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LEADING TOPIC

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Different blood-brain-barrier disruption profiles in multiple sclerosis, neuromyelitis optica spectrum disorders, and neuropsychiatric systemic lupus erythematosus

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ABSTRACT

Aim of the study. To assess differences in BBB damage profiles by measuring serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble platelet endothelial cell adhesion molecule-1 (sPECAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), and S100 calcium-binding protein B (S100B) in relapsing-remitting multiple sclerosis (RRMS), neuromyelitis optica spectrum disorders (NMOsd), and neuropsychiatric systemic lupus erythematosus (NPSLE) patients.

Clinical rationale for the study. Blood-brain-barrier (BBB) disruption is one of the key pathological processes involved in various demyelinating diseases of the central nervous system (CNS) and is associated with shedding of cell adhesion molecules and S100B into the serum compartment. Therefore, making an assessment of serum levels of the above-mentioned molecules could provide information about disease pathogenesis, severity of BBB disruption, and disease activity.

Material and methods. We recruited 42 RRMS, 19 NMOsd and 35 NPSLE patients. Subjects were treated with beta-interferons or glatiramer acetate (RRMS), oral steroids and/or azathioprine (NMOsd, NPSLE), other immunosuppressants (NPSLE), or antimalarials (NPSLE). The clinical condition of the patients was assessed using the Kurtzke Expanded Disability Status Scale for MS and NMOsd, and the Systemic Lupus Erythematosus Disease Activity Index for NPSLE. Serum levels of sVCAM-1, sPECAM-1, sICAM-1 and S100B were determined using enzyme-linked immunosorbent assay (ELISA).

Results. We found the lowest levels of sPECAM-1, sICAM-1 and S100B in sera from NMOsd patients. The highest levels of sPECAM-1 and sICAM-1 were observed in NPSLE, and in NPSLE and MS, respectively. There were no statistically significant differences in sVCAM-1 levels between the examined groups. In MS and NMOsd, there was a negative correlation between the EDSS score and the following molecules: sPECAM-1, sICAM-1 and S100B.

Conclusions and clinical implications. We conclude that there is a different profile of blood-brain-barrier disruption reflected by cell adhesion molecules shedding in the spectrum of autoimmune CNS disorders with disseminated white matter lesions. These molecules could become new biomarkers to be used in CNS demyelinating diseases differential diagnoses and monitoring disease activity, but further studies on larger groups of patients are necessary.

Key words: blood-brain barrier breakdown, multiple sclerosis, systemic lupus erythematosus, neuromyelitis optica spectrum disorders, adhesion molecules

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Introduction

The spectrum of demyelinating disorders of the central nervous system (CNS) covers a large group of disorders where myelin destruction is a key pathological finding, with different pathomechanisms leading to it. The main diseases to consider in an adult patient with a neurological syndrome and disseminated white matter lesions are: multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOsd), and neuropsychiatric systemic lupus erythematosus (NPSLE). The differential diagnosis between the above-mentioned diseases is based on clinical, laboratory and neuroimaging findings. In MS, no biomarker has been identified to date, and the diagnosis is made according to the revised 2017 McDonald criteria [1]. Although a biomarker for NMOsd has been found, namely anti-aquaporin 4 antibodies (AQP4-IgG) [2], the diagnostic process remains challenging, especially in seronegative cases, in which seropositivity for antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG) could occur [3]. Additionally, antibodies against aquaporin 1 (AQP1-Ab) have been found in NMOsd, but also in MS patients, in whom even higher serum levels were observed in a single study by our group [4]. As for systemic lupus erythematosus (SLE), which is a prototypic disease driven by serum antinuclear autoantibodies (ANA), in patients with neuropsychiatric involvement anti-double-stranded DNA (anti-dsDNA) antibodies are not always present [5]. Moreover, ANA are found in 2.5–81% of MS patients [6] and in approximately half of NMOsd patients [7]. Finally, oligoclonal bands, although typical for MS, can also be found in up to 60% of NPSLE [8,9] and in 15–30% of NMOsd patients [10,11].

Therefore, there remains a need for additional markers differentiating these three conditions. One area worth exploring in this context is blood-brain-barrier (BBB) disruption, which is a common and essential step in the development of CNS autoimmune diseases. BBB permeability can be classically assessed using albumin CSF/serum ratio, which provides information about the extent of BBB dysfunction and is significantly higher in NMOsd than in MS, but it is not specific for one particular CNS disorder [12, 13]. Because BBB breakdown results in the shedding of cell adhesion molecules (CAMs) and S100B into the serum compartment, assessing their levels might be useful in clinical practice. Several studies have been published on adhesion molecules in serum and in the cerebrospinal fluid (CSF) in demyelinating CNS disorders, and their correlations with disease activity, magnetic resonance imaging (MRI), and treatment [14–17].

CAMs participate in various biological processes, primarily allowing cell-cell and cell-extracellular matrix interactions as well as forming an 'adhesion cascade', and they play an essential role in the inflammatory response [18, 19]. The importance of immunoglobulin superfamily CAMs in inflammation, particularly in leukocytes recruitment to the CNS, has been highlighted [19]. Vascular cell adhesion molecule-1 (VCAM-1), which is present on the surface of activated endothelial cells, interacts with very late antigen (VLA) on leukocytes and mediates leukocyte-endothelial cell adhesion [18, 20]. Intercellular adhesion molecule-1 (ICAM-1), expressed on endothelial cells, binds leukocytes through lymphocyte function-associated antigen 1 (LFA-1), and mediates their adhesion to epithelial cells and facilitates trans-endothelial migration [18, 21]. Platelet endothelial cell adhesion molecule-1 (PECAM-1), found on platelets (in large amounts), most subtypes of leukocytes, and in endothelial cell intercellular junctions, plays a role in trans-endothelial migration of leukocytes [18, 22, 23].

S100 calcium-binding protein B (S100B) is present mainly in astrocytes and other glial cells. It participates in cell proliferation, migration, differentiation and apoptosis. [24, 25]. S100B is thought to be a biological marker of some disorders, as it can be detected in blood, CSF or other biological fluids in various pathological conditions, including MS, NMOsd, NPSLE and other neurological and non-neurological diseases [25, 26]. In neurological disorders, elevated serum S100B can be the result of BBB disruption as well as of brain damage [27].

