Cerebrospinal fluid neurofilament light chains and CXCL13 as predictive factors for clinical course of multiple sclerosis

Pavel Hradilek[1,2], Kamila Zondra Revendova[1,2], Jana Horakova[2], Radovan Bunganic[1,2], Ondrej Pelisek[3], David Zeman[4], Pavla Hanzlikova[5], Pavlina Kusnierova[6,7]

Aim. The aim of this study was to identify whether NfL and CXCL13 cerebrospinal fluid (CSF) concentrations at diagnostic lumbar puncture can predict the course of multiple sclerosis (MS) in terms of relapses, higher expanded disability status scale (EDSS) and magnetic resonance imaging (MRI) activity.
Methods. We conducted a single-centre prospective observational cohort study at the MS center, University Hospital Ostrava, Czech Republic. CSF NfL (cNfL) and CXCL13 concentrations were examined (ELISA method) in patients with clinically isolated syndrome (CIS) and relapsing-remitting MS (RRMS) at the time of diagnostic lumbar puncture.
Results. A total of 44 patients with CIS or early RRMS were enrolled, 31 (70.5%) of whom were women. The median age at the time of CSF sampling was 31.21 years (IQR 25.43–39.32), and the follow-up period was 54.6 months (IQR 44.03–59.48). In the simple and multiple logistic regression models, CXCL13 levels did not predict relapses, MRI activity or EDSS > 2.5. Similarly, cNfL concentrations did not predict relapses or MRI activity in either model. In the multiple regression, higher cNfL levels were associated with reaching EDSS > 2.5 (odds ratio [OR] 1.002, 95% confidence interval [CI] 1.000 to 1.003).
Conclusions. Our data did not confirm cNfL and/or CXCL13 CSF levels were predictive factors for disease activity such as relapses and MRI activity at the time of diagnostic lumbar puncture in patients with RRMS. While cNfL CSF levels predicted higher disability only after adjustment for other known risk factors, elevated CSF CXCL13 did not predict higher disability at all.

Key words: multiple sclerosis, biomarkers, neurofilament light chain, CXCL13, clinical course

Received: October 25, 2022; Revised: December 20, 2022; Accepted: January 5, 2023; Available online: January 25, 2023
https://doi.org/10.5507/bp.2023.002
© 2023 The Authors; https://creativecommons.org/licenses/by/4.0/

[1]Department of Clinical Neurosciences, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic
[2]Department of Neurology, University Hospital Ostrava, Ostrava, Czech Republic
[3]Faculty of Medicine, Masaryk University, Brno, Czech Republic
[4]Department of Laboratory Medicine, University Hospital Brno, Brno, Czech Republic
[5]Department of Imaging Methods, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic
[6]Department of Clinical Biochemistry, Institute of Laboratory Medicine, University Hospital Ostrava, Ostrava, Czech Republic
[7]Institute of Laboratory Medicine, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic
Corresponding author: Pavel Hradilek, e-mail: pavel.hradilek@fno.cz

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disease of the central nervous system. Inflammatory process leading to focal destruction of myelin, astrogliosis and axonal loss are the main pathophysiological features[1]. MS is the major cause of non-traumatic disability in young adults[2]. Several clinical trials proved that early modulation of MS by specific treatment can successfully delay sustained disability and reach No Evidence of Disease Activity (NEDA) (ref.[3,4]). However, we are still lacking reliable markers for monitoring the therapeutic response to be assured that an individual patient really profits from our therapeutic approach. Evaluation of biomarker levels in either serum or cerebrospinal fluid (CSF) of MS patients seems to be one of the promising possibilities how to monitor disease activity and therapeutic response. Neurofilaments, cytoskeletal proteins important for maintaining morphologic integrity of axons represent a promising biomarker of neurodegeneration. According to the molecular weight of particular subunits there are three types of neurofilament chains: light (NfL, molecular weight 68 kDa), intermediate (NfM, 160 kDa) and heavy (NfH, 200 kDa). As a result of axonal injury, axonal content is released into interstitial space and proteins and NfL diffuse into the CSF. Elevated concentrations of NfL were found in various diseases characterized by axonal injury (i.e. dementia, amyotrophic lateral sclerosis or spinal cord injury) (ref.[5-10]). NfL seem to be a useful biomarker and predictor of disease course in early MS. Elevated concentrations of NfL in the CSF (cNfL) are already present in patients with clinically isolated syndrome (CIS), optic neuritis (ON) or radiologically isolated syndrome (RIS) (ref.[11,12]). Elevated concentration can even be detected several years prior to first MS symptoms[13]. In progressive MS the degree of

