF, determination of free light chains by ELISA, which has less accuracy compared to nephelometry.

Conclusion. The obtained results indicate an increase in the concentration of kappa free light chains of immunoglobulins in the CSF of patients with MS, having high specificity for this pathology. The conducted study, in our opinion, has created theoretical prerequisites for further study of kappa free light chains of immunoglobulins in MS, which in the future may serve as an additional diagnostic marker for MS and may be included in the diagnostic criteria.

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Conflict of Interest Statement

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