Neurofilament Light Chain as a Biomarker in Multiple Sclerosis

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Abstract

Structure of Neurofilament proteins: light (NF-L), medium (NF-M), and heavy (NF-H) which belongs to Type IV intermediate filament family (IF), are neuron-specific cytoskeletal components, they are axonal structural components and integral components of synapses, which are important for neuronal electric signal transmissions along the axons . Due to the heterogeneity of MS, with a big variation in disease activity and prognosis, and with an increasing number of medications with different mechanisms of action, it would be a great advantage to have biomarkers that could facilitate disease activity assessments and individualized treatment.Neurofilament light chain levels in serum/plasma has shown consistent promise in MS, and unlike cytokines and chemokines biomarkers, the only source of NFL is neurons.As regards MS diagnosis and NfL, without considering any additional clinical context, sNfL alone is insufficient for a diagnosis of MS or for differentiating it from other neuroinflammatory disorders with neuroaxonal damage and elevated sNfL levels

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Introduction

Structure of Neurofilament proteins: light (NF-L), medium (NF-M), and heavy (NF-H) which belongs to Type IV intermediate filament family (IF), are neuron-specific cytoskeletal components, they are axonal structural components and integral components of synapses, which are important for neuronal electric signal transmissions along the axons. **(1)**

Although neurofilament protein chains differ significantly from one another in terms of molecular mass, they share a common structure with about 46 nm long central α-helical coiled region (the rod domain) composed of approximately 310 amino acids ,separating non-helical amino-and carboxy-terminal regions (called the head and tail domains). (2)

