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# CORRELATION OF IgG AND IgM OLIGOCLONAL BANDS IN CSF AND SERUM WITH PROGRESSION AND DEGREE OF DISABILITY IN PATIENTS WITH MULTIPLE SCLEROSIS

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**Abstract**

Objective: Our aim was to determine whether there was a correlation between IgG and IgM oligoclonal bands in CSF and serum and the EDSS score in patients with multiple sclerosis (MS).

Methods**:** The material for this clinical, retrospective, and prospective, observational study consisted of patients with MS, treated at the University Clinic for Neurology, and outpatients followed in different stages of the disease.

This study included 58 patients with confirmed MS diagnosis only. MS diagnosis was established according to widely accepted and revised McDonald's criteria for MS (MRI of the brain and spine, EP, routine laboratory blood tests) and who had a lumbar puncture during the diagnostic protocol. This paper included patients with various clinical forms of MS (RRMS, SPMS, PPMS). From all patients diagnosed with MS, demographic (age of onset of the first symptoms and sex) and clinical data (clinical course of the disease, duration of symptoms, degree of disability) were taken. The degree of disability was measured using a measurement scale of disability Kurz (EDSS). Matched cerebrospinal fluid (CSF) and plasma samples were analyzed using isoelectric focusing and IgG specific immunofixation to test for the presence of intrathecal specific OCB.

Results: OCB are important biomarkers that can support MRI diagnostics and help to avoid false-positive MS diagnoses. Therefore, the revised McDonalds criteria have increased the importance of OCB. Using both cross-sectional samples and serial sampling in a subgroup of patients we found that the presence of CSF-restricted IgM OCB (but not of IgG OCB) was associated with an active inflammatory disease phenotype in PPMS patients. The presence of CSF IgM OCB may be a biomarker for a subset of PPMS patients with more active inflammatory disease, who may benefit from immune-directed treatments.

***Key words****:* oligoclonal bands, EDSS score, multiple sclerosis

## Introduction

Multiple sclerosis (MS) is a chronic heterogeneous inflammatory disease of the central nervous system (CNS) that leads to demyelination and loss of neurons [1,2]. It is one of the leading causes of neurological disability in young people worldwide. Multiple sclerosis is a multifactorial disease whose etiology and pathogenesis of the disease have not been fully investigated despite a number of clinical studies. Numerous potential risk factors have recently been identified, such as genetic predisposition, EB virus infections, herpes viruses, and vitamin D deficiency. Significant attention is paid to the first clinical signs, the number of affected systems at the onset of the disease and its changes, environmental factors, genetic, and the impact of immune factors on the course of the disease [3,4]. There is a general consensus that early treatment is useful in delaying the accumulation of disability [5] and new, more aggressive therapies show greater efficacy. Symptoms and course of the disease can vary dramatically between patients [6]. As always, treatment with disease-altering therapies (DMT), especially more aggressive treatments, increases the risk of side effects. In addition, the long-term risks of adverse side effects have not yet been fully elucidated. Examination of cerebrospinal fluid (CSF), when diagnosed in patients with suspected multiple sclerosis (MS), has returned to the latest version of the criteria for diagnosing MS due to its good diagnostic accuracy and increased problems with misdiagnosis of MS [7].

A feature of MS-specific changes in CSF is the detection of oligoclonal bands (OCBs) that

occur in the vast majority of patients with MS. OCB deficiency has a very high negative predictive value indicating a red flag at the time of diagnosis, and alternative diagnoses should be considered in such patients [8,9,10]. Additional CSF analyses can help support the diagnosis of MS, improve the differential diagnosis of MS subtypes, and predict the course of the disease, thus selecting the optimal therapy for each patient. The presence of oligoclonal bands (OCBs) in the CSF is a qualitative measure of intrathecal IgG synthesis and is a known risk factor for the progression of multiple sclerosis (MS) to a progressive form. Some studies also suggest that patients with IgG or IgM OCB in their CSF may have a higher risk of developing a disability, which has not been confirmed [11].