axonal loss increases during the disease course, which correlates with clinical disability. The cNfL are elevated during MS relapse and correlate with both neurological disability and new lesions on brain magnetic resonance imaging (MRI) (ref.[11]). It also correlates with the presence of active and mixed white matter MS lesions with foamy microglia showing high acute axonal damage[14]. Serum NfL (sNfL) are suggested to be implemented into diagnostic and therapeutic considerations in MS patients[15]. However, the real predictive value of sNfL on the long-term prognosis of MS still remains unclear. Apart from biomarkers of neurodegeneration or tissue injury also markers of inflammation can be found in the CSF. One of these candidate biomarkers is CXCL13, a homeostatic chemokine that is importantly involved in B-cell activation. Intrathecal production of CXCL13 is a sign of acute inflammation in the CNS. In MS CXCL13 is found in early active demyelinating lesions, not in inactive lesions, and correlates with disease activity. Concentration of CXCL13 in the CSF is elevated in patients with active MS (ref.[16]). Although increased CXCL13 CSF concentrations are not specific for MS it can predict conversion form CIS to MS (ref.[17]) or disease activity in MS patients[18].

The primary aim of our study was to determine whether cNfL and CXCL13 levels at the time of lumbar puncture are the predictors of the second MS relapse and MRI activity in the cohort of RRMS patients. Secondarily we explored, whether the concentrations are associated with the risk of higher disability.

MATERIALS AND METHODS

Study design and participants

We conducted a single-center prospective observational study at MS center, University Hospital Ostrava, Ostrava, Czech Republic. Patients were enrolled into the study between 1st January 2017 and 31st December 2019. The end of the follow-up was 30th June 2022. The inclusion criteria were: i) age ≥ 18 years, ii) diagnosis of CIS or RRMS according to the 2017 revision McDonald criteria[19], and iii) diagnostic lumbar puncture. Exclusion criteria were: i) a progressive course of MS, ii) control lumbar puncture, and iii) treatment with disease-modifying therapies (DMTs) at the time of CSF sampling.

The diagnosis of MS was established according to the 2010 revision of the McDonald criteria[20] and reclassified according to the last 2017 revision[19].

DMTs were categorized into moderately effective DMTs (all interferons, dimethyl fumarate, glatiramer acetate and teriflunomide) and highly effective DMTs (HET: alemtuzumab, cladribine, fingolimod, natalizumab, ofatumumab, ocrelizumab, and autologous stem cell transplantation). EDSS was assessed by treating neurologist, who was blinded to laboratory results[21]. Relapse was defined as a manifestation of any new or recurrent neurological symptoms lasting more than 24 h in the absence of fever or infectious disease[22].

MRI scans

The brain and spinal cord MRI scans were performed with a Magnetom Avanto 1.5 T Syngo B19 (Siemens, Erlangen, Germany). The standard MS protocol for the brain is comprised of the T2W TSE axial, axial fluid-attenuated inverse recovery (T2 TSE FLAIR), axial diffusion-weighted imaging (DWI) sequences with b value 0, 500, 1000, and with the automatically computed map of apparent diffusion coefficient (ADC). All sequences were performed in 5 mm layers, gap 20%, T1 GRE MPRAGE 3D and T2 TSE SPACE FLAIR 3D with the acquisition in isometric voxel 1 mm, primarily in the sagittal plane[23,24]. T2 TSE sagittal, T2 GRE medic axial, and T2 TSE Stir sagittal sequences with a layer thickness of 3 mm were acquired on the C spine as a gold standard[24,25]. MRI activity was defined as an evident diffusion-restricted lesion at b 1000 with a correlation on the ADC map-hypersignal margin at b 1000 with a correlation in the hyposignal margin at the ADC, a lesion without explicit restriction or a facilitated diffusion lesion was not evaluated as an active lesion[24,26]. Another marker of MRI activity was defined as progression in time or space according to McDonald and MAGNIMS criteria of a new lesion or enlargement of any existing lesion[19,27].

