European Review for Medical and Pharmacological Sciences 2022; 26: 3933-3941

# Correlation of selected serum protein levels with the degree of disability and NEDA-3 status in multiple sclerosis phenotypes

P. LAMANCOVÁ, P. URBAN, J. MAŠLANKOVÁ, M. RABAJDOVÁ, M. MAREKOVÁ

Department of Medical and Clinical Biochemistry, P.J. Šafárik University, SNP 1, Košice, Slovakia

**Abstract. – OBJECTIVE: Multiple sclerosis (MS) is a multifactorial disease that begins in 80-85% of patients as a relapsing-remitting form (RRMS), and about 50% of patients gradually develop a secondary progressive form (SPMS). Approximately 10-20% of patients are affected primarily by the progressive form (PPMS) of this disease, which is characterised by a progressive course. This work focuses on the detection of potential protein biomarkers (CHI3L1, sNfL, CXCL13, MCP-1, MMP-2, and MMP-9) in the serum of patients with multiple sclerosis, divided according to phenotype.**

**PATIENTS AND METHODS: We detected serum (RRMS: n=40, SPMS: n=25, PPMS: n=15) concentrations of selected markers of demyelination and inflammation using ELISA and zymographic determination for accurate and reproducible recognition of individual forms of MS, as well as a comparison of their levels with a worsening of no evidence of disease activity (NEDA-3) status and patients' disability.**

**RESULTS: We detected that concentrations of sNfL in the blood of patients with PMS were higher than in those with RRMS (about 46%, $p<0.001$). The association with a worsening of NEDA-3 status was confirmed in the RRMS group by positive correlation of sNfL and the expanded disability status scale (EDSS) score (r=0.579, $p<0.01$). The levels of MCP-1 protein were not significantly different in patients with the RRMS to the progressive form of MS (r=0.58, $p=0.02$), while the levels of CHI3L1 in both the RRMS and PMS groups were significantly increased in groups with evidence of disease activity (RRMS about 76%, $p<0.001$ and PMS about 62%, $p<0.001$).**

**CONCLUSIONS: Earlier and non-invasive detection of serum biomarkers and their correlations with neurological disability can help to recognise the transition from RRMS to progressive forms of MS and complement the results of clinical and radiological follow-up of the patient and potentially help in monitoring the patient's response to the treatment.**

*Key Words:*
Multiple sclerosis, Serum markers, Demyelination, Serum markers, ELISA.

## Introduction

Demyelinating diseases have been a studied and widely discussed area of research for several decades. The most common demyelinating disease is multiple sclerosis (MS)[1]. MS is a multifactorial disease; the causes are individual, depending on genetic predisposition, as well as environmental and epigenetic factors. In the case of acute demyelinating disease, the myelin sheath can completely regenerate, after which it thins out. In chronic demyelinating diseases, repeated demyelination and remyelination cause Schwann cell proliferation, nerve fibre thickening and a loss of axons[2].

From a clinical point of view, MS begins in 80–85% of patients as a relapsing remitting form (RRMS), in which clinical relapses occur and functional recovery usually occurs. About 50% of patients with RRMS develop secondary progressive MS (SPMS)[3]. The other 10–20% of patients are affected by the primary progressive form of the disease (PPMS), which is characterised by a progressive course from the beginning of the disease[4]. The diagnosis of MS depends on the integration of clinical, imaging and laboratory findings. Primary diagnostic tests include magnetic resonance imaging (MRI), blood tests and spinal fluid lumbar puncture analysis. Another method is a CNS test, in which the presence of antibodies and proteins may signal an abnormal immune response, such as, e.g., the presence of oligoclonal bands (IgG)[5]. Secondary diagnostic criteria include evoked potential (EP) testing, optical coherence tomography and cognitive testing.

*Corresponding Author:* Peter Urban, Ph.D; e-mail: peter.urban@upjs.sk

Complementary tests include eye examinations, hearing tests, electrophysiology and cardiovascular examinations[6].