The aim of this study was to investigate the possible association of IgG and IgM intrathecal synthesis in CSL correlation with the degree of disability (EDSS) in disease progression.

**Material and methods**

The group of patients who were analyzed for the purposes of this paper was taken from the database of the Department of Developmental Neurology at the University Clinic for Neurology in the period from 01.01.2013 to 01.10.2019. This paper included 58 patients who met the diagnostic McDonald's criteria for MS (positive history, typical clinical presentation, clinical examination, neurophysiological and radiological diagnosis) and had different clinical forms of MS (RRMS, SPMS, PPMS). From all patients diagnosed with MS, demographic (age of onset of the first symptoms and sex) and
clinical data (clinical course of the disease, duration of symptoms, time of occurrence of secondary progression in STDs, number of exacerbations (relapses), degree of disability) were taken. All patients are properly informed about the study.

In all patients was done the following procedures:

- MRI of the brain and spine (with contrast),
- EP (WEP, SEP, and BAEP),
- Routine laboratory blood tests and serological analysis of serum (ANA, ANCA Anti DNA, BAB, Borellia, CMV, HSV, HZV)
- LP with electrophoresis of liquor and serum (number of cells, IgG index and IgG synthesis, oligoclonal bands)
- EDSS Disability Scale per Kurz

Lumbar puncture in all patients was performed in a hospital setting during the diagnostic protocol with electrophoresis of the liquor and serum. The degree of disability from the beginning of diagnosis was determined in all patients. The degree of disability was measured using the Kurz (EDSS) degree of disability measurement scale. Relapse within the disease was defined as the onset or worsening of symptoms of neurological dysfunction that lasted for more than 24 hours and ended with partial or complete remission. Symptoms that occurred during the same month were part of the same relapse. Fatigue and short-term deterioration of symptoms associated with febrile seizures were not considered relapses. The secondary progressive stage was defined as initially as relapse remittent form of the disease accompanied by progression with or without relapses and with an increase in EDDS score by at least one point during one year from the onset of the disease.

**Statistical analysis**

The results were analyzed using standard statistical methods, with the statistical software IBM SPSS Statistics 21. Namely, from the tracked variables a database was created which was systematically analyzed. All numerical series were tested for normality in value distribution using the Kolmogorov – Smirnov test for normality, the Lilliefors test and the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965; Razali and Vah, 2011), as well as through visual analysis of histograms of distribution. In doing so, an approximately normal distribution of values was estimated if the p-value for all of the three tests was higher than 0.05. The values for all parameters that followed normal distribution were

represented by the mean (measurement for the central tendency) SD (measure for the dispersion of values). One-Way ANOVA, followed by the Tukey-HSD Post-Hoc test, was used to compare the significant differences between the groups.
The values for those parameters which distribution deviated significantly from normal were represented by their median, and as a measure of the dispersion of values we took the intercurrent rank (IQR) of the data. Bivariate statistical analyses were performed using nonparametric correlations with the Spearman r coefficient. In all cases, the statistical significance level was defined at $p < 0.050$, i.e. for high significance $p < 0.001$.

**Results**

**Table 1.** Basic demographic and clinical characteristics of patients

| **Parameters** | **Number of patients, (%)** |
|---|---|
| **Total number of patients (n)** | 58 |
| **Gender structure**<br>Men<br>Women | <br>18 (31.03%)<br>40 (68.97%) |
| **Adult group**<br><20 yrs.<br>20-30 years.<br>> 30 years. | <br>9 (15.52%)<br>31 (53.45%)<br>18 (31.03%) |
| **Initial symptoms**<br>visual motor<br>sensitive<br>mixed | <br>15 (25.86%)<br>18 (31.05%)<br>11 (18.96%)<br>14 (24.15%) |
| **Presence of oligoclonal IgG bands in CSF**<br><br>Positive<br>Negative | <br><br>35 (60.35%)<br>15 (39.65%) |