Samples and analytical methods

CSF samples were collected into the polypropylene tubes (Sarstedt, Nümbrecht, Germany). CSF samples were centrifuged at 390 x g for 10 min at room temperature, and the supernatants for determination of CXCL13 and cNfL were aliquoted into at least 3 vials (0,3 ml per vial) and stored at – 70 °C until analyzed. The concentrations of CXCL13 (CXCL13 ELISA, REF EQ 6811-9601-L, Euroimmun AG) and cNfL (NF-light ELISA, REF 10-7001, IVD CE, UmanDiagnostics AB, Umeå, Sweden) were determined by ELISA. Measurements were performed in duplicates using 50 µL of undiluted cell-free CSF per well for CXCL13 and 100 µLof diluted (1+1) cell-free CSF per well. The detection limit was 33 ng/L for cNfL and 4.0 ng/L for CXCL13. Intra-assay coefficients of variation were below 5% and inter-assay coefficients of variation were below 10% for both methods.

Statistical methods

The study data were analyzed using the Stata Statistical Software: Release 17, College Station, TX: StataCorp LLC. Descriptive statistics were used to describe our data. The categorical variables were evaluated using frequency tables. The continuous and integer variables were reported as medians and interquartile ranges (IQR) because of a non-normal distribution. Logistic regression analysis was used to estimate association between CXCL13 and cNfL levels and outcome variables (relapse, MRI activity, and reaching EDSS > 2.5). Associations were reported using odds ratio (OR) and 95% confidence interval (CI).

Compliance with ethical standards

This study was approved by the Ethical Committee of University Hospital Ostrava as a part of the project "CSF biomarkers of MS" (reference number 400/2017).

RESULTS

We performed 76 lumbar punctures in patients with CIS or MS from January 2017 to December 2019. We excluded patients with progressive course of disease (n = 11), with reclassified diagnosis (n = 1), control lumbar puncture (n = 6), missing follow-up data (n = 5), and missing laboratory results (n = 9). The flowchart of the study participants is presented in Fig. 1.

A total of 44 patients with RRMS were enrolled in the final analysis of which were 31 (70.5%) women. The median age at the time of CSF sampling was 31.21 years (IQR 25.43–39.32), and the follow-up period was 54.6 months (IQR 44.03–59.48). The median of cNfL levels was 652 ng/L (IQR 510.5–1169.5), and the median of CXCL13 concentration was 19.65 ng/L (IQR 1.7–60.1). The baseline characteristics of the study participants are presented in Table 1.

The second relapse occurred in 19 (43.2%) patients during the follow-up period. The median time from a lumbar puncture to the second relapse was 1167 days (IQR 339–1644). The MRI activity developed in 20 (45.5%) patients during the follow-up. Using simple logistic regression, cNfL and CXCL13 levels did not increase the risk of the second relapse (95% CI 0.999 to 1.001 for cNfL and 95% CI 0.988 to 1.008 for CXCL13). After adjustment for age, sex, first EDSS, and DMTs before the second relapse, the results did not change (95% CI 0.998 to 1.001, and 95% CI 0.983 to 1.012 for cNfL and CXCL13, respectively). Similarly, cNfL and CXCL13 levels did not influence the risk of development of MRI activity in the simple and multiple logistic regression model (adjusted for age, sex, and ongoing DMTs before MRI activity) (95% CI 0.999 to 1.002, and 95% CI 0.999 to 1.002 for cNfL and 95% 0.996 to 1.017, and 95% 0.995 to 1.016 for CXCL13) (Table 2).

Table 1. Characteristics of the study participants.