Clinical rationale for the study

We assessed serum levels of sVCAM-1, sICAM-1, sPECAM-1 and S100B in patients with MS, NMOsd and NPSLE as well as in healthy controls (HC). Because BBB breakdown results in shedding of cell adhesion molecules and S100B into the serum compartment, we hypothesised that assessing their serum levels could provide more information about demyelinating diseases' pathogeneses and could be helpful in making differential diagnoses, evaluating the severity of BBB disruption, and monitoring disease activity.

Material and methods

Study design

Patients were recruited in the Department of Neurology and the Department of Rheumatology, Rehabilitation and Internal Medicine at Poznan University of Medical Sciences, Poland and in Poznan private rheumatological practice between April 2014 and August 2017. Nineteen patients with diagnosed NMOsd according to the International Consensus Diagnostic Criteria for NMOsd [28] and seropositive for AQP4-IgG were recruited for the study from those hospitalised in the Department of Neurology or evaluated for AQP4-IgG in the Division of Neurochemistry and Neuropathology at Poznan University of Medical Sciences. A total of 42 patients with relapsing-remitting MS (RRMS) hospitalised in the Department of Neurology, who met the revised 2010 McDonald criteria [29], were incorporated into the study. Forty-nine patients with SLE, who fulfilled the American College of Rheumatology (ACR) criteria [30], were referred from the two Rheumatology Clinics in Poznan, of whom 35 with CNS

Table 1. Adhesion molecules and S100B in patients with NMOsd, MS, NPSLE and HC

	sVCAM1 (ng/mL)				sPECAM1 (ng/mL)			
	HC	MS	NMOsd	NPSLE	HC	MS	NMOsd	NPSLE
Maximum	9,774.2	4,586.9	4,462.6	4,037.5	157.9	186.2	110.8	186.2
Median	1,359.2	1,121.3	1,024.3	1,130.6	93.9	102.2	84.3	123.7
Minimum	376.6	289.9	469.8	537.4	62.9	51.1	70.2	50.2
	sICAM (ng/mL)				S100B (pg/mL)			
	HC	MS	NMOsd	NPSLE	HC	MS	NMOsd	NPSLE
Maximum	725.4	732.0	574.8	794.1	56.3	822.9	0.0	106.8
Median	402.8	462.3	319.2	466.6	0.0	0.0	0.0	0.0
Minimum	109.4	250.8	191.7	287.9	0.0	0.0	0.0	0.0

HC — healthy controls; NMOsd — neuromyelitis optica spectrum disorders; MS — multiple sclerosis; NPSLE — neuropsychiatric systemic lupus erythematosus; sVCAM-1 — soluble vascular cell adhesion molecule-1; sPECAM-1 — soluble platelet endothelial cell adhesion molecule-1; sICAM-1 — soluble intercellular adhesion molecule-1; S100B — S100 calcium-binding protein B

involvement were included in the final analysis. Thirty-nine HC without any known acute or chronic diseases were recruited on a random basis.

Inclusion criteria were: age ≥ 18 years, NMOsd with seropositivity for AQP4-IgG, relapsing-remitting type of MS, SLE with neurological manifestations, ability to walk without/with assistance or to move in a wheelchair. Active inflammation was an exclusion criterion for participating in the study.

Patient clinical evaluations using the Kurtzke Expanded Disability Status Scale (EDSS) for MS and NMOsd, and the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) for NPSLE was performed to assess the clinical severity of the disease. Blood samples were collected for laboratory analyses, and the results were assessed using statistical methods as described below.

The study protocol was approved by the Internal Review Ethics Board at the Poznan University of Medical Sciences. All subjects gave written consent to study participation.

Laboratory analyses

Serum samples were obtained from the study subjects and then frozen at –80°C. Serum soluble VCAM-1 (sVCAM-1), soluble PECAM-1 (sPECAM-1), soluble ICAM-1 (sICAM-1) and S100B levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (BioVendor Laboratory Medicine Inc., Czech Republic and R&D Systems Inc., Minneapolis, MN, USA). The levels of sVCAM1, sPECAM1, and sICAM1 were calculated from standard curves in nanograms per millilitre (ng/mL). The concentration of S100B was expressed in picograms per millilitre (pg/mL).

Statistical analysis

Statistical analyses were performed with the use of StatSoft STATISTICA version 13 (TIBCO Software Inc. 2017), Statistica (data analysis software system) version 13 (http://statistica.io), MedCalc for Windows version 15.8 (MedCalc software, Ostend, Belgium, https://www. medcalc.org), and JASP version 0.14.1 (JASP Team 2020, https://jasp-stats.org).

The values are reported as either means or medians with 95% confidence intervals. A p value ≤ 0.05 was considered statistically significant.

Firstly, a D'Agostino-Pearson test was used to determine distribution of the measurements within each group and, depending on distribution, either a Levene's or a Brown-Forsythe test to assess the homogenicity of variances. Due to confirmation of non-normally distributed variables or uneven variances, intergroup differences were examined with a Kruskal-Wallis test, with a Dunn's multiple comparisons post-hoc test for results with $p < 0.05$. For correlations, a Kendall rank correlation coefficient was employed.

Results

Patient characteristics

A total of 135 patients were incorporated into the study: 39 healthy individuals (27 women, 12 men), 19 patients with NMOsd (18 women, one man), 42 with MS (38 women, four men), and 35 with NPSLE (34 women, one man). A higher EDSS score was observed in the NMOsd than in the MS group (median 4.0 *vs.* 1.0, respectively) (Fig. 1C) and higher scores were observed in older patients. The median SLEDAI score in the NPSLE group was 17 in the baseline, and 6 in the follow-up examination after one year. Clinical characteristics of the patients' groups are set out in Supplementary Table 1 and Figures 1A–1C.

All MS patients were treated with standard immunomodulation (beta-interferons or glatiramer acetate). Eleven of the 19 NMOsd patients were on low doses of oral steroids or/and azathioprine and the other eight were untreated. NPSLE patients received oral or pulse steroid therapy, alone or with immunosuppressants (azathioprine, cyclophosphamide, mycophenolate mofetil or methotrexate), and/or with antimalarials (chloroquine or hydroxychloroquine). The doses of oral steroids ranged from 2.5 mg to 30.0 mg in the NMOsd group, and from 2.5 mg to 15.0 mg in the NPSLE group (prednisone equivalent doses). Treatments of the study participants are set out in Supplementary Table 2.