Molecular biomarkers are easily identifiable and can complement magnetic resonance imaging and clinical characteristics. In MS detection, almost all established biomarkers are proteins, often antibodies. However, a number of potential biomarkers at different stages of testing are promising[7,8]. Light chains of the neurofilaments (NfL) are the predominant components of the cytoskeleton. They are involved in the growth of axons, maintaining their stability and mediating the intracellular transport of molecules along axons[9]. In neuroaxonal damage during neurodegeneration, NfLs are released into the interstitial space, then into the cerebrospinal fluid (CSF) and finally into the blood[10]. Serum NfL (sNfL) appears to be the most promising biomarker in patients with MS[11]. The B cell chemoattractant (CXCL13, chemokine C-X-C motif ligand 13) is chemotactic for B cells belonging to subclasses B-1 and B-2. Elevated levels of this chemokine occur in MS patients, and it is hypothesised that early neutralisation of CXCL13 could interfere with the organisation and function of meningeal tertiary lymphoid organs, thus modifying and reducing inflammation in patients with CNS and MS[12]. Monocyte chemoattractant protein 1 (MCP-1) is a β-chemokine that, by binding to a CCR2 receptor, directly activates monocytes and other immune cells, such as memory T cells and natural killer cells, which promote inflammation. MCP-1 also induces the expression of adhesion molecules as well as IL-1, IL-6, TNF-α and other intracellular signal transduction pathways[13]. With special chemokine and chemokine receptor interference, it can prevent inflammatory cell infiltration, control the inflammation cascade, prevent demyelinating damage, and promote remyelination, which offers new hope in the treatment of MS[14].

Matrix metalloproteinases (MMPs) are Zn-dependent endopeptidases with proteolytic activity. They are involved in tissue remodelling under physiological and pathological conditions. In RRSM, they are involved in almost all pathological processes. These processes include disruption of the blood-brain barrier (BBB), perivascular lymphocyte infiltration and an enhanced chemotactic gradient, focal myelin damage leading to typical lesions and axonal disruption[15].

In MS patients treated with disease-modifying therapy (DMT) optimal treatment is measured using the No Evident Disease Activity (NEDA) status[16]. Three-compound No Evident Disease Activity (NEDA-3) status takes into account the absence of relapse, brain magnetic resonance imaging (MRI) activity and worsening disability.

The detection of MS is usually invasive, based on CSF collection, with imaging techniques and neurological assessment being the exceptions. This article focuses on the assessment of correlations of the concentration of multiple plasmatic proteins (CHI3L1, sNfL, CXCL13, MCP-1, MMP-2 and MMP-9) with the progression of MS and disease activity as defined by the concept NEDA-3 in a cohort of patients with the relapse-remitting and progressive forms of the disease.

## Patients and Methods

### Patients and Sampling

The set of patients comprised an experimental group of patients (n=80) with different phenotypes of multiple sclerosis (40 with relapse-remitting form, 25 with secondary progressive and 15 with primary progressive form). Blood samples were collected at the Department of Neurology of Louis Pasteur University Hospital in Košice using BD Vacutainer® EDTA test tubes (Becton Dickinson, Brea, CA, USA). Samples were processed by centrifugation at 3500 rpm/3 min/room temperature for serum separation, which was then stored at -70°C until measurements. Blood samples were pseudonymised and analysed without clinical data. Patients in the experimental group were divided into groups according to the MS phenotype (Table I). The study was approved by the Louis Pasteur University Hospital Ethics Committee (2020/EK/06044) and was performed in accordance with the Good Clinical Practice standard and the Declaration of Helsinki.

The inclusion criteria were: (1) diagnosis of relapse-remitting MS, primary or secondary-progressive MS phenotype according to the classification[17], (2) patients older than 18 years, (3) the ability to sign written informed consent. Exclusion criteria were the occurrence of severe comorbidities like depression, anxiety, cardiovascular diseases, and certain autoimmune disorders such as diabetes, thyroid disease, and inflammatory bowel disease. The baseline visit took place 12 months before the follow-up visit. Elapsed time was homogenous in all study participants. The baseline visit included compliance with the inclusion criteria, demographic data, and clini-