Variable	
Age at the time of lumbar puncture in years (median, IQR)	31.2 (25.4–39.3)
Women (n, %)	31 (70.5)
Follow-up in months (median, IQR)	54.6 (44.0–59.5)
Second relapse during follow-up (n, %)	19 (43.2)
Time to the second relapse in days (median, IQR)	1167 (339–1644)
DMTs before the second relapse (n, %)	
Moderately effective DMTs	32 (93.2)
High effective DMTs	3 (6.8)
No treatment	9 (21.5)
Escalation of DMTs (n, %)	13 (29.6)
MRI activity during follow-up (n, %)	20 (45.5)
First EDSS (median, IQR)	1.5 (1.5–2.5)
Last EDSS (median, IQR)	2.0 (1.5–3.5)
Reaching EDSS > 2.5 (n, %)	17 (38.6)
cNfL in ng/L (median, IQR)	651 (511–1170)
CXCL13 in ng/L (median, IQR)	18.7 (1.70–60.1)

IQR, interquartile ranges; n, number of samples; DMTs, treatment with disease-modifying therapies; EDSS, expanded disability status scale; cNfL, cerebrospinal fluid neurofilament light chain; CXCL13, cerebrospinal fluid CXCL13.

Table 2. Neurofilament light chain and CXCL13 levels and the risk of the second relapse, MRI activity, reaching EDSS > 2.5, and escalation.

	Simple			Multiple		
	OR	95% CI	*P*	aOR	95% CI	*P*
(A) The second relapse						
cNfL (ng/L)	1.000	0.999 to 1.001	0.426	0.999	0.998 to 1.001	0.428
CXCL13 (ng/L)	0.998	0.988 to 1.008	0.651	0.997	0.983 to 1.012	0.726
(B) MRI activity						
cNfL (ng/L)	1.001	0.999 to 1.002	0.113	1.001	0.999 to 1.002	0.171
CXCL13 (ng/L)	1.006	0.996 to 1.017	0.226	1.006	0.995 to 1.016	0.277
(C) Reaching EDSS > 2.5						
cNfL (ng/L)	1.000	0.999 to 1.002	0.076	1.002	1.000 to 1.003	0.009
CXCL13 (ng/L)	0.989	0.975 to 1.003	0.128	0.994	0.979 to 1.009	0.411

OR, odds ratio; 95% CI: 95% confidence interval; cNfL, cerebrospinal fluid neurofilament light chain; MRI, magnetic resonance imaging; EDSS, expanded disability status scale.

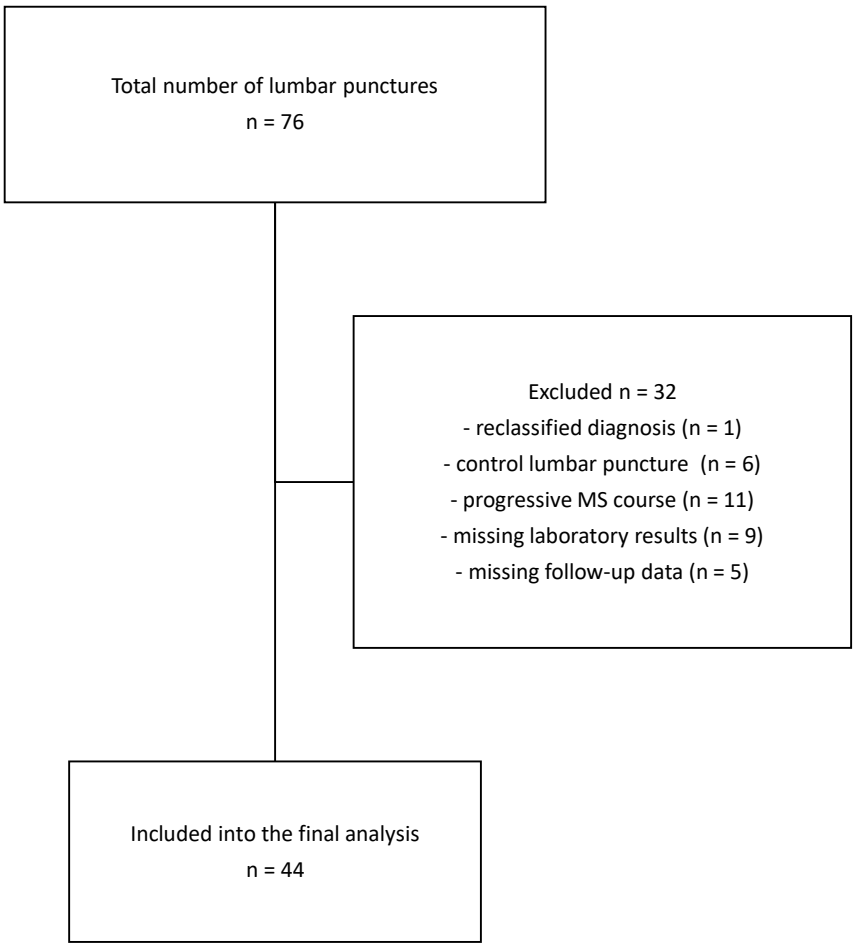

Fig. 1. The flowchart of the study participants.