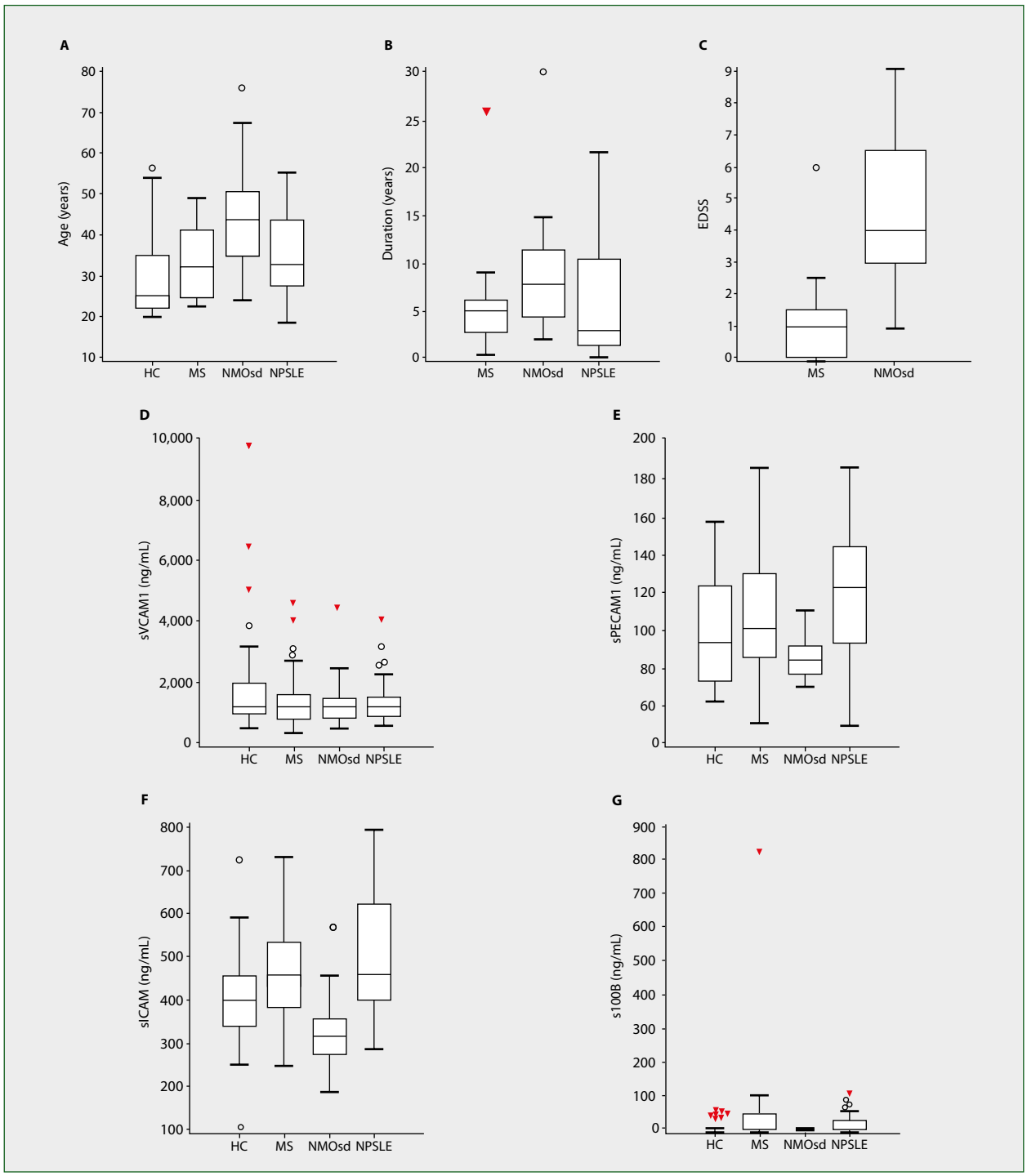

Figure 1. Clinical parameters, including age (**A**), disease duration (**B**), EDSS score (**C**), and levels of sVCAM-1 (**D**), sPECAM-1 (**E**), sICAM-1 (**F**) and S100B (**G**) in healthy controls (HC) group and in patients with neuromyelitis optica spectrum disorders (NMOsd), multiple sclerosis (MS), and neuropsychiatric systemic lupus erythematosus (NPSLE)

Adhesion molecules and S100B in evaluated groups

Significant differences between the groups were found for all the assessed molecules, except for sVCAM-1 (Tab. 1).

There were no statistically significant differences of sVCAM-1 levels between all the examined groups. The highest levels were found in HC (median 1,359.2 ng/mL) and the lowest in NMOsd (median 1,024.3 ng/mL). The levels of

Table 2. Correlations between adhesion molecules, S100B and clinical parameters

Kendall's Tau Correlations		Kendall's tau B	P-value
Age (years)	Duration	0.201 *	0.018
	EDSS	0.360 ***	< 0.001
	sVCAM1 (ng/mL)	0.075	0.211
	sPECAM1 (ng/mL)	–0.003	0.962
	sICAM (ng/mL)	–0.060	0.314
	S100B (pg/mL)	–0.005	0.940
Duration (years)	EDSS	0.129	0.220
	sVCAM1 (ng/mL)	–0.013	0.876
	sPECAM1 (ng/mL)	–0.069	0.406
	sICAM (ng/mL)	–0.089	0.283
	S100B (pg/mL)	–0.061	0.512
EDSS	sVCAM1 (ng/mL)	–0.024	0.809
	sPECAM1 (ng/mL)	–0.204 *	0.044
	sICAM (ng/mL)	–0.296 **	0.003
	S100B (pg/mL)	–0.418 ***	< 0.001
sVCAM1 (ng/mL)	sPECAM1 (ng/mL)	–0.040	0.496
	sICAM (ng/mL)	–0.015	0.797
	S100B (pg/mL)	–0.152 *	0.022
sPECAM1 (ng/mL)	sICAM (ng/mL)	0.280 ***	< 0.001
	S100B (pg/mL)	0.272 ***	< 0.001
sICAM (ng/mL)	S100B (pg/mL)	0.346 ***	< 0.001

*p < 0.05; **p < 0.01; ***p < 0.001; EDSS — Kurtzke Expanded Disability Status Scale; sVCAM-1 — soluble vascular cell adhesion molecule-1; sPECAM-1 — soluble platelet endothelial cell adhesion molecule-1; sICAM-1 — soluble intercellular adhesion molecule-1; S100B — S100 calcium-binding protein B

sVCAM-1 were comparable in MS and NPSLE (medians 1,121.3 ng/mL and 1,130.6 ng/mL, respectively) (Fig. 1D).