**Comparison of IgG concentration in CSF between MS patients and patients without CNS inflammatory processes**

The Figure 1 shows the histograms of the value distribution in two groups of patients. As it can be seen from the visual analysis of these histograms, there is an approximate deviation from the normal distribution of values in both groups. Because Kolmogorov – Smirnov, Shapiro-Wilk, and Lilliefors tests for normality confirmed that IgG concentration of CSF in MS patients did not differ significantly from normal IgG concentration of CSF in patients without an inflammatory process ($p > 0.05$), for further statistics we used parametric statistics.

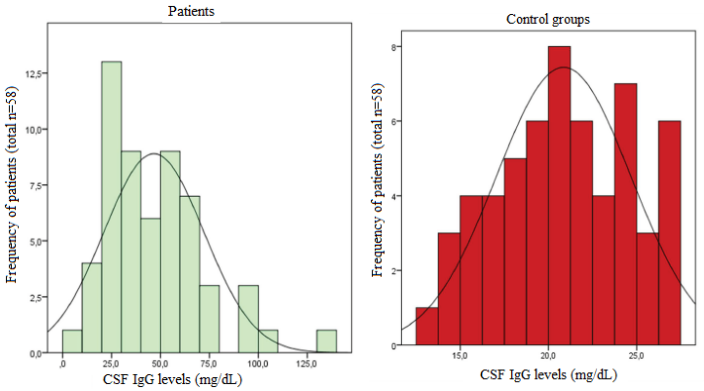



**Figure 1.** Distribution histogram of values for IgG concentration in CSF within the two groups. Values follow a normal distribution.

Our results showed that MS patients had much higher values for CSF IgG concentration (46.65 ± 25.55) compared to the control group (20.89 ± 3.82) (Figure 2). Statistical analyses confirmed highly significant differences between the means of both groups (ANOVA: F = 58,136; p = 7.94 - 10-12).

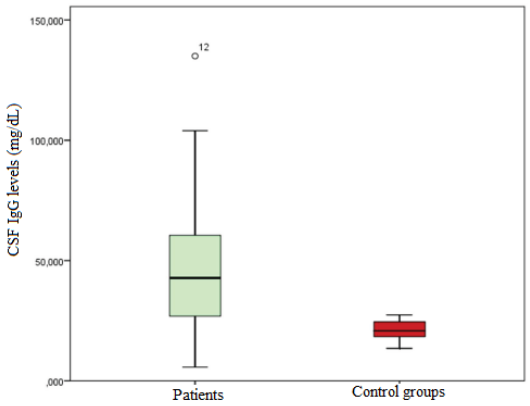



**Figure 2.** Mean IgG concentration of CSF in MS patients, compared to patients without CNS inflammatory processes.

The results are presented as the average ± SD. Patients had statistically significantly higher values of IgG concentration than healthy controls (*p <0.001) (One-way ANOVA).

**Comparison of EDSS score and presence of oligoclonal IgG bands in patients with MS**

Further statistical analysis was aimed at determining the dependence of EDSS score on the presence of oligoclonal IgG bands in CSF in patients with MS.

Our results showed the absence of statistically significant differences in the EDSS score depending on the presence of oligoclonal IgG and CSF IgG concentration (p> 0.05, n.s.) (Figure 3).