The median first EDSS was 1.5 (IQR 1.5–2.5), and during the follow-up 17 (38.6%) patients reached EDSS > 2.5. The levels of cNfL did not predict reaching EDSS > 2.5 in the simple logistic regression (95% CI 0.999 to 1.002). After adjustment for age, sex, ongoing DMTs, higher cNfL levels were associated with reaching EDSS > 2.5 with OR 1.002 (95% CI 1.000 to 1.003) (Fig. 2). However, CXCL13 levels did not affect the risk of reaching EDSS > 2.5 (Table 2).

DISCUSSION

Lot of trials tried to identify biomarkers of MS that could improve diagnostics of the disease, predict disease progression and also enable to individualize specific treatment[28]. Lot of markers are known to be a general marker of inflammatory process, but there is still lack of markers specific for MS. Current research is focused on finding of such appropriate markers[29]. Disanto et al. found that elevated serum concentrations of NfL were observed in patients with MS relapse and progression of disability and also correlated with EDSS (ref.[30]). The fact that NfL could be a useful prognostic marker in early RRMS also shows Salzers cohort. In that trial NfL concentrations in the CSF were evaluated in early MS and its correlation with clinical status of the patients. The diagnostic lumbar puncture was performed in 99 patients and 95 of them were followed up for next 14 years. Significant correlations of NfL concentrations in the CSF with Multiple Sclerosis Severity Scale were found in all patients, in RRMS patients and patients with recent relapse[31]. It was unambiguously proved that elevated NfL concentrations in the CSF were associated with worse prognosis of MS (ref.[32]). Modvig also investigated predictive attributes of the CSF biomarkers. NfL and glycoprotein Chitinase 3-like-1 protein were important predictors of physical and psychical disability and conversion to CDMS in patients after optic neuritis as the first demyelinating event[33]. Another longitudinal study suggests that elevated NfL levels in the CSF predict conversion into clinically definitive MS within 1 year after CIS (ref.[34]). Elevated concentrations of NfL have been also proved to negatively correlate with the length of DMTs in MS patients[30,35-37]. In our cohort we did not find elevated cNfL and CXCL13 as risk factors of relapse at follow up. As well we did not find elevated CSF CXCL13 concentration as a risk factor for EDSS > 2.5 at follow up. However, after adjustment for age, sex and ongoing DMTs after diagnostic LP, elevated cNfL predicted reaching EDSS > 2.5 at follow up. We

suggest, that more biomarkers together with other independent variables should be considered to investigate to better assess prognosis of MS clinical course at follow up.

CONCLUSIONS

In our cohort we did not find elevated CSF cNfL and/or CXCL13 at the time of diagnostic LP to be a predictor of future clinical outcome in MS patients. However, after adjustment for age, sex and ongoing DMTs after diagnostic LP, elevated cNfL predicted earlier worsening of neurological status. Thus, having found elevated cNFL at diagnostic LP, we should be more vigilant about therapeutic considerations at disease onset to prevent disability accumulation at follow up. Larger cohorts with longer follow up are necessary to better prove clinical significance of predictive value of various biomarkers to be able to more individualize therapeutic approach in MS patients.

Achknowledgement: The study was supported in part by an institutional grant from the Ministry of Health, Czech Republic (12/RVO-FNOs/2020).

Author contributions: All authors brought substantial intellectual contributions to the study. PHr, JH, DZ: contributed to data collection; PHr, KZR, JH: performed the initial literature review and wrote first draft of the manuscript; RB, OP: contributed to data processing; PHa: analyzed MRI data; DZ, PK: supervised the study; KZR, PK: performed statistical analysis; PK, DZ: performed and revised laboratory tests, contributed to revisions. All authors critically reviewed the text of the manuscript and approved its final version.