For sPECAM-1, concentrations were the highest in NPSLE (median 123.7 ng/mL) and in MS (median 102.2 ng/mL), followed by HC (median 93.9 ng/mL), and were the lowest in NMOsd (median 84.3 ng/mL). Moreover, statistically significant differences were observed between NPSLE and both HC and NMOsd, as well as between NMOsd and both NPSLE and MS (Fig. 1E).

The lowest values of sICAM-1 occurred again in NMOsd (median 319.2 ng/mL), while both MS (median 462.3 ng/mL) and NPSLE (median 466.6 ng/mL) had significantly higher concentrations. A trend for higher values was also present in HC (median 402.8 ng/mL) (Fig. 1F). Importantly, the only significant differences occurred between NMOsd and MS, as well as between NMOsd and NPSLE.

S100B levels were the highest in MS (mean 40.8 pg/mL), and results greater than 0.0 pg/mL were found in 20 of 42 (47.6%) patients. The odds ratio for S100B concentrations greater than 0.0 pg/mL equalled 3.52 (95% CI: 1.32 to 9.44; p = 0.0122) for MS compared to HC. This was extremely high compared to NMOsd (OR = 35.53, 95% CI: 2.01 to 626.86; p = 0.0148), and reached the borderline of significance when compared to NPSLE (OR = 2.62; 95% CI: 0.99 to 6.93; p = 0.0511). In the NPSLE group, S100B levels reached mean 16.5 pg/mL and values greater than 0.0 pg/mL were found in nine of 35 (25.7%) patients. In HC, S100B levels were the lowest (mean 9.6 pg/mL), and values greater than 0.0 were found only in a few subjects (8/39; 20.5%). None of the NMOsd patients had a S100B concentration greater than 0.0 pg/mL (Fig. 2G). The only significant difference existed between NMOsd and MS.

Correlations between adhesion molecules, S100B and clinical parameters

In all the examined patients, disease duration correlated positively with age, but not with the evaluated molecules' levels. Positive correlations were observed between sICAM-1 and sPECAM-1, as well as between S100B and both sPECAM-1 and sICAM-1. Moreover, there was a negative correlation between S100B and sVCAM-1.

In HC, sPECAM-1 positively correlated with sICAM-1 and S100B. There was also a correlation between sICAM-1 and S100B. In the NMOsd group, there was a fairly strong correlation between sVCAM-1 and patient age. In the MS group, sPECAM-1 positively correlated with sICAM-1 and S100B. Additionally, there was a correlation between sICAM-1 and S100B. In the NPSLE group, there was a positive correlation between sICAM-1 and sPECAM-1. Furthermore, S100B positively correlated with sICAM-1 and negatively with sVCAM-1.

In MS and NMOsd, we observed a negative correlation between the EDSS score and the following molecules: sPECAM-1, sICAM-1 and S100B. The higher EDSS was found in older patients (Fig. 2A–D).

In NPSLE, we did not find a correlation between the SLEDAI score (in both the baseline and the follow-up examination) and serum levels of adhesion molecules or S100B. Nevertheless, we found a negative correlation between sVCAM-1 serum levels and the SLEDAI score change from baseline to follow-up (i.e. the higher the level of sVCAM-1, the greater the improvement in the SLEDAI score over time) (Suppl. Fig. 1).

The correlations between evaluated molecules and clinical parameters are set out in Table 2.

Summary of results

We found that the levels of sPECAM-1, sICAM-1 and S100B were the lowest in the sera of patients with NMOsd. The highest levels of sPECAM-1 were observed in NPSLE, and the highest levels of sICAM-1 were found in NPSLE and MS. In turn, there were no statistically significant differences in sVCAM-1 levels between all the examined groups. Interestingly, in MS and NMOsd there was a negative correlation between the EDSS score and the following molecules: sPECAM-1, sICAM-1 and S100B.

