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Serum neurofilament level as a biomarker in multiple sclerosis

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Abstract

Background & Objective: Currently, MS disease activity and neurodegeneration are assessed mainly through clinical evaluation and magnetic resonance imaging. These measures lack sensitivity and specificity, leading to a constant search for a new biomarker. Several markers have been studied, and the most promising to date is the neurofilament. Our objective was to examine the relationship between serum neurofilament levels and multiple sclerosis parameters. *Methods:* Fifty-six adults who fulfilled the McDonald's criteria 2017 for the diagnosis of MS, and forty-four healthy controls were enrolled in this study. Serum samples were collected to assess neurofilament light and heavy chain (NfL & NfH) levels using the ELISA method, the results were compared for MS patients and controls; and were also correlated to the type of MS, disease duration, EDSS (expanded disability status scale), MSSS (MS severity score) and disease activity. *Results:* The mean serum NfL and NfH levels for cases were 133.3 pg/ml and 3654.5 pg/ml respectively, which are significantly higher than that for controls (NfL = 80 pg/ml; and NfH = 408.8 pg/ml; p-value < 0.001). There were no significant correlations between serum neurofilament levels and EDSS, MSSS, disease activity, type, and duration.
Conclusions: In our study, we could not find a significant role for the ELISA neurofilament serum level as a biomarker for MS disease activity and severity.

Keywords: Multiple sclerosis, neurofilament light chain, neurofilament heavy chain, serum, ELISA, disability, biomarker.

INTRODUCTION

Multiple sclerosis (MS) is a chronic disease characterized by inflammation and demyelination of the central nervous system (CNS) associated with variable degrees of axonal and neuronal damage.[1] Although demyelination is the hallmark of MS, axonal injury is present, even from the earliest stages of the disease, and appears to be an important contributor to symptoms and disability.[2] The evaluation of axonal degeneration is still a major challenge in MS, and with the advent of newer and more aggressive disease-modifying therapies (DMTs) that can delay disability, there is a real need for a reliable test to measure disease status. Most of the algorithms guiding disease monitoring and treatment switching today are based on the occurrence of clinical relapses, disability progression, MRI activity, and more recently brain atrophy[3], however, many of these parameters are retrospective, imprecise, and often fail to predict individual disease progression and therapy response. Therefore, a new biomarker that can reflect tissue damage and allow monitoring of subclinical disease activity is highly desirable.[4]

A notable example of a fluid biomarker that is already in clinical use in MS is the oligoclonal bands.[5] Yet, no fluid biomarker has established clinical use in routine disease monitoring and prediction in MS. Because of this unmet need, many studies have been performed towards this purpose, among these are the studies that evaluate neurofilament's role in MS, which has shown some promising results.[6,7] Neurofilaments are neuronal-specific heteropolymers that are particularly abundant in axons. Their functions include the provision of structural support and maintaining the size, shape, and caliber of the axons. Neurofilaments consist of a triplet of light (NfL), medium (NfM), and heavy (NfH) chains. The nomenclature-light (~68 kDa), -medium

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(~145 kDa), and heavy (~200 kDa) refer to the molecular weight of the filaments.[8]

Disruption to the axonal membrane releases Nf into the interstitial fluid and eventually into the cerebrospinal fluid (CSF) and, subsequently blood.[9,10] Hence, measuring intracellular cytoskeletal proteins like NfL and NfH seems to be a comprehensive way to assess the extent of axonal damage within the central nervous system. In humans, neurofilaments were first used as indicators of neuronal damage in amyotrophic lateral sclerosis (ALS) and Alzheimer's disease.[11] Subsequently, neurofilaments levels were found to be higher in CSF of MS patients compared to controls, suggesting these proteins could also be a potential biomarker for MS disease activity.[12,13,14] Recent studies have focused on sampling Nf in serum instead of the relatively invasive CSF, and fortunately, a lot of them have shown promising results for associating serum Nf levels with outcomes related to MS disease activity, progression, treatment response, and prognosis.[15,16,17,18]

Our study was done to establish the value of serum Nf as a biomarker in MS, we aim to examine the effects of disease duration, activity (clinical and radiological), disability, and treatment on serum NfL and NfH levels, using available resources in our region.