**Table I.** Distribution of patients in the experimental group according to type of MS.

| | RRMS | SPMS | PPMS |
|---|---|---|---|
| **Demographic characteristics** | | | |
| Sample size, n | 40 | 25 | 15 |
| Female, n (%) | 24 (60) | 10 (40) | 7 (47) |
| Age (years), mean (SD) | 39 (9.9) | 49 (9.8) | 48 (9) |
| **Clinical characteristics** | | | |
| Disease duration (years), median, IQR | 11.1 (10.2-13.6) | 16.5 (14.39-21.1) | 10.5 (8.9-13.1) |
| EDSS, median, IQR | 3.5 (1.5-6) | 5 (4-7.5) | 5.5 (5-7) |
| Proportion of patients with last year relapse, n (%) | 11 (27.5) | 3 (12) | 3 (20) |
| Proportion of patients with EDSS worsening, n (%) | 11 (27.5) | 8 (20) | 7 (47) |
| **NEDA-3 status** | | | |
| Proportion of patients with NEDA-3, n (%) | 21 (52.5) | 22 (55) | |
| Proportion of patients with EDA-3, n (%) | 19 (47.5) | 18 (45) | |
| **Therapy** | | | |
| Proportion of patients with first line DMT, n (%) | 25 (62.5) | 10 (40) | 5 (33.3) |
| Proportion of patients with interferon-beta n (%) | 3 (7.5) | 2 (8) | 1 (6.7) |
| Proportion of patients with teriflunomide, n (%) | 4 (10) | 4 (16) | 2 (13.3) |
| Proportion of patients with dimethyl fumarate, n (%) | 18 (45) | 4 (16) | 2 (13.3) |
| Proportion of patients on second line DMT, n (%) | 15 (37.5) | 15 (60) | 10 (66.7) |
| Proportion of patients with fingolimod n (%) | 3 (7.5) | 2 (8) | 1 (6.7) |
| Proportion of patients with ocrelizumab, n (%) | 2 (5) | 3 (12) | 2 (13.3) |
| Proportion of patients with cladribine, n (%) | 8 (20) | 6 (24) | 5 (33.3) |
| Proportion of patients with alemtuzumab, n (%) | 2 (5) | 4 (16) | 2 (13.3) |

cal data (disease duration, EDSS score, current DMT). Collected blood samples were used for common biochemical examination including IgG index and sNfL determination. The follow-up visit included blood collection for biomarkers detection, history of relapse in the last 12 months, EDSS score and a change in the last 12 months of relapse. Evidence of disease activity (EDA) status was defined as unfilled NEDA status.

### Definition of NEDA-3

The NEDA-3 status was determined using data from the last 12 months. NEDA-3 status was based on several parameters, such as the absence of relapse, a worsening EDSS score and MRI activity. The worsening of EDSS was defined as an increase in the EDSS score of 1.5 points, if the previous EDSS score was (a baseline score) 0; an increase of 1.0 point, if EDSS $\leq$5.0; or an increase of 0.5 points if EDSS $\geq$5.5, confirmed at 6 months[18].

### ELISA

For enzyme-immunological tests, we used the commercial kits Human CHI3L1/Chitinase-3-like Protein 1 ELISA kit (Sigma-Aldrich, Saint-Louis, MO, USA), the NEFL High Sensitivity ELISA kit (Human) (Aviva Systems Biology Corporation, San Diego, CA, USA), the Human BCA1 ELISA Kit (CXCL13) (Abcam, Cambridge, UK) and the Human MCP1 ELISA Kit (Abcam, Cambridge, UK). The samples were measured according to the manufacturer's protocols and diluted with sample buffer in the following proportions (Table II). Absorbance was detected at 450 nm on a standard microplate reader. All samples were triplicated.

### Zymographic Determination

Patient serum samples were diluted 20-fold with sample buffer. The samples were fractionated on 10% polyacrylamide gels containing 1% gelatine by electrophoresis at 120 V. After electrophoresis, the gels were washed with 2.5% Triton X-100 for 2 x 30 minutes. While stirring in a shaker, the gels were incubated in buffer (10 mM CaCl 2, 0.005 mM ZnCl 2, 100 mM Tris-HCl pH 7.4) for 39 h at 37°C. After incubation, the gels were stained with 0.5% Coomassie Brilliant Blue G-250 in a solution of 40% isopropanol and 10% acetic acid for 1 h at room temperature and decolorised for 4 h in a solution of 40% methanol

**Table II.** Dilution ratio of individual markers.

| **Marker** | CHI3L1 | sNfL | MCP-1 | CXCL13 |
|---|---|---|---|---|
| **Dilution** | 1:149 | 1:9 | 1:2 | 1:1 |

and 10% acetic acid and then in a solution of 5% methanol and 10% acetic acid for 20 hours. The proteolytic activity of MMP-9 on the gel was visualised as clear white bands at 92 kDa on a blue background and MMP-2 at 72 kDa. Proteolytic activities were quantified densitometrically using Image J software (Wayne Rasband, Bethesda, MD, USA).