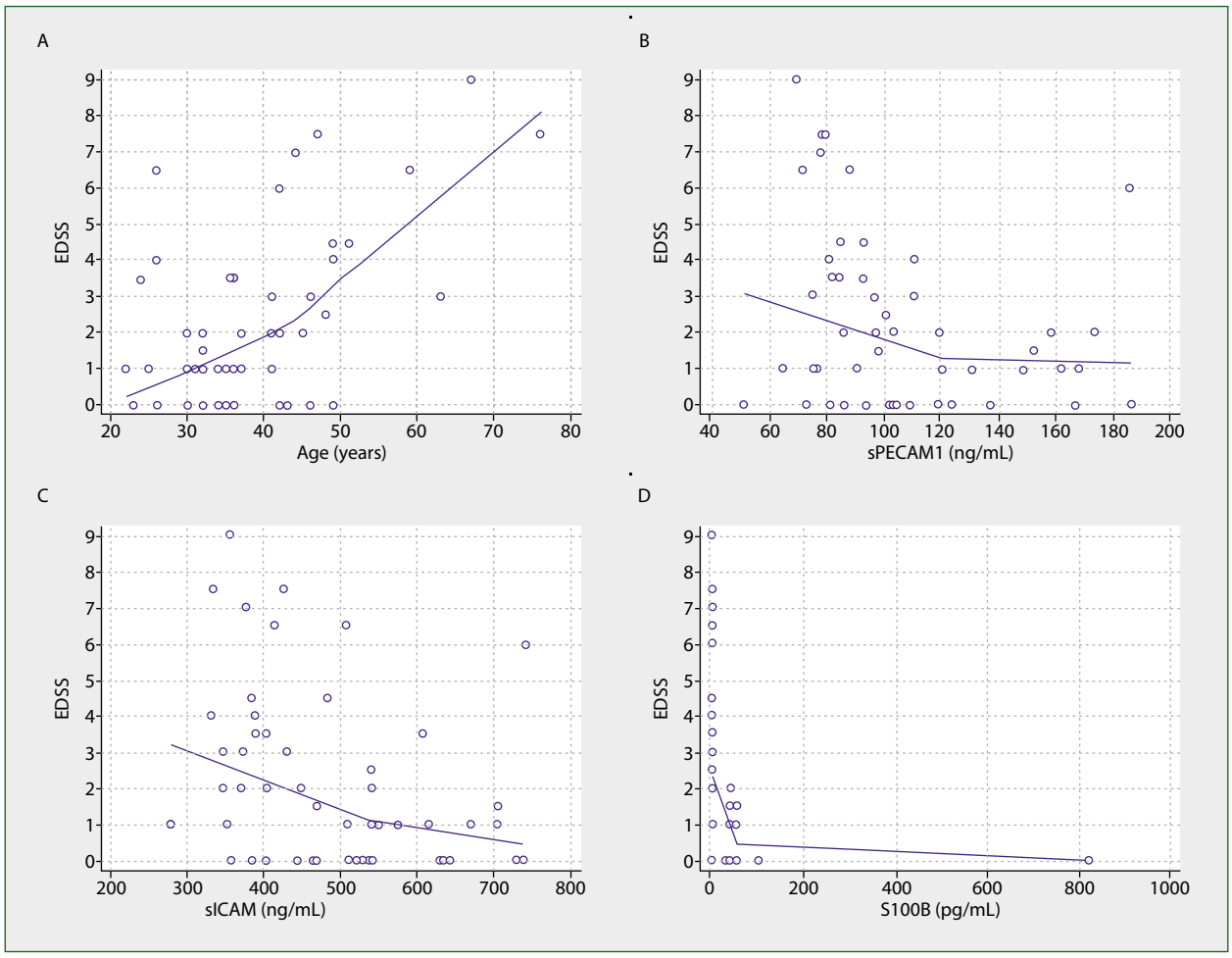

Figure 2. Correlations between age (**A**), levels of sPECAM-1 (**B**), sICAM-1 (**C**) or S100B (**D**) and Kurtzke Expanded Disability Status Scale (EDSS) score

Discussion

In our study, we observed that serum sPECAM-1 concentrations were significantly lower in patients with NMOsd than with MS and in the HC. This was in accordance with the findings published by Chang et al. [31]. However, our results differed for sICAM-1 and sVCAM-1, in that they found higher concentrations of VCAM-1 in NMOsd than in MS and HC, and higher ICAM-1 levels in NMOsd than in HC (but not in MS) [31]. Uzawa et al. showed that sICAM-1 serum levels were elevated in patients with NMO compared to MS [32]. We in turn found that levels of sICAM-1 were lower in the NMOsd than in the SLE, MS and HC groups. In fact, we noted that sICAM-1 was higher in the MS than in the HC group (just as in the Uzawa et al. study [32]), but the difference was statistically insignificant. Moreover, in our cohort there were no statistical differences in sVCAM-1 serum levels, which were the highest in HC, followed by SLE and MS, and the lowest in NMOsd. These results are different to those from the above-mentioned studies [31, 32].

Such differences could be due to various factors. Firstly, we tested MS patients treated with beta-interferons or glatiramer acetate (which were, at the time, the most common first-line treatments in Polish MS patients [33]), oral steroids and/or azathioprine (NMOsd and NPSLE), and/or other immunosuppressants (NPSLE), while Chang et al. evaluated patients before therapy with steroids, immunoglobulins or plasmapheresis [31]. The impact of treatment on adhesion molecules levels was analysed in previous studies which showed that sVCAM-1 serum levels decrease in patients with RRMS after intravenous methylprednisolone or that sVCAM-1 serum levels increase in MS patients treated with beta-interferon, which in turn correlated with a decrease in MRI activity [34, 35]. Secondly, our patients were in remission, while Chang et al. evaluated patients within two weeks of symptoms onset or acute relapse [31]. Levels of adhesion molecules might depend on disease activity, as in MS patients serum levels of sICAM-1, sVCAM-1 or PECAM-1 correlate with the presence of gadolinium-enhancing lesions on MRI [15, 35–39].

Our results suggest that the BBB damage is more severe in NPSLE than in MS and NMOsd, as we noted significantly higher serum concentrations of sPECAM-1 in NPSLE than in the other groups. This resembles the observation by Santos et al. that serum levels of PECAM-1 were higher in SLE than in HC [40]. However, contrary to the above-mentioned research, we found higher sVCAM-1 levels in HC than in NPSLE (but without statistical significance) [40]. The differences in sVCAM-1 levels in our study as opposed to other studies could be attributable to several factors, e.g. disease activity and treatment. It has been noted that sVCAM-1 concentrations correlate with disease activity, as they are higher in the sera of SLE patients during relapse and become lower during remission [41]. Nonetheless, serum levels of sICAM-1, although elevated in SLE patients, do not reflect disease activity [41]. Treatment could influence the levels of adhesion molecules, as a decrease of ICAM-1 and an increase of E-selectin concentrations have been observed on mycophenolate mofetil therapy [40]. On the other hand, there was no significant influence of steroids (prednisolone), immunosuppressives or antimalarials on serum levels of VCAM-1, ICAM-1, PECAM-1, E-selectin and P-selectin [40].

Interestingly, we found that in MS and NMOsd there was a negative correlation between the EDSS score and the following molecules: sPECAM-1, sICAM-1 and S100B. Chang et al. also observed a negative correlation between serum levels of PECAM-1 and the EDSS score in NMOsd patients [31]. Santos et al. noted an association between serum concentration of PECAM-1 and the SLEDAI score in SLE patients [40]. Based on the literature and on clinical observations, it is known that the EDSS score increases with disease duration. This negative correlation could be the result of immunopathogenesis of the CNS demyelinating disorders and the intensity of inflammatory response in different disease stages. For example, in MS the inflammatory response is particularly expressed in the first few years of the disease and then gradually decreases, as neurodegeneration starts to play an increasing role [42].