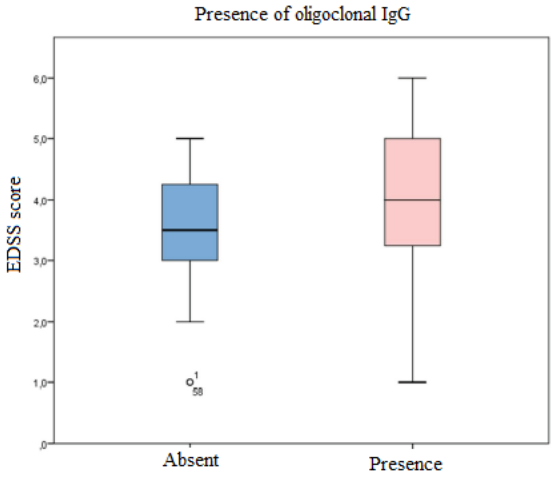



**Figure 3.** EDSS effect on the presence of oligoclonal IgG bands in CSF

The results are presented as the average ± SD. There was no statistically significant difference between the EDSS score and the presence of oligoclonal IgG bands in CSF. Statistical analyses did not confirm highly significant differences between the means of the two groups (ANOVA: F = 3,499; p = 0.0667).

**Determination of clinical variables**

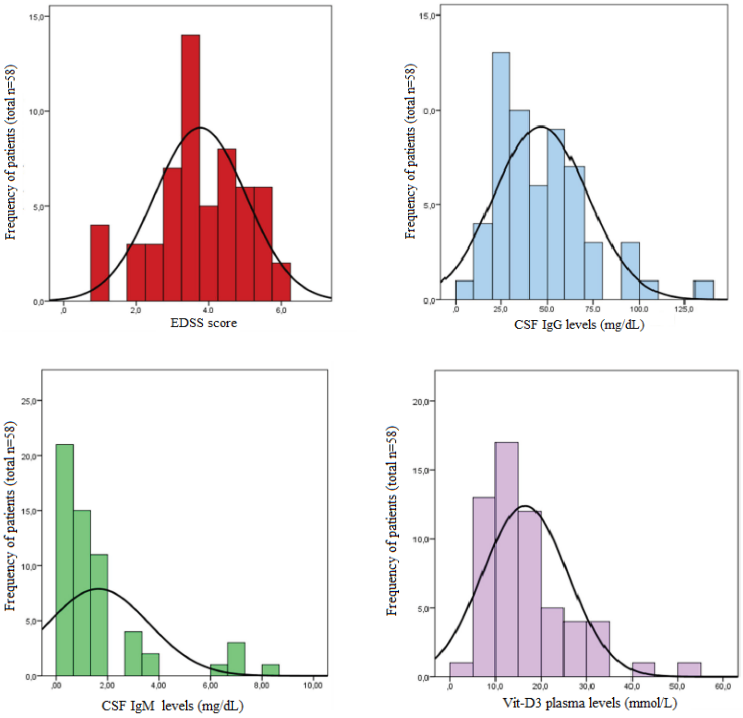



**Figure 4.** Distribution histograms of the values for the observed clinical variables in MS patients.

Kolmogorov – Smirnov, Shapiro-Wilk and Lilliefors tests of normality showed that the values for all tracked clinical variables (EDSS score, IgG, and IgM concentration in CSF, plasma vitamin D concentration) deviated significantly from normal, due to normality ($p < 0.05$).

Non-parametric statistics was used for further analysis. The histograms of the distribution of the values of the stated clinical variables are presented in Figure 4.

As it can be seen from the histograms, most of the patients in the current study were characterized by lower plasma concentrations of vitamin D, while higher values were obtained for the CSF concentration of IgG.

Table 1 shows the measures for the central tendencies and the dispersion of the values for the variables (through the median and the interval range).

**Table 2.** Comparison of clinical parameters in patients

| **Parameters** | **Median** | **IQR** |
|---|---|---|
| EDSS score | 3.77 | 1.27 |
| CSF IgG concentration | 46.52 | 25.35 |
| CSF IgM concentration | 1.64 | 1.96 |
| Plasma vitamin D concentration | 16.49 | 9.34 |
| | | |

**Neurological status in patients with MS when receiving EDSS score**

Kolmogorov – Smirnov, Shapiro-Wilk and Lilliefors tests of normality showed that the values for EDSS score at the time of admission and after the last examination (2-5 years) deviated significantly from normal ($p < 0.05$), so the results are presented through their media, and IQR was taken as a measure of the dispersion of values.