### Statistical Analysis

Differences between groups were then assessed using the nonparametric Kruskal Wallis H-test and Mann-Whitney U tests to determine pairs that differed. Statistically significant results were considered at $p<0.05$. Spearman's correlation analysis was used to find interdependencies. Statistical analysis was performed using IBM SPSS Statistics 26 (Armonk, NY, USA).

## Results

### Differences Between Phenotypes

The concentrations of sNfL increased significantly (by about 46%, $p < 0.001$) between patients with the RR and SP forms of MS (Figure 1). The mean concentration of sNfL in RR patients was 428.94 ± 108.13 pg/ml compared to the SP form with 793.67 ± 180.80 pg/ml. The mean concentration was also higher in the PP form (680.94.15 ± 102.2 pg/ml). For the second marker, MCP-1, the mean concentrations of the values (Figure 1) showed a significant increase (about 30%, $p < 0.01$) between the RRMS (91.5 ± 27.4 pg/ml) and PP (130.5 ± 25.7 pg/ml) groups. In patients with the SP type of MS, the mean concentration was 119.8 ± 23.5 pg/ml.

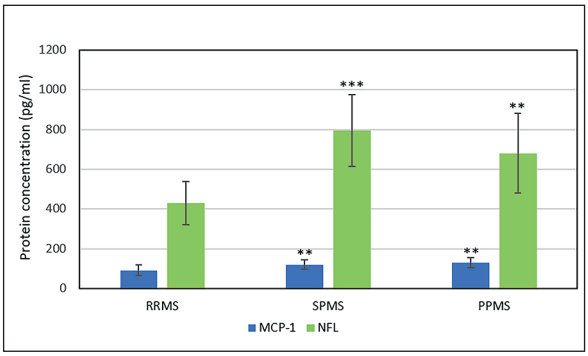


**Figure 1.** sNfL and MCP-1 protein levels in patients with different phenotypes of multiple sclerosis. RRMS – relapse-remitting MS, SPMS – secondary progressive MS, PPMS – primary progressive MS. Values are presented as the mean ± SD, $^{**}p<0.01$, $^{***}p \leq 0.001$ means statistical significance.

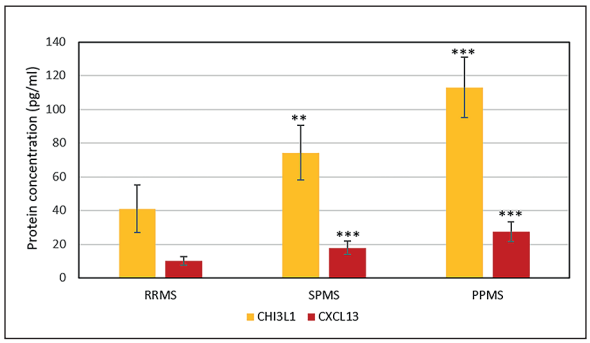


**Figure 2.** .CXCL13 and CHI3L1 protein levels in patients with different phenotypes of multiple sclerosis. RRMS – relapse-remitting MS, SPMS – secondary progressive MS, PPMS – primary progressive MS. Values are presented as the mean ± SD, $^{**}p<0.01$, $^{***}p \leq 0.001$ means statistical significance.

The highest mean of CXCL13 concentration, at 27.92 ± 5.85 pg/ml, was found in samples from patients with PPMS (Figure 2), which was about 64% ($p<0.001$) higher than the value in RRMS patients (10.15 ± 1.6 pg/ml). The mean of CXCL13 in the SP type MS was 17.79 ± 4.1 pg/ml. The CHI3L1 concentrations showed similar tendencies, with significant differences occurring between RRMS in comparison to both progressive forms.