Another important factor to consider is disease activity, as during disease relapse BBB permeability increases, which is connected with the appearance of new gadolinium-enhancing lesions in the CNS [43]. Moreover, it was noted in a Polish MS cohort of older patients that EDSS tends to be higher (as in our study) but the presence of gadolinium-enhancing lesions is lower, so BBB permeability could change with age [44]. It is worth underlining that our patients with NMOsd had higher EDSS scores than those with MS (medians 4.0 and 1.0, respectively). In comparison, in a recently described Polish MS population, the median EDSS score was 1.5 for the first-line and 3.0 for the second-line DMT [33]. We must stress that the profile of adhesion molecules and S100B serum levels presented in our research applied to low disability MS, and in the future the above-mentioned parameters should also be assessed in a severely disabled MS cohort.

We found that S100B protein levels were the highest in the sera of MS, followed by NPSLE patients, and that the risk for S100B release was the highest in the MS group. Several studies have revealed elevated concentrations of S100B in the CSF and serum during MS relapse [45, 46]. Missler et al. observed that in MS serum levels of S100B were significantly increased during disease deterioration, and lower in remission [46]. Bartosik-Psujek et al. found that S100B concentrations were elevated in both the CSF and serum of MS patients, and decreased significantly after mitoxantrone therapy [47]. Increased concentrations of S100B have also been observed in sera and in the CSF of NPSLE patients [48, 49]. It is thought-provoking that we did not detect S100B in any of the subjects with NMOsd, which could be the result of the low activity of the disease (remission) or treatment. Fujii et al. showed elevated CSF and serum levels of S100B during relapse in NMOsd and observed higher values in sera of AQP4-IgG positive compared to seronegative patients [50]. On the other hand, some healthy individuals participating in our study had detectable S100B levels in their sera. Nevertheless, elevated serum levels of S100B were in fact observed in physiological conditions including physical (e.g. running, swimming) or mental activity or stress [25]. Due to the variable results of S100B, its measurements should be interpreted with caution, and further studies are necessary.

Our study has several limitations. Firstly, the number of patients in each evaluated group was relatively small to establish an exact profile of BBB disruption in MS, NMOsd and NPSLE. Nevertheless, NMOsd is an orphan disease, particularly rare in Caucasians, and our study reflects a single centre experience. Because of the limited sample size in our cohort, we were unable to provide satisfactory statistical analyses to assess the relation between the molecules levels and other factors including: disease activity (relapse or remission, Annualised Relapse Rate), treatment status, and/or type of therapy and concomitant disorders. Besides, because of the lack of blood samples collected before treatment, we had no opportunity to evaluate the potential impact of treatment on the investigated parameters. Also, it would definitely be valuable to correlate our laboratory results with MRI activity, especially the presence of gadolinium-enhancing lesions. Unfortunately, our study design did not include contrast administration as patients with NPSLE were often burdened with nephropathy and consequently, as this was a voluntary scientific study, additional risk was unacceptable.

In future studies, especially with the participation of patients diagnosed *de novo* where a full diagnostic work up is necessary, contrast administration would be justified. Moreover, analysis of the CSF levels of the investigated parameters would provide interesting and important insights into the relationship between adhesion molecules and S100B levels and BBB disruption. Nevertheless, as our patients were typically already diagnosed at the time of study inclusion, there was no medical necessity to repeat CSF sampling, which would

necessitate hospitalisation and add the traumatic aspect of a lumbar puncture and a risk of adverse events. We also did not analyse the previous CSF results in the context of adhesion molecules serum levels, because it would not reflect a true relationship due to the various (and sometimes long) time among taking the CSF and blood samples.

Conclusions and future directions

The results of our study point to the following conclusions:

1. There is a distinct BBB disruption profile in patients with MS, NMOsd and NPSLE;
2. Serum levels of adhesion molecules reflect these differences;
3. BBB damage is more severe in NPSLE than in MS and NMOsd.

We believe that the above-mentioned particles could become new biomarkers used in association with other known markers such as AQP4-IgG or MOG-IgG and ANA in differential diagnoses between demyelinating diseases of the CNS.

Nevertheless, further studies on larger cohorts and with broader study protocols, including CSF sampling and gadolinium administration on MRI, are necessary. This would allow detailed assessment of the relationship between adhesion molecules, S100B and clinical factors. Obviously, evaluation of the molecules at treatment initiation and during therapy is warranted. Future research on BBB disruption markers in MS, NMOsd and NPSLE will broaden our knowledge about the immunopathogenesis of demyelinating diseases of the CNS, and potentially allow us to use these molecules in diagnostic procedures, to monitor disease activity and response to treatment, as well as to find new therapeutic options.