**Table 3.** Neurological status in patients with MS on admission and after the last examination (2-5 years)

| **Parameters** | **Values** |
|---|---|
| EDSS score first examination | 3.77 (IQR = 1.6) |
| EDSS after last examination (2-5 years) | 3.59 (IQR = 1.5) |

**Correlations between the concentration of IgG, IgM in CSF and vitamin D in blood plasma with clinical variability - EDSS score**

Correlations between IgG, IgM in CSF and vitamin D in blood plasma with the clinical variable were performed using the nonparametric Spearman R coefficient, due to the fact that not all follow-up clinical variables followed a normal distribution of values.

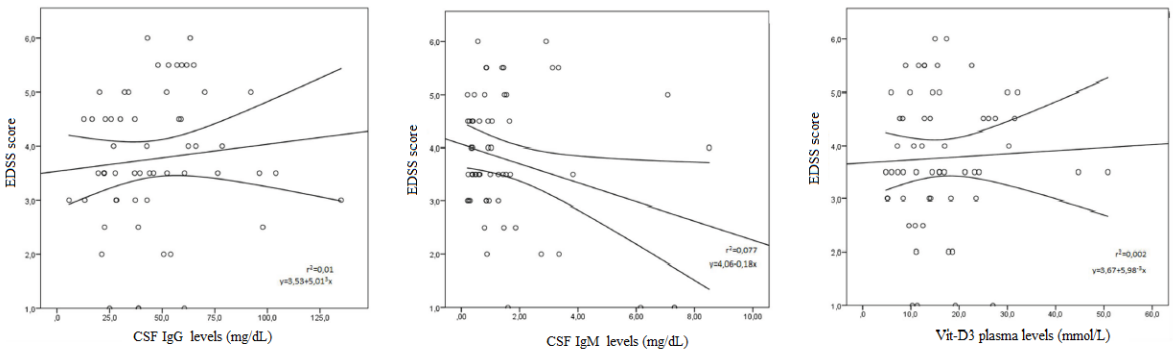
Our results are summarized in Table 4.

**Table 4.** Correlations between the concentration of IgG, IgM in CSF and vitamin D in blood plasma with the clinical variable

| **Parameters** | **Spearman parameter** | **p--value** |
|---|---|---|
| IgG concentration in CSF | 0.189 | 0.1534 |
| IgM concentration in CSF | -0.113 | 0.4 |
| Plasma vitaminD concentration | 0.088 | 0.5124 |

As can be seen, our results showed a positive and moderate correlation between IgG concentration and EDSS score on admission (p = 0.189), and a moderate degree of significance (p<0.001).

Dependencies between the EDSS score and the concentration of IgG, IgM in CSF and vitamin D in blood plasma are also graphically represented in the following images, using linear regression with a 95% confidence interval. In addition, the graphical representation itself presents the values for r2 of the linear regression and the equation that describes the dependence of the two variables, in the standard format y = kx + n.



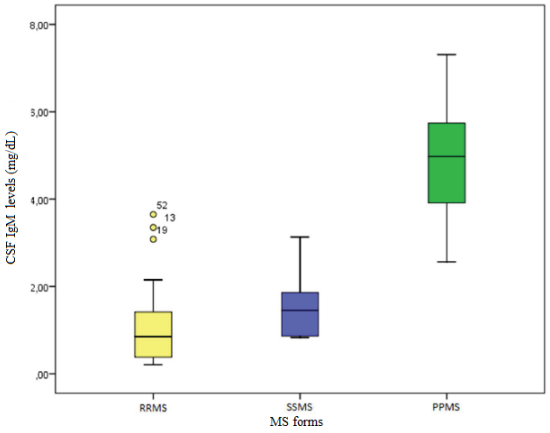




**Figure 5.** Difference between MS forms (SPMS, RRMS, and PPSM) and IgM concentration determined in CSF

The most liquefied biochemical markers showed similar values between patients with SPMS and RRMS form of MS, levels of total CSL proteins, albumins, IgM, and daily IgG synthesis and did not differ significantly between groups. However, patients with a primary progressive form of MS were characterized by significantly higher IgM levels in the liquor (Figure 5).