CHI3L1 concentrations reached a maximum in the PPMS group (113.17 ± 17.76 pg/ml), where it was significantly higher, by about 63% ($p<0.001$), than in the RRMS group (41.1 ± 14.1 pg / ml), and about 34% ($p<0.001$) higher than in the SPMS group (74.37 ± 16.1 pg/ml) (Figure 2).

Other markers measured were MMP-2 and 9, which are related to the BBB damage and progressive inflammation. The activity of both matrix metalloproteinases was performed by gelatine zymography. The relative activity of MMP-9 reached a maximum in PPMS patients (Figure 3). Therefore, the relative value of the activity of MMP-9 was about 81.2% lower in the RR form compared to the PP form of MS ($p<0.001$). In the SPMS form, we detected the lowest activity, decreased by about 98% in comparison to the PP form of MS ($p<0.001$). The activity of MMP-2 increased in the RR form of MS by 31.2% compared to the SPMS form ($p<0.001$).

### Correlation Analysis

We used the Spearman correlation for a description of reciprocal interdependencies between the concentrations of the detected proteins and the individual phenotypes of MS (Table III).

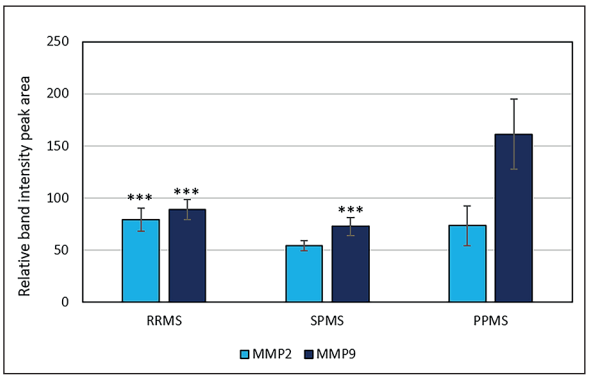


**Figure 3.** MMP-2 and MMP-9 activity in patients with different phenotypes of multiple sclerosis. RRMS – relapse-remitting MS, SPMS – secondary progressive MS, PPMS – primary progressive MS. Values are presented as the mean ± SD, ***$p \leq 0.001$ means statistical significance.

Several interesting relationships were found in the RRMS group, as sNfL concentrations showed strong positive correlation with EDSS values (r = 0.579, $p$ <0.01), which suggests that increased levels of sNfL correspond to a worsening of MS. Protein CXCL13 showed strong positive correlations with both CHI3L1 (r=0.687, $p$ <0.01) and MCP-1 (r=0.730, $p$ <0.01). In the secondary progressive group of MS, it was revealed that increasing levels of sNfL are positively correlated with CH3L1 (r=0.682, $p$ <0.01). Levels of CXCL13 positively correlate with the CHI3L1 (r=0,605, $p$ <0.01) and EDSS values (r=0.525, $p$ <0.01). In the primary progressive form of MS, a strong positive correlation was found between sNfL and MCP-1 (r=0.829, $p$ <0.01).

When detecting the activity of matrix metalloproteinases (Table III), we found higher values of MMP-9 compared to MMP-2 in all types of MS. In addition to that, all phenotypes of MS express a negative correlation between MMP-9 and MMP-2 (RRMS: rs = -0.526, $p \leq 0.05$, SPMS: rs = -0.757, $p$ <0.01 and PPMS: rs = -0.910, $p$ <0.01).

### *Differences Between NEDA and EDA Status*

For better evaluation of differences in rising concentrations of detected proteins when comparing to a worsening of the EDSS score, the RRMS group as well as the progressive MS group (PMS, made up of both SPMS and PPMS due to the lower numbers of samples and disease characteristics) were divided according to NEDA-3 status into two groups: NEDA and EDA (evidence of disease activity) (Table IV).

From the Table IV it is obvious that levels of sNfL are dramatically elevated between the EDA and NEDA groups in both RRMS (about 43%, $p$ <0.001) and PMS (about 51%, $p$ <0.001). The levels of protein MCP-1 were significantly elevated only in the PMS group, whereas EDA samples showed a significant increase in comparison to the NEDA group (about 24%, $p$ <0.01). Another difference – in the CHI3L1 levels – was found in both the RRMS and PMS groups, where an extreme increase was detected in the EDA group (RRMS about 76%, $p$ <0.001 and PMS about 62%, $p$ <0.001).