METHODS

A case-control study was conducted in the Middle-Euphrates-Neuroscience Centre, Al-Najaf, over a period of 4 months from June to October 2019.

Inclusion criteria

Fifty six cases aged (16-45 years) with RRMS (no.=49), SPMS (no.=6), and PPMS (no.=1) were included in the study. All patients fulfilled the revised McDonald's criteria (2017) for the diagnosis of MS.[19]

Fourty-four age and gender-matched, healthy volunteers with no previous chronic disease were included as controls. All the participants in the study voluntarily provided verbal informed consent.

Exclusion criteria

The exclusion criteria were: 1. Patients with neurologic (other than MS), inflammatory, and psychiatric conditions; 2. Patients with co-morbid cardiovascular risk factors such as DM and hypertension[20]; 3. In Patients with pseudorelapse[19] a pseudorelapse is defined as the recurrence of symptoms from a previous clinical relapse or a subclinical lesion; 4. Extremes of age; as age can physiologically increase Nf levels by 2.2% per year, and above the age of 60 sNf levels increase greatly.[21]

Clinical assessment

History and clinical examinations were performed for all participants in the MS clinic unit. Age, gender, type of MS, duration since the onset of symptoms, disease severity, disability, and type of treatment were recorded for each case. Clinical neurological assessment was done using the revised McDonald's criteria (2017) for diagnosis of cases[19] and EDSS[22] for assessment of disability. MS disease severity was calculated by using the EDSS score and disease duration for each patient applied in MSSS.[23] Patients were also assessed for clinical relapse in the last 3 months.[24]

Blood sampling

For MS cases and controls, venous blood samples were drawn into an additive-free plastic blood collection tube and allowed to clot for a minimum of 30 minutes and a maximum of 120 minutes at room temperature. The serum was removed after centrifuging, aliquoted, and stored at -20°C. Neurofilament light and heavy chains were measured by sandwich ELISA method (Human neurofilament light polypeptide and Human phosphorylated neurofilament heavy polypeptide ELISA kit, Elabscience Biotechnology Inc.) in a board-certified lab in Al-Najaf city.

Radiological assessment

A standard MRI protocol for MS with IV gadolinium (Gd)[25] (using 1.5 tesla MRI scanner, Siemens Medical Systems, Germany) was done within 3 weeks[26] of the venous blood sampling for each patient and was analyzed for the presence and number of gadolinium lesions by an experienced radiologist.

Statistical analysis

The normality of the distribution of continuous variables was tested by the one-sample Kolmogorov-Smirnov test. Means and standard deviations (SDs) were given for the normally distributed variables, while median, minimum, and maximum values were given for variables that were not normally distributed. Frequencies and percentages were used to present categorical

Table 1: Demographic characteristics of MS patients and healthy controls

Variables	Cases N=56	Control N=44	P value
Age			
Mean age in years (SD)	34.3 (9.1)	33.7 (7.2)	0.81
Gender			
Male N (%)	14 (23.7)	14 (29.8)	0.45
Female N (%)	42 (71.2)	30 (63.8)	

variables. The differences between NfL and NfH levels in cases and controls were assessed using independent samples t-test assuming unequal variances. The Mann Whitney U test was used to test the NfL and NfH levels in patients with positive and negative MRI-enhanced gadolinium lesions as well as to compare those levels for patients in remission to the levels of patients with clinical relapse. The Spearman rho test was used to test whether different variables correlate with NfL and NfH levels because the samples weren't normally distributed. The two-tailed p-value was 0.05 for all the tests used. All the tests were made using SPSS 26.0, IBM, NY, US.

RESULTS

Demographical data of study and control group

Fifty-six cases were enrolled in the study, the mean age was 34.3 (SD = 9.1) years with a median disease duration of 3 (0 – 14) years. Forty-four subjects were included as controls with a mean age of 33.7 (SD = 7.2). The male:female ratio was 1:3 for the cases and 1:2 for controls. Other patient characteristics are demonstrated in Tables 1 and 2.