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References

1. 1. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018; 17(2): 162–173, doi: 10.1016/S1474-4422(17)30470-2, indexed in Pubmed: 29275977.
2. Lennon VA, Kryzer TJ, Pittock SJ, et al. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med. 2005; 202(4): 473–477, doi: 10.1084/jem.20050304, indexed in Pubmed: 16087714.
3. Sato DK, Callegaro D, Lana-Peixoto MA, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. Neurology. 2014; 82(6): 474–481, doi: 10.1212/WNL.0000000000000101, indexed in Pubmed: 24415568.
4. Jasiak-Zatońska M, Michalak S, Osztynowicz K, et al. Relationship between blood-brain permeability and antibodies against aquaporins in neuromyelitis optica spectrum disorders and multiple sclerosis patients. Neurol Neurochir Pol. 2022 [Epub ahead of print], doi: 10.5603/PJNNS.a2022.0007, indexed in Pubmed: 35029294.
5. Mostafa GA, Ibrahim DH, Shehab AA, et al. The role of measurement of serum autoantibodies in prediction of pediatric neuropsychiatric systemic lupus erythematosus. J Neuroimmunol. 2010; 227(1-2): 195–201, doi: 10.1016/j.jneuroim.2010.07.014, indexed in Pubmed: 20724007.
6. Magro Checa C, Cohen D, Bollen EL, et al. Demyelinating disease in SLE: is it multiple sclerosis or lupus? Best Pract Res Clin Rheumatol. 2013; 27(3): 405–424, doi: 10.1016/j.berh.2013.07.010, indexed in Pubmed: 24238696.
7. Wingerchuk D. Neuromyelitis spectrum disorders. Continuum: Lifelong Learning in Neurology. 2010; 16: 105–121, doi: 10.1212/01.con.0000389937.69413.15.
8. Unterman A, Nolte JES, Boaz M, et al. Neuropsychiatric syndromes in systemic lupus erythematosus: a meta-analysis. Semin Arthritis Rheum. 2011; 41(1): 1–11, doi: 10.1016/j.semarthrit.2010.08.001, indexed in Pubmed: 20965549.
9. Mok MoY, Chan EYT, Wong WS, et al. Intrathecal immunoglobulin production in patients with systemic lupus erythematosus with neuropsychiatric manifestations. Ann Rheum Dis. 2007; 66(6): 846–847, doi: 10.1136/ard.2006.061069, indexed in Pubmed: 17513577.
10. Wingerchuk DM, Hogancamp WF, O'Brien PC, et al. The clinical course of neuromyelitis optica (Devic's syndrome). Neurology. 1999; 53(5): 1107–1114, doi: 10.1212/wnl.53.5.1107, indexed in Pubmed: 10496275.
11. Wingerchuk D, Lennon V, Lucchinetti C, et al. The spectrum of neuromyelitis optica. The Lancet Neurology. 2007; 6(9): 805–815, doi: 10.1016/s1474-4422(07)70216-8.
12. Wang Y, Zhu M, Liu C, et al. Blood Brain Barrier Permeability Could Be a Biomarker to Predict Severity of Neuromyelitis Optica Spectrum Disorders: A Retrospective Analysis. Front Neurol. 2018; 9: 648, doi: 10.3389/fneur.2018.00648, indexed in Pubmed: 30131763.
13. Tomizawa Y, Yokoyama K, Saiki S, et al. Blood-brain barrier disruption is more severe in neuromyelitis optica than in multiple sclerosis and correlates with clinical disability. J Int Med Res. 2012; 40(4): 1483–1491, doi: 10.1177/147323001204000427, indexed in Pubmed: 22971500.
14. Elovaara I, Lällä M, Spåre E, et al. Methylprednisolone reduces adhesion molecules in blood and cerebrospinal fluid in patients with MS. Neurology. 1998; 51(6): 1703–1708, doi: 10.1212/wnl.51.6.1703, indexed in Pubmed: 9855526.
15. Rieckmann P, Altenhofen B, Riegel A, et al. Soluble adhesion molecules (sVCAM-1 and sICAM-1) in cerebrospinal fluid and serum correlate with MRI activity in multiple sclerosis. Ann Neurol. 1997; 41(3): 326–333, doi: 10.1002/ana.410410307, indexed in Pubmed: 9066353.
16. Losy J, Niezgoda A, Wender M. Increased serum levels of soluble PECAM-1 in multiple sclerosis patients with brain gadolinium-enhancing lesions. J Neuroimmunol. 1999; 99(2): 169–172, doi: 10.1016/s0165-5728(99)00092-2, indexed in Pubmed: 10505971.