## Discussion

As MS is an incurable disease, research focuses on the identification and characterisation of new biochemical and molecular biomarkers to detect the onset of the disease as soon as possible and to try to eliminate the symptoms of the disease as much as possible. During the development of MS, substances pass through the damaged BBB, which can also be detected from the blood, by relatively non-invasive sampling.

**Table III.** Spearman correlation coefficient (rs) between protein concentrations (*$p$ < 0.05, **$p$ < 0.01).

| | | CHI3L1 | MCP1 | MMP-2 | EDSS |
|---|---|---|---|---|---|
| RR | sNfL | 0.229 | | | 0.579** |
| | CXCL13 | 0.687** | 0.730** | | 0.866* |
| | MMP-9 | | | -0.526* | |
| SP | sNfL | 0.682** | | | 0.420 |
| | CXCL13 | 0.605** | | | 0.525* |
| | MMP-9 | | | -0.757** | |
| PP | sNfL | 0.396 | 0.829** | | 0.354 |
| | MCP1 | 0.274 | | | 0.265 |
| | MMP-9 | | | -0.910 ** | |

**Table IV.** Comparison of protein concentrations with NEDA-3 status (mean ± SD, $^{**}p < 0.01$, $^{**}p < 0.001$).

| | RRMS | | PMS | |
|---|---|---|---|---|
| | NEDA | EDA | NEDA | EDA |
| sNfL (pg/ml) | 373.7 ± 97.1 | 654.1 ± 132.1*** | 340.9 ± 82.5 | 691.1 ± 103.5*** |
| CXCL3 (pg/ml) | 9.4 ± 2.6 | 15.7 ± 3.2 | 21.6 ± 4.9 | 31.4 ± 4.6 |
| MCP1 (pg/ml) | 112.9 ± 17.4 | 158.7 ± 20.1 | 121.5 ± 10.3 | 150.5 ± 4.4 ** |
| CHI3L1 (pg/ml) | 50.5 ± 9.1 | 210.4 ± 31.5*** | 40.1 ± 10.2 | 106.1 ± 10.7*** |

As specific demyelinating marker for neuronal cells, sNfLs are secreted into the CSF and are also detectable at lower concentrations in peripheral blood. In diseases that cause neuronal damage, sNfL concentrations are elevated. We detected that the concentrations of sNfL in the blood of patients with PMS were higher than in those with RRMS (about 46%, $p < 0.001$). The association with a worsening of NEDA-3 status was confirmed in the RRMS group by the positive correlation of sNfL and EDSS status ($r = 0.579$, $p < 0.01$). An article by Szilasiova et al[19] confirms our result by using Quanterix Simoa assay, where they showed in the PMS group significantly higher levels in the EDA group than in the NEDA group. Our results also showed that sNfL concentrations were positively correlated with CHI3L1 concentrations in the SPMS group ($r=0.682$, $p< 0.01$). A study published by Gil-Perotin et al[20] found similar correlations between sNfL and CHI3L1 ($r=0.58$, $p = 0.02$) in the RR form of MS, of which 50% of cases prograde to the SPMS type. There is a presumption that high levels of sNFL and CHI3L1 could predict the transition of RRMS to SPMS. It is hypothesised that the combination of both mentioned biomarkers may have value not only in identifying different types of MS but also in predicting an increase in disability and further diagnosis of ongoing progression in patients with RRMS. However, to confirm this theory the collection of a sufficient number of patients in the PMS group is needed.

Most studies report a reduction in MCP1 in RRMS compared to healthy controls. We did not use healthy subjects as controls in our work, but we focused on comparing the average protein concentration between individual MS phenotypes. However, when comparing our average concentrations with the concentrations in the mentioned studies, our results are comparable, and we found that MCP-1 levels are not significantly different from RRMS to the progressive form ($r=0.58$, $p = 0.02$). Multiple authors have found that MCP-1 levels are reduced mainly during relapses, confirming our results[21-23].