**Table 5.** Comparison of MS form and IgM concentration in CSF

| **Form of MS** | **Media** | **IQR** |
|---|---|---|
| **RRMS (relapse remission)** | **0.85** | **1.05** |
| **SPMS (secondary progressive)** | **1.45** | **1.52** |
| **PPMS (primary progression)** | **4.97** | **2.56** |

**Discussion**

The course of the disease in patients with MS is very variable and there are no reliable biomarkers for disease prognosis and degree of disability [12]. In this study, we analyzed the possible associations of standard CSF parameters with the degree of deterioration presented through the EDSS score in patients with RRMS, SPMS, and PPMS. CSF analysis is a standard diagnostic procedure in patients with MS and may be an important tool for finding treatment solutions and determination of the course of the disease. The presence of OCT in CSLs appears to be independently associated with conversion to progressive forms of MS [13,14]. Accordingly, diagnostic criteria for MS that have recently been revised include OCT as a parameter of time dissemination. The results obtained in this paper showed that IgG intrathecal production was increased in MS patients relative to the control group. The presence of OCB in the CSF as an indication for local IgG production in the central nervous system has also been associated with a higher risk of disability in patients with MS [15,16]. While we observed an association of intrahepatic IgG synthesis, established quantitative testing of IgG results showed that there was no statistically significant difference between the EDSS score and the presence of IgG in the CSF. This may be due to the small number of patients with negative OCB status (39.65%) in this group, which differed from the prevalence of patients who were OCB negative in the mentioned studies [17,18]. The low rate of patients with negative OCBs go hand in hand with low potency until detection of an association with worsening of EDSS in this study; hence, much larger groups will be needed to see this outcome. The EDSS score at the time of admission and at the last examination deviated from normal. There was a moderate correlation between the EDSS score on admission and IGG with a moderate degree of negligence ($p < 0.001$).

Most of the studies have found that intrathecal IgM is a negative prognostic marker associated with a worse clinical picture and MRI results. Six studies [19,20] show an association between IgM and a more aggressive course of the disease. However, the largest of these studies was the study of MS patients with RRMS and CIS (i.e. 503RRMS [19,20] and 566 CIS). Gasperi *et al.* [20] focused on the association between intrathecal IgG synthesis and the progression of the EDSS score. The presence of IgM in CSF in some MS patients has long been recognized, and the role of intrathecal IgM as a prognostic factor for progression and MS has been assessed in studies covering nearly 3 decades. In our paper, most cerebrospinal biochemical markers showed similar values

between patients with SPMS and RRMS form of MS, levels of total CSL proteins, albumins, IgM, and daily IgG synthesis and did not differ significantly between groups. However,

patients with a primary progressive form of MS were characterized by significantly higher IgM levels in the liquor.

**Conclusion**

The combined study results of MS patients in the literature suggest that intrathecal IgM production is associated with an increased risk of developing STDs and a shorter time until the first relapse. Furthermore, intrathecal IgM is associated with worse clinical course and worse disease outcomes in the first years after diagnosis of MS, as well as worse long-term prognosis, with faster progression of EDSS and possibly earlier progression to SPMS. However, several studies, some with large populations, have reported that it controls the results of the MS forecast.

Further studies will be needed to determine the potential value of intrathecal IgM as a marker of response to treatment. Developing a standardized method for detecting intrathecal IgM production can generate a useful tool when combined with clinical and MRI findings, can identify patients with STMS and RRME who are at higher risk and have a more aggressive course of the disease, and may benefit early start of DMT. Such a tool can also help in making decisions and making choices in treating patients with MS who may benefit from newer, more aggressive therapies. Further research is needed to determine a more accurate assessment of the effect of intrathecal IgM on the course of MS.