17. Dore-Duffy P, Washington R, Dragovic L. Expression of endothelial cell activation antigens in microvessels from patients with multiple sclerosis. Adv Exp Med Biol. 1993; 331: 243–248, doi: 10.1007/978-1-4615-2920-0_38, indexed in Pubmed: 8333339.
18. Albelda S, Buck C. Integrins and other cell adhesion molecules. The FASEB Journal. 1990; 4(11): 2868–2880, doi: 10.1096/fasebj.4.11.2199285.
19. Albelda S, Smith C, Ward P. Adhesion molecules and inflammatory injury. The FASEB Journal. 1994; 8(8): 504–512, doi: 10.1096/fasebj.8.8.8181668.
20. Elangbam CS, Qualls CW, Dahlgren RR. Cell Adhesion Molecules—Update. Veterinary Pathology. 2016; 34(1): 61–73, doi: 10.1177/030098589703400113.
21. Yang L, Froio RM, Sciuto TE, et al. ICAM-1 regulates neutrophil adhesion and transcellular migration of TNF-alpha-activated vascular endothelium under flow. Blood. 2005; 106(2): 584–592, doi: 10.1182/blood-2004-12-4942, indexed in Pubmed: 15811956.
22. Muller WA, Weigl SA, Deng X, et al. PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med. 1993; 178(2): 449–460, doi: 10.1084/jem.178.2.449, indexed in Pubmed: 8340753.
23. Woodfin A, Voisin MB, Nourshargh S. PECAM-1: a multi-functional molecule in inflammation and vascular biology. Arterioscler Thromb Vasc Biol. 2007; 27(12): 2514–2523, doi: 10.1161/ATVBAHA.107.151456, indexed in Pubmed: 17872453.
24. Donato R, Cannon BR, Sorci G, et al. Functions of S100 proteins. Curr Mol Med. 2013; 13(1): 24–57, indexed in Pubmed: 22834835.
25. Michetti F, Corvino V, Geloso MC, et al. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. J Neurochem. 2012; 120(5): 644–659, doi: 10.1111/j.1471-4159.2011.07612.x, indexed in Pubmed: 22145907.
26. Sedaghat F, Notopoulos A. S100 protein family and its application in clinical practice. Hippokratia. 2008; 12(4): 198–204, indexed in Pubmed: 19158963.
27. Marchi N, Cavaglia M, Fazio V, et al. Peripheral markers of blood-brain barrier damage. Clin Chim Acta. 2004; 342(1-2): 1–12, doi: 10.1016/j.cccn.2003.12.008, indexed in Pubmed: 15026262.
28. Wingerchuk DM, Banwell B, Bennett JL, et al. International Panel for NMO Diagnosis. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology. 2015; 85(2): 177–189, doi: 10.1212/WNL.0000000000001729, indexed in Pubmed: 26092914.
29. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011; 69(2): 292–302, doi: 10.1002/ana.22366, indexed in Pubmed: 21387374.
30. Nived O, Sturfelt G, Liang MH, et al. The ACR nomenclature for CNS lupus revisited. Lupus. 2003; 12(12): 872–876, doi: 10.1191/0961203303lu495oa, indexed in Pubmed: 14714904.
31. Chang BL, Ro LS, Chen CM, et al. Serum levels of cell adhesion molecules in patients with neuromyelitis optica spectrum disorder. Ann Clin Transl Neurol. 2020; 7(10): 1854–1861, doi: 10.1002/acn3.51167, indexed in Pubmed: 32860355.
32. Uzawa A, Mori M, Masuda S, et al. Markedly elevated soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1 levels, and blood-brain barrier breakdown in neuromyelitis optica. Arch Neurol. 2011; 68(7): 913–917, doi: 10.1001/archneurol.2011.148, indexed in Pubmed: 21747031.
33. Kapica-Topczewska K, Collin F, Tarasiuk J, et al. Clinical and epidemiological characteristics of multiple sclerosis patients receiving disease-modifying treatment in Poland. Neurol Neurochir Pol. 2020; 54(2): 161–168, doi: 10.5603/PJNNS.a2020.0020, indexed in Pubmed: 32219813.
34. Elovaara I, Ukkonen M, Leppäkynnäs M, et al. Adhesion molecules in multiple sclerosis: relation to subtypes of disease and methylprednisolone therapy. Arch Neurol. 2000; 57(4): 546–551, doi: 10.1001/archneur.57.4.546, indexed in Pubmed: 10768630.
35. Rieckmann P, Altenhofen B, Riegel A, et al. Correlation of soluble adhesion molecules in blood and cerebrospinal fluid with magnetic resonance imaging activity in patients with multiple sclerosis. Mult Scler. 1998; 4(3): 178–182, doi: 10.1177/135245859800400317, indexed in Pubmed: 9762670.
36. Giovannoni G, Lai M, Thorpe J, et al. Longitudinal study of soluble adhesion molecules in multiple sclerosis: correlation with gadolinium enhanced magnetic resonance imaging. Neurology. 1997; 48(6): 1557–1565, doi: 10.1212/wnl.48.6.1557, indexed in Pubmed: 9191766.
37. Hartung HP, Reiners K, Archelos JJ, et al. Circulating adhesion molecules and tumor necrosis factor receptor in multiple sclerosis: correlation with magnetic resonance imaging. Ann Neurol. 1995; 38(2): 186–193, doi: 10.1002/ana.410380210, indexed in Pubmed: 7544573.
38. Minagar A, Jy W, Jimenez JJ, et al. Elevated plasma endothelial microparticles in multiple sclerosis. Neurology. 2001; 56(10): 1319–1324, doi: 10.1212/wnl.56.10.1319, indexed in Pubmed: 11376181.
39. Minagar A, Alexander JS. Blood-brain barrier disruption in multiple sclerosis. Mult Scler. 2003; 9(6): 540–549, doi: 10.1191/1352458503ms965oa, indexed in Pubmed: 14664465.
40. da Rosa Franchi Santos LF, Stadtlober NP, Costa Dall'Aqua LG, et al. Increased adhesion molecule levels in systemic lupus erythematosus: relationships with severity of illness, autoimmunity, metabolic syndrome and cortisol levels. Lupus. 2018; 27(3): 380–388, doi: 10.1177/0961203317723716, indexed in Pubmed: 29400123.
41. Janssen BA, Luqmani RA, Gordon C, et al. Correlation of blood levels of soluble vascular cell adhesion molecule-1 with disease activity in systemic lupus erythematosus and vasculitis. Br J Rheumatol. 1994; 33(12): 1112–1116, doi: 10.1093/rheumatology/33.12.1112, indexed in Pubmed: 7528085.
42. Lassmann H. Pathology and disease mechanisms in different stages of multiple sclerosis. J Neurol Sci. 2013; 333(1-2): 1–4, doi: 10.1016/j.jns.2013.05.010, indexed in Pubmed: 23735777.
43. Shimizu F, Nishihara H, Kanda T. Blood-brain barrier dysfunction in immuno-mediated neurological diseases. Immunol Med. 2018; 41(3): 120–128, doi: 10.1080/25785826.2018.1531190, indexed in Pubmed: 30938273.
44. Małecka I, Przybek-Skrzypecka J, Kurowska K, et al. Clinical and laboratory parameters by age for patients diagnosed with multiple sclerosis between 2000 and 2015. Neurol Neurochir Pol. 2021; 55(4): 387–393, doi: 10.5603/PJNNS.a2021.0055, indexed in Pubmed: 34355789.
45. Massaro AR, Michetti F, Laudisio A, et al. Myelin basic protein and S-100 antigen in cerebrospinal fluid of patients with multiple scle-

rosis in the acute phase. Ital J Neurol Sci. 1985; 6(1): 53–56, doi: 10.1007/BF02229218, indexed in Pubmed: 2581917.

46. Missler U, Wandinger KP, Wiesmann M, et al. Acute exacerbation of multiple sclerosis increases plasma levels of S-100 protein. Acta Neurol Scand. 1997; 96(3): 142–144, doi: 10.1111/j.1600-0404.1997.tb00256.x, indexed in Pubmed: 9300065.
47. Bartosik-Psujek H, Psujek M, Jaworski J, et al. Total tau and S100b proteins in different types of multiple sclerosis and during immunosuppressive treatment with mitoxantrone. Acta Neurol Scand. 2011; 123(4): 252–256, doi: 10.1111/j.1600-0404.2010.01393.x, indexed in Pubmed: 20597867.
48. Schenatto CB, Xavier RM, Bredemeier M, et al. Raised serum S100B protein levels in neuropsychiatric lupus. Ann Rheum Dis. 2006; 65(6): 829–831, doi: 10.1136/ard.2005.048330, indexed in Pubmed:16699054.
49. Yang Xy, Lin J, Lu Xy, et al. Expression of S100B protein levels in serum and cerebrospinal fluid with different forms of neuropsychiatric systemic lupus erythematosus. Clin Rheumatol. 2008; 27(3): 353–357, doi: 10.1007/s10067-007-0722-y, indexed in Pubmed: 17955279.
50. Fujii C, Tokuda T, Ishigami N, et al. Usefulness of serum S100B as a marker for the acute phase of aquaporin-4 autoimmune syndrome. Neurosci Lett. 2011; 494(1): 86–88, doi: 10.1016/j.neulet.2011.02.063, indexed in Pubmed: 21371524.