Another protein detected was CXCL13, whose levels are elevated in both CSF and blood in MS. An increase of this chemokine often occurs during active and acute CNS inflammatory processes and is likely associated with B cell-related immune activation. Most studies have focused on the detection of this protein in CSF because it is found in very low amounts in serum. We compared the average concentrations of CXCL13 between the different types of MS. Iwanowski et al[24] found no significant differences in the concentrations of this protein in RRMS compared to PPMS, which does not correlate with our findings, because we showed significantly higher levels in the PPMS group (by about 63%, $p <0.001$) than the RRMS group. However, they did not report CXCL13 values in the SPMS group or in association with levels of CHI3L1 ($r=0.605$, $p <0.01$) as well as worsening of EDSS status ($r = 0.512$, $p <0.05$). The mean concentrations in our experimental study were comparable to the concentrations detected in other studies[25-27].

Canto et al[28] detected increased levels in serum protein CHI3L1 between the RRMS and SPMS groups (approximately 69%), which supports our findings, though our difference reached only about a 45% higher concentration in the RRMS group. We also evaluated differences between the EDA and NEDA groups in both the RRMS and PMS phenotypes and showed an intensive increase in the EDA group (RRMS by about 76%, $p <0.001$ and PMS by about 62%, $p <0.001$).

MMPs have a crucial role in BBB permeability and leukocyte invasion of the CNS during MS. Using gelatine zymography, we determined the MMP activity in the sera of MS patients. In our findings, increased mean MMP-9 activities correlated with decreased levels of MMP-2 ac-

tivities in all three types of MS. Our findings on MMP-2 levels are consistent with previous studies, which found an increased expression of this protein in patients with the RRMS compared to SPMS form[29,30]. However, Sanchooli et al[31], when comparing the concentrations between MS types, found lower MMP-2 values in PPMS compared to other forms, which correspond with our results. In MS, the activity of MMPs in tissues is the result of a balance between MMPs and their tissue inhibitors (TIMPs). MMP-9 predominates in acute MS lesions and is inhibited by TIMP-1, while MMP-2 may be involved in extracellular matrix (ECM) remodelling, for example, in chronic disease, and is inhibited by TIMP-2. Previous studies have provided conflicting views on the level of MMP-2 in MS. These differences may be reflected in the CSF and serum. There are several limitations of our study: our cohort is small, and the follow-up was relatively short; prospective study with a larger cohort of PMS patients could better explain serum markers validity in individual PMS patient´s disease course. We did not consider all of the patients' comorbidities which may affect serum levels of selected biomarkers.

## Conclusions

Multiple sclerosis is an autoimmune, inflammatory demyelinating disease that mainly affects people of reproductive age. The clinical manifestations of the disease vary, from a few specific to severe disabilities. Early diagnosis is crucial for the patient, as the available treatment works particularly in the early stages of the disease. Current research is bringing about more and more options that, although they cannot cure the disease, they can slow its progression. This work provides an overview of selected potential biomarkers, suitable for the recognition of progression from relapsing forms of MS even before the neurological status of patients worsens. The most promising candidates that we examined and for which we demonstrated their potential usefulness for differential diagnosis were sNfl, CXCL13 and CHI3L1, with a significant positive correlation to the value of EDSS in both RRMS and PMS. However, our findings should be confirmed using a larger cohort of patients to determine their sensitivity and specificity. Earlier and non-invasive detection of serum biomarkers and their correlations with neurological disability can help recognise the transition from the RRMS form to progressive forms of MS, complement the results of clinical and radiological follow-up of the patient, and prospectively be used for monitoring a patient's response to treatment.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Ethics Approval

The study was approved by the Louis Pasteur University Hospital Ethics Committee (2020/EK/06044).

### Informed Consent

All patients signed informed consent.

### Funding

The work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic, project VEGA 1/0540/2020.

### Authors' Contribution

PL, PU contributed to the conception and design. PL, JM, MR performed the material preparation, data collection and analysis. The first draft of the manuscript was written by PU and PL, and all authors commented on previous versions of the manuscript. All authors have read and approved the final version of the manuscript.

### ORCID ID

Petra Lamancová 0000-0001-5322-1905; Peter Urban 0000-0003-2749-7953; Janka Mašlanková 0000-0003-0465-7950; Miroslava Rabajdová 0000-0001-9562-5756; Mária Mareková 0000-0002-9103-4710.

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