Conflict of interest statement: PHr has received personal compensations for consulting and lectures from Biogen, Merck, Novartis, Roche, Sanofi and TEVA. KZR has no potential conflict of interest. JH has no potential conflict of interest. RB has no potential conflict of interest. OP has no potential conflict of interest. DZ has no potential conflict of interest. PHa has no potential conflict of interest. PK has no potential conflict of interest.

REFERENCES

1. Lassmann H, Brück W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. Brain pathology 2007;17(2):210-18.
2. Dobson R, Giovannoni G. Multiple sclerosis – a review. Eur J Neurol 2019; 6(1):27-40.
3. Kappos L, Edan G, Freedman MS, Montalban X, Hartung HP, Hemmer B & BENEFIT Study Group. The 11-year long-term follow-up study from the randomized BENEFIT CIS trial. Neurology 2016;87(10):978-87.
4. Rotstein DL, Healy BC, Malik MT, Chitnis T, Weiner HL. Evaluation of no evidence of disease activity in a 7-year longitudinal multiple sclerosis cohort. JAMA neurology 2015;72(2):152-8.
5. Malmeström C, Haghighi S, Rosengren L, Andersen O, Lycke J. Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS. Neurology 2003;61(12):1720-5.
6. Norgren N, Rosengren L, Stigbrand T. Elevated neurofilament levels in neurological diseases. Brain research 2003;987(1):25-31.
7. Trentini A, Comabella M, Tintoré M, Koel-Simmelink MJ, Killestein J, Roos B, Teunissen CE. N-acetylaspartate and neurofilaments as biomarkers of axonal damage in patients with progressive forms of multiple sclerosis. J Neurol 2014;261(12):2338-43.
8. Villar LM, Picón C, Costa-Frossard L, Alenda R, García-Caldentey J, Espiño M, Álvarez-Cermeño JC. Cerebrospinal fluid immunological biomarkers associated with axonal damage in multiple sclerosis. Eur J Neurol 2015;22(8):1169-75.
9. Kuhle J, Gaiottino J, Leppert D, Petzold A, Bestwick JP, Malaspina A, Casha S. Serum neurofilament light chain is a biomarker of human spinal cord injury severity and outcome. J Neurol Neurosurg Psychiatry 2015;86(3):273-9.
10. Lu CH, Macdonald-Wallis C, Gray E, Pearce N, Petzold A, Norgren N, Malaspina A. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology 2015;84(22):2247-57.
11. Kuhle J, Barro C, Disanto G, Mathias A, Soneson C, Bonnier G, Granziera C. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler 2016t;22(12):1550-9. doi: 10.1177/1352458515623365
12. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci 2005;233(1-2):183-98. doi: 10.1016/j.jns.2005.03.015
13. Bjornevik K, Munger KL, Cortese M, Barro C, Healy BC, Niebuhr DW, Ascherio A. Serum neurofilament light chain levels in patients with presymptomatic multiple sclerosis. JAMA Neurol 2020;77(1):58-64. doi: 10.1001/jamaneurol.2019.3238
14. van den Bosch A, Fransen N, Mason M, Rozemuller AJ, Teunissen C, Smolders J, Huitinga I. Neurofilament Light Chain Levels in Multiple Sclerosis Correlate With Lesions Containing Foamy Macrophages and With Acute Axonal Damage. Neurol Neuroimmunol Neuroinflamm 2022;9(3):e1154. doi: 10.1212/NXI.0000000000001154
15. Bittner S, Oh J, Havrdová EK, Tintoré M, Zipp F. The potential of serum neurofilament as biomarker for multiple sclerosis. Brain 2021;144(10):2954-63. doi: 10.1093/brain/awab241
16. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, Malmeström C, Lycke J. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. J Neurochem 2017;141(2):296-304. doi: 10.1111/jnc.13881
17. Khademi M, Kockum I, Andersson ML, Iacobaeus E, Brundin L, Sellebjerg F, Olsson T. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult Scler 2011;17(3):335-43. doi: 10.1177/1352458510389102
18. DiSano KD, Gilli F, Pachner AR. Intrathecally produced CXCL13: A predictive biomarker in multiple sclerosis. Mult Scler J Exp Transl Clin 2020;6(4):2055217320981396. doi: 10.1177/2055217320981396
19. Thompson AJ, Banwell BL, Barkhof F, Carroll,W, Coetzee T, Comi G, Cohen JA. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17(2):162-73. doi: 10.1016/S1474-4422(17)30470-2
20. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69(2):292-302. doi: 10.1002/ana.22366
21. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33(11):1444-52. doi: 10.1212/wnl.33.11.1444
22. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Tourtellotte WW. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13(3):227-31. doi: 10.1002/ana.410130302
23. Vidal-Jordana A, Pareto D, Sastre-Garriga J, Auger C, Ciampi E, Montalban X, Rovira A. Measurement of cortical thickness and volume of subcortical structures in multiple sclerosis: agreement between 2D spin-echo and 3D MPRAGE T1-weighted images. AJNR Am J Neuroradiol. 2017;38(2):250-6. doi: 10.3174/ajnr.A4999
24. Revendova K, Zeman D, Kusnierova P, Bunganic R, Hanzlikova P, Karasova K, Volny O, Bar M. Prognostic value of intrathecal IgM synthesis determined by various laboratory methods in patients with early multiple sclerosis - a prospective observational study. Mult Scler Relat Disord 2022;63:103847. doi: 10.1016/j.msard.2022.103847
25. Abolhasani Foroughi A, Salahi R, Nikseresht A, Heidari H, Nazeri M, Khorsand A. Comparison of diffusion-weighted imaging and enhanced T1-weighted sequencing in patients