**References**

1. Goldenberg MM. Multiple sclerosis review. *Pharmacol Ther*. 2012.doi:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3351877/.

2. Oksenberg JR, Baranzini SE, Sawcer S, Hauser SL. The genetics of multiple sclerosis: SNPs to pathways to pathogenesis. *Nat Rev Genet*. 2008. doi:10.1038/nrg2395.

3. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol*. 2015. doi:10.1038/nri3871.

4. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. *Ann Neurol*. 2011. doi:10.1002/ana.22366

5. Aktas O, Wattjes MP, Stangel M, Hartung HP. Diagnosis of multiple sclerosis: revision of the McDonald criteria 2017. *Nervenarzt*. 2018.

6. Sand IBK, Lublin FD. Diagnosis and differential diagnosis of multiple sclerosis.*Contin Lifelong Learn Neurol*. 2013. doi:10.1212/01.CON.0000433290.15468.21.

7. Dobson R, Ramagopalan S, Davis A, Giovannoni G. Cerebrospinal fluid oligoclonal bands in multiple sclerosis and clinically isolated syndromes: A meta-analysis of prevalence, prognosis and effect of latitude. *J Neurol Neurosurg Psychiatry*. 2013. doi:10.1136/jnnp-2012-304695.

8. Dujmovic I. Cerebrospinal Fluid and Blood Biomarkers of Neuroaxonal Damage in Multiple Sclerosis. *Mult Scler Int*. 2011;2011:1-18. doi:10.1155/2011/767083.

9. Andersson M, Alvarez-Cermeco J, Bernardi G, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report.J Neurol Neurosurg Psychiatry. 1994;57(8):897-902. doi:10.1136/jnnp. 57.8.897

10. Freedman MS, Thompson EJ, Deisenhammer F, et al. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. Arch Neurol. 2005;62(6): 865-870. doi:10.1001/archneur.62.6.865

11. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018;17(2):162-17.

12. Rudick RA, Lee JC, Cutter GR, et al. Disability progression in a clinical trial of relapsing-remitting multiple sclerosis: eight-year follow-up. Arch Neurol. 2010;67(11):1329-1335. doi:10.1001/archneurol. 2010.150.

13. Alharbi FM. Update in vitamin D and multiple sclerosis. *Neurosciences*. 2015. doi:10.17712/nsj.2015.4.20150357.

14. Wingerchuk DM. Smoking: Effects on multiple sclerosis susceptibility and disease progression. *Ther Adv Neurol Disord*. 2012. doi:10.1177/1756285611425694.

15. Ferraro D, Simone AM, Bedin R, et al. Cerebrospinal fluid oligoclonal IgM bands predict early conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome.J Neuroimmunol. 2013;257(1-2):76-81. doi:10.1016/j.jneuroim.2013.01.011.

16. Magraner MJ, Bosca I, Simy-Castelly M, et al. Brain atrophy and lesion load are related to CSF lipid-specific IgM oligoclonal bands in clinically isolated syndromes. Neuroradiology. 2012;54(1):5- 12. doi:10.1007/s00234-011-0841-7.

17. Harris VK, Tuddenham JF, Sadiq SA. Biomarkers of multiple sclerosis: current findings. Degener Neurol Neuromuscul Dis. 2017;7(7):19-29.

18. Vrethem M, Fernlund I, Ernerudh J, Ohman S. Prognostic value of cerebrospinal fluid IgA and IgG in multiple sclerosis. Mult Scler. 2004;10(4):469-471. doi:10.1191/1352458504ms1052sr.

19. Katsavos S, Anagnostouli M. Biomarkers in Multiple Sclerosis: An Up-to-Date Overview. *Mult Scler Int*. 2013;2013(ii):340508. doi:10.1155/2013/340508.

20. Tremlett H, Paty D, Devonshire V. Disability progression in multiple sclerosis is slower than previously reported. *Neurology*. 2006.