Eighteen patients (32.1%) were on treatment with interferon beta-1a, and the same number of patients were on interferon beta-1b. The rest of the patients were on natalizumab (5 patients, 8.9%), fingolimod (3 patients,5.4%), or no treatment (12 patients, 21.4%).

Most MS cases (n=48; 85.7%) had no enhancing gadolinium lesions on MRI as demonstrated in Table 2. Forty-four patients (74.6%) were in remission at the time of data collection, and twelve patients (25.4%) were in clinical relapse.

Results of serum NfL and NfH

The mean NfL level for cases was 133.3 (SD = 62.6) pg/ml which is significantly higher than that for controls (Mean = 80 pg/ml; SD = 22.5; p-value <0.001). The mean NfH level was 3654.5 (SD = 567.8) pg/ml for cases which is also significantly higher than the levels obtained from healthy subjects (408.8 pg/ml; SD = 343.5; p-value < 0.001) as shown in Table 3.

Table 2: Disease characteristics among MS patients

Characteristics	Value
Mean EDSS (SD)	1.77 (1.8)
Mean MSSS (SD)	3.2 (2.8)
MS Type:	
RR MS, N (%)	49 (87.5)
SP MS, N (%)	6 (10.7)
PP MS, N (%)	1 (1.7)
Number of gadolinium lesions, N (%)	
No lesion	48 (85.7%)
1	4 (7.1%)
2	1 (1.8%)
3	2 (3.6%)
> 3	1 (1.8%)

EDSS = expanded disability status scale; n = number; MS = multiple sclerosis; MSSS = multiple sclerosis severity score RR = relapsing-remitting; SP = secondary progressive; PP = primary progressive; SD = standard deviation.

Table 3: Differences in NfL and NfH between cases and healthy subjects

Variables	Cases N=56	Control N=44	P value
NfL Mean (SD)	143.96 (106.7)	80.02 (22.5)	<0.001
NfH Mean (SD)	3654.5 (567.8)	408.77 (343.5)	<0.001

N = number; NfL = Neurofilament light (pg/ml); NfH = Neurofilament heavy (pg/ml); SD = standard deviation

Serum Nf correlation to image findings

The mean NfL and NfH levels were not significantly different between patients with negative and positive gd-enhanced MRI lesions, as shown in Table 4.

Serum Nf correlation to MS severity and other demographic data

Correlation analysis, using Spearman rho statistic, did not demonstrate significant correlations between NfL levels and EDSS, MSSS, age, or duration of the disease. The same goes for NfH levels, as shown in Table 5.

There was no significant association between clinical disease activity and NfH (p = 0.75) or NfL (p = 0.71) levels as demonstrated in Figure 1.

Serum Nf correlation to MS treatment status

There was no significant difference between NfL and NfH levels and different treatment groups (Interferon beta-1-a, interferon beta-1-b, natalizumab, fingolimod and no treatment) in MS patients (NfL: P value = 0.8; NfH: P-value = 0.73). Boxplots of NfL and NfH in different treatment groups are shown in Figure 2.

DISCUSSION

This study evaluates serum NfL level as a biomarker in MS by comparing these levels in a sample of Iraqi MS patients to that of age and gender-matched controls using commercially available ELISA. Moreover, it compares serum NfL to NfH levels and investigates the relationship between these 2 assays and disability, brain MRI activity, clinical disease activity, severity, and treatment status. Importantly, our method allows us to make use of the readily available patient blood samples, instead of the relatively difficult CSF sampling.

The male-to-female ratio in the study sample was 1:3 which is reflective of the female predominance seen in MS.[27] The duration of the disease in this study did not correlate with serum NfL and NfH levels. Meta-analyses of case-control studies reported limited data in subgroup analysis to reach a consensus regarding whether NfL and NfH levels were correlated with disease duration.[28]

As for MS type, there is heterogeneity in reports comparing Nf levels in PMS with that of RRMS cases, however, some studies have reported NfL to be higher or increase more rapidly in PMS compared to RRMS[29]; whilst others have found no such correlation.[30] In this study, NF levels were not different between patients with RRMS and PMS, and this could be attributed, at least in part, to the fact that most patients in this study were in the RRMS subgroup while only seven patients collectively had the progressive subtype.

We have found that serum NfL and NfH levels were significantly higher in MS cases than in controls. These findings are consistent with a recent meta-analysis by Cai *et al*.[31] and other studies.[32,33]

Table 4: Differences in NfL and NfH levels according to gadolinium lesions

Variables	Gadolinium lesions		P value
	Negative N=48	Positive N=8	
NfL Mean	129.2	157.4	0.42
NfH Mean	3,635.5	3,768.8	0.76

N = number; NfL = Neurofilament light (pg/ml); NfH = Neurofilament heavy (pg/ml) Gad = Gadolinium

Table 5: Correlations between NfL and NfH levels and other variables.

Variable	Coefficient	P- value
NfL		
EDSS	-0.06	0.7
MSSS	-0.07	0.59
Age	-0.11	0.4
Duration	-0.19	0.14
NfH		
EDSS	-0.06	0.63
MSSS	-0.13	0.32
Age	0.1	0.46
Duration	-0.1	0.94

Siller *et al*. found that serum NfL levels correlated significantly with T2 lesion volume and with the presence and number of Gd-enhancing lesions; Kuhle and colleagues also have reached a similar conclusion.[34] Unfortunately, the software that analyzes the volume of T2 lesions and brain atrophy is not available in our center so we could not assess that correlation, however, we compared serum NfL & NfH levels for different gd-enhanced MRI results, and in this regard, we could not

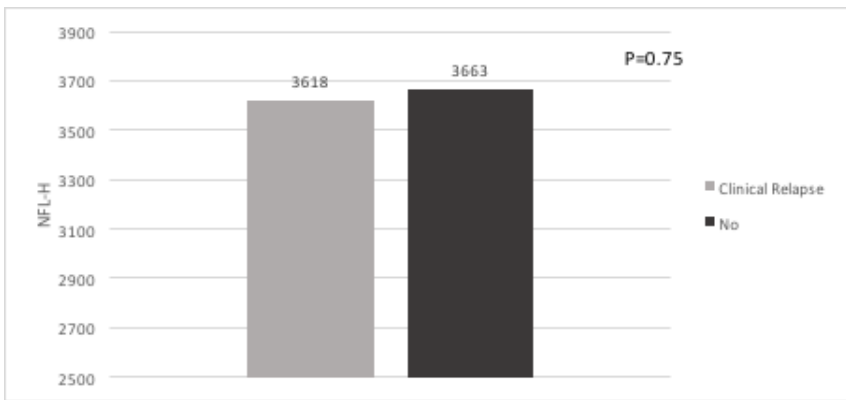

Figure 1 A: Mean NFL-H in pg/ ml among MS patients with clinical relapse in comparison to patients in remission.

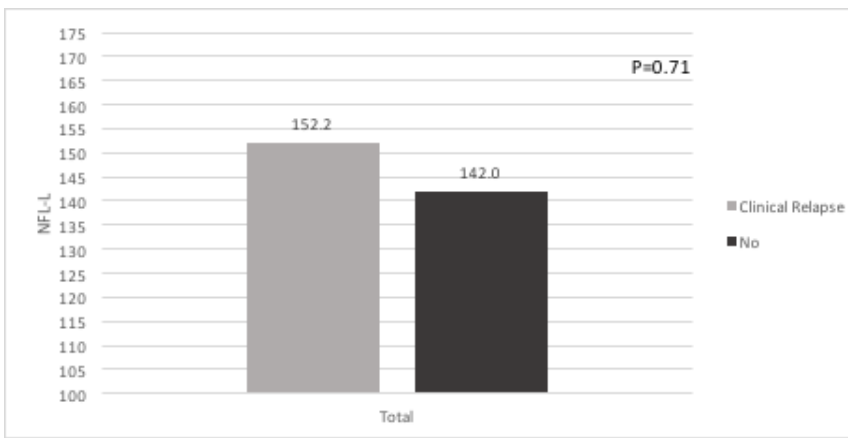

Figure 1 B: Mean NFL-L in pg/ml among MS patients with clinical relapse in comparison to patients in remission.

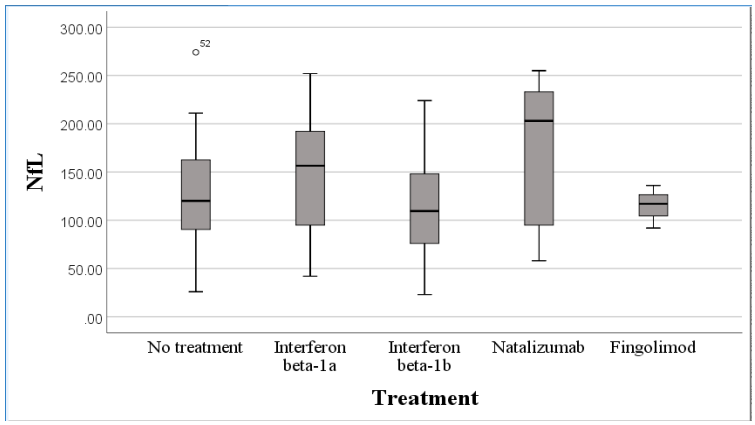

Figure 2 A: Boxplots of NfL levels in pg/ ml for different treatment groups in MS patients.

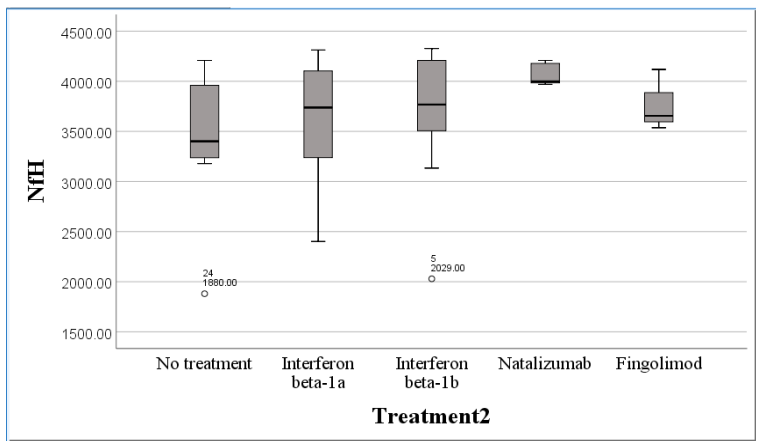

Figure 2 B: Boxplots of NfH levels in pg/ ml for different treatment groups in MS patients.

detect a significant association between serum Nf levels and MRI findings; however, the presence of significantly higher NfL and NfH levels in patients without Gd-enhanced lesions on MRI compared to those in healthy controls, suggests that Nf levels may reflect ongoing neuronal damage and loss, independent of detectable inflammatory activity.[35] Damasceno *et al*. and Gresle *et al*. have reached a similar conclusion in their analysis.[36,33]

Existing data on the relation between Nf and clinical outcomes in MS are limited to relatively short-term correlations. Disanto *et al*. showed that serum NfL was independently associated with EDSS assessments.[17] Other studies also showed that serum and CSF NfL levels were correlated with disability scores over time.[36,34] More recently, using large nationwide population-based data, Manouchehrinia *et al*. found that elevated NfL levels at the time of MS diagnosis are associated with the risk of long-term sustained disability development.[37] However, we were unable to reach a similar finding in our study; serum NfL and NfH could not be proved to correlate with EDSS or MSS scores. However, our results go in line with a study by Arrambide and colleagues which demonstrated that serum NfL levels were not correlated with disability in MS.[38] As for clinical disease activity, our study did not have the power to identify a significant difference in Nf levels between patients in remission and those with clinical relapse. On the other hand, many recent studies have consistently reported higher serum NfL levels in MS cases with disease activity compared to MS cases in remission. However, Cantó *et al*. reported no such association between serum NfL and disease activity in MS.[16]

There could be a few explanations for these inconsistencies: First, the sampling in our study was done at a single point in time and some reports suggest that Nf may be released periodically rather than continuously, so, cross-sectional sampling could underestimate the proportion of patients with intermittently high Nf serum titers; Secondly, the relatively small sample size and the

fact that many patients were clinically stable or on treatment, could be implicated in these results; And finally, the analysis of neurofilament levels in our study was undertaken by commercially available ELISA kits unlike most of the recent studies that have used SIMOA method for serum sample assay; which is more sensitive than ELISA[39], unfortunately, SIMOA is not available in our region.

Regarding treatment effects, longitudinal NfL reductions have been reported for most established treatments for relapsing and progressive MS, these include dimethyl fumarate[40], fingolimod[41], natalizumab[42] siponimod[43], ocrelizumab[44], and ofatumumab[45] among others. However, in this analysis, NfL and NfH levels were not significantly different between newly diagnosed patients and those already on treatment or between various treatment groups. This controversy in results could be justified by the fact that the participants in this study have been sampled only once, while a considerable treatment effect on Nf would be rather apparent if a frequent sampling method had been adopted instead.

In conclusion, in this study, we could not find a significant correlation between serum NfL levels and disease parameters in MS including disability, and clinical and radiological disease activity; however, serum Nf levels were significantly higher in MS cases compared to healthy controls.

DISCLOSURE

Ethics: This study was approved by the Institutional Review Board at Kufa University. Written informed consent from the patients was obtained.

Financial support: None

Conflict of interest: None

REFERENCES

1. Brownlee WJ, Hardy TA, Fazekas F, Miller DH. Diagnosis of multiple sclerosis: progress and challenges. *Lancet* 2017;389(10076):1336-46. DOI: 10.1016/S0140-6736(16)30959-X
2. Wootla B, Eriguchi M, Rodriguez M. Is multiple sclerosis an autoimmune disease? *Autoimmune Dis* 2012;2012:969657. DOI: 10.1155/2012/969657
3. Giovannoni G, Turner B, Gnanapavan S, Offiah C, Schmierer K, Marta M. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? *Mult Scler Relat Disord* 2015;4(4):329-33. DOI: 10.1016/j.msard.2015.04.006
4. Comabella M, Montalban X. Body fluid biomarkers in multiple sclerosis. *Lancet Neurol* 2014;13(1):113-26. DOI: 10.1016/S1474-4422(13)70233-3
5. 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):865-70. DOI: 10.1001/archneur.62.6.865
6. Delcoigne B, Manouchehrinia A, Barro C, *et al*. Blood neurofilament light levels segregate treatment effects in multiple sclerosis. *Neurology* 2020;94(11): e1201-12. DOI: 10.1212/WNL.0000000000009097
7. Gunnarsson M, Malmeström C, Axelsson M, *et al*. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. *Ann Neurol* 2011 ;69(1):83-9. DOI: 10.1002/ana.22247
8. Fuchs E, Cleveland DW. A structural scaffolding of intermediate filaments in health and disease. *Science* 1998;279(5350):514-9. DOI: 10.1126/science.279.5350.514
9. Bomont P. Degradation of the intermediate filament family by gigaxonin. *Methods Enzymol* 2016; 569:215-31. DOI: 10.1016/bs.mie.2015.07.009
10. Carare RO, Bernardes-Silva M, Newman TA, *et al*. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. *Neuropathol Appl Neurobiol* 2008;34(2):131-44. DOI: 10.1111/j.13652990.2007.00926.x
11. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelsø C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. *J Neurochem* 1996;67(5):2013-8. DOI: 10.1046/j.1471-4159.1996.67052013.x
12. Deisenhammer F, Egg R, Giovannoni G, *et al*. EFNS guidelines on disease-specific CSF investigations. *Eur J Neurol* 2009;16(6):760-e163. DOI: 10.1111/j.1468-1331.2009.02595.x
13. Lycke JN, Karlsson JE, Andersen O, Rosengren LE. Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1998;64(3):402-4. DOI: 10.1136/jnnp.64.3.402
14. Kuhle J, Plattner K, Bestwick JP, *et al*. A comparative study of CSF neurofilamen light and heavy chain protein in MS. *Mult Scler* 2013 ;19(12):1597-603. DOI: 10.1177/1352458513482374
15. Chitnis T, Gonzalez C, Healy BC, *et al*. Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. *Ann Clin Transl Neurol* 2018;5(12):1478-91. DOI: 10.1002/acn3.638
16. Cantó E, Barro C, Zhao C, *et al*. Association between serum neurofilament light chain levels and long-term disease course among patients with multiple sclerosis followed up for 12 years. *JAMA Neurol* 2019;76(11):1359-66. DOI: 10.1001/jamaneurol.2019.2137
17. Disanto G, Barro C, Benkert P, *et al*. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. *Ann Neurol* 2017;81(6):857-70. DOI: 10.1002/ana.24954
18. Varhaug KN, Barro C, Bjørnevik K, *et al*. Neurofilament light chain predicts disease activity in relapsing-remitting MS. *Neurol Neuroimmunol Neuroinflamm* 2018;5(1): 1-7. DOI: 10.1212/NXI.0000000000000422

19. Thompson AJ, Banwell BL, Barkhof F, *et al*. 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. Thebault S, Booth RA, Freedman MS. Blood neurofilament light chain: the neurologist's troponin? *Biomedicines* 2020;8(11):523-64. DOI: 10.3390/biomedicines8110523
21. Khalil M, Pirpamer L, Hofer E, *et al.* Serum neurofilament light levels in normal aging and their association with morphologic brain changes. *Nat Commun* 2020 ;11(1):812. DOI: 10.1038/s41467-020-14612-6
22. 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
23. Roxburgh RH, Seaman SR, Masterman T, *et al.* Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. *Neurology* 2005;64(7):1144-51. DOI: 10.1212/01.WNL.0000156155.19270.F8
24. Bergman J, Dring A, Zetterberg H, *et al*. Neurofilament light in CSF and serum is a sensitive marker for axonal white matter injury in MS. *Neurol Neuroimmunol Neuroinflamm* 2016 ;3(5):271-7. DOI: 10.1212/NXI.0000000000000271
25. Traboulsee A, Simon JH, Stone L, et al. Revised recommendations of the consortium of MS centers task force for a standardized MRI protocol and clinical guidelines for the diagnosis and follow-up of multiple sclerosis. *Am J Neuroradiol* 2016;37(3):394-401. DOI: 10.3174/ajnr.A4539
26. Cotton F, Weiner HL, Jolesz FA, Guttmann CR. MRI contrast uptake in new lesions in relapsing-remitting MS followed at weekly intervals. *Neurology* 2003;60(4):640-6. DOI: 10.1212/01.wnl.0000046587.83503.1e
27. Steinman L. Multiple sclerosis: a two-stage disease. *Nat Immunol* 2001;2(9):762-4. DOI: 10.1038/ni0901-762
28. Martin SJ, McGlasson S, Hunt D, Overell J. Cerebrospinal fluid neurofilament light chain in multiple sclerosis and its subtypes: a meta-analysis of case–control studies. *J Neurol Neurosurg Psychiatry* 2019;90(9):1059-67. DOI: 10.1136/jnnp-2018-319190
29. Thebault S, Abdoli M, Fereshtehnejad SM, Tessier D, Tabard-Cossa V, Freedman MS. Serum neurofilament light chain predicts long term clinical outcomes in multiple sclerosis. *Sci Rep* 2020;10:10381. DOI: 10.1038/s41598-020-67504-6
30. Gafson AR, Jiang X, Shen C, *et al*. Serum neurofilament light and multiple sclerosis progression independent of acute inflammation. *JAMA Netw Open* 2022;5(2): e2147588. DOI:10.1001/jamanetworkopen.2021.47588
31. Cai L, Huang J. Neurofilament light chain as a biological marker for multiple sclerosis: a meta-analysis study. *Neuropsychiatr Dis Treat* 2018; 14:2241. DOI: 10.2147/NDT.S173280
32. Reinert MC, Benkert P, Wuerfel J, *et al*. Serum neurofilament light chain is a useful biomarker in pediatric multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* 2020;7(4): e749. DOI: 10.1212/NXI.0000000000000749
33. Gresle MM, Liu Y, Dagley LF, *et al*. Serum phosphorylated neurofilament-heavy chain levels in multiple sclerosis patients. *J Neurol Neurosurg Psychiatry* 2014;85(11):1209-13. DOI: 10.1136/jnnp-2013-306789
34. Siller N, Kuhle J, Muthuraman M, *et al.* Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. *Mult Scler* 2019;25(5):678-86. DOI: 10.1177/1352458518765666
35. Kuhle J, Nourbakhsh B, Grant D, *et al.* Serum neurofilament is associated with progression of brain atrophy and disability in early MS. *Neurology* 2017;88(9):826-31. DOI: 10.1212/WNL.0000000000003653
36. Barro C, Benkert P, Disanto G, *et al*. Serum neurofilament light chain as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. *Brain* 2018: 141; 2382-91. DOI: 10.1093/brain/awy154
37. Damasceno A, Dias-Carneiro RP, Moraes AS, *et al*. Clinical and MRI correlates of CSF neurofilament light chain levels in relapsing and progressive MS. *Mult Scler Relat Disord* 2019; 30:149-53. DOI: 10.1016/j.msard.2019.02.004
38. Novakova L, Axelsson M, Khademi M, *et al*. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. *J Neurochem* 2017; 141:296–304. DOI: 10.1111/jnc.13881
39. Manouchehrinia A, Stridh P, Khademi M, *et al*. Plasma neurofilament light levels are associated with risk of disability in multiple sclerosis. *Neurology* 2020;94(23): e2457-67. DOI: 10.1212/WNL.0000000000009571
40. Arrambide G, Espejo C, Eixarch H, *et al*. Neurofilament light chain level is a weak risk factor for the development of MS. *Neurology* 2016;87(11):1076-84. DOI: 10.1212/WNL.0000000000003085
41. Novakova L, Zetterberg H, Sundström P, *et al*. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. *Neurology* 2017;89(22):2230-7. DOI: 10.1212/WNL.0000000000004683
42. Kuhle J, Kropshofer H, Haering DA, *et al*. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. *Neurology* 2019;92(10): e1007-15. DOI: 10.1212/WNL.0000000000007032
43. Kuhle J, Barro C, Andreasson U, *et al*. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med* 2016;54(10):1655-61. DOI: 10.1515/cclm-2015-1195
44. Sejbaek T, Nielsen HH, Penner N, *et al*. Dimethyl fumarate decreases neurofilament light chain in CSF and blood of treatment naïve relapsing MS patients. *J Neurol Neurosurg Psychiatry* 2019;90(12):1324-30. DOI: 10.1136/jnnp-2019-321321

45. Piehl F, Kockum I, Khademi M, *et al*. Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. *Mult Scler* 2018;24(8):1046-54. DOI: 10.1177/1352458517715132
46. Kapoor R, Sellebjerg F, Hartung HP, *et al*. Natalizumab reduced serum levels of neurofilament light chain in secondary progressive multiple sclerosis patients from the phase 3 ASCEND study. *Mult Scler* 2018; 24:988. DOI: https://doi.org/10.1177/1352458518799980
47. Kuhle J, Kropshofer H, Barro C, et al. Siponimod reduces neurofilament light chain blood levels in secondary progressive multiple sclerosis patients. *Neurology* 2018; 90 (15 Supplement) (S8. 006). DOI: 10.1212/WNL.90.15_supplement.S8.006
48. Bar-Or A. Blood neurofilament light levels are lowered to a healthy donor range in patients with RMS and PPMS following ocrelizumab treatment. ECTRIMS Online Library. Dec 9, 2019.
49. Hauser SL, Bar-Or A, Cohen JA, *et al*. Ofatumumab versus teriflunomide in multiple sclerosis. *N Eng J Med* 2020;383(6):546-57. DOI: 10.1056/NEJMoa1917246