with multiple sclerosis. Neuroradiol J 2017;30(4):347-51. doi: 10.1177/1971400916678224
26. Osborn AG. Essentials of Osborn's Brain E-Book: A Fundamental Guide for Residents and Fellows. Elsevier Health Sciences; 2019.
27. Filippi M, Rocca MA, Ciccarelli O, De Stefano N, Evangelou N, Kappos L, MAGNIMS Study Group. MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. Lancet Neurol 2016;15(3):292-303.
28. Housley WJ, Pitt D, Hafler DA. Biomarkers in multiple sclerosis. Clin Immunol 2015;161(1):51-8.
29. Verbeek MM, De Jong D, Kremer HPH. Brain-specific proteins in cerebrospinal fluid for the diagnosis of neurodegenerative diseases. Ann Clin Biochem 2003;40(1):25-40.
30. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, Vehoff J. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017;81(6):857-70.
31. Roxburgh RH, Seaman SR, Masterman T, Hensiek AE, Sawcer SJ, Vukusic S, Compston DA. Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. Neurology 2005;64(7):1144-51.
32. Salzer J, Svenningsson A, Sundström P. Neurofilament light as a prognostic marker in multiple sclerosis. Mult Scler 2010;16(3):287-92.
33. Modvig S, Degn M, Roed H, Sørensen TL, Larsson HB, Langkilde AR, Sellebjerg F. Cerebrospinal fluid levels of chitinase 3-like 1 and neurofilament light chain predict multiple sclerosis development and disability after optic neuritis. Mult Scler 2015;21(14):1761-70.
34. Martínez MA, Olsson B, Bau L, Matas E, Calvo ÁC, Andreasson U, Zetterberg H. Glial and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. Mult Scler 2015;21(5):550-61.
35. Gunnarsson M, Malmeström C, Axelsson M, Sundström P, Dahle C, Vrethem M, Lycke J. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann Neurol 2011;69(1):83-9.
36. Kuhle J, Disanto G, Lorscheider J, Stites T, Chen Y, Dahlke F, Francis G, Shrinivasan A, Radue EW, Giovannoni G, Kappos L. Fingolimod and CSF neurofilament light chain levels in relapsing-remitting multiple sclerosis. Neurology 2015;84(16):1639-43.
37. Romme Christensen J, Ratzer R, Börnsen L, Lyksborg M, Garde E, Dyrby TB, Siebner HR, Sorensen PS, Sellebjerg F. Natalizumab in progressive MS: results of an open-label, phase 2A, proof-of-concept trial. Neurology 2014;82(17):1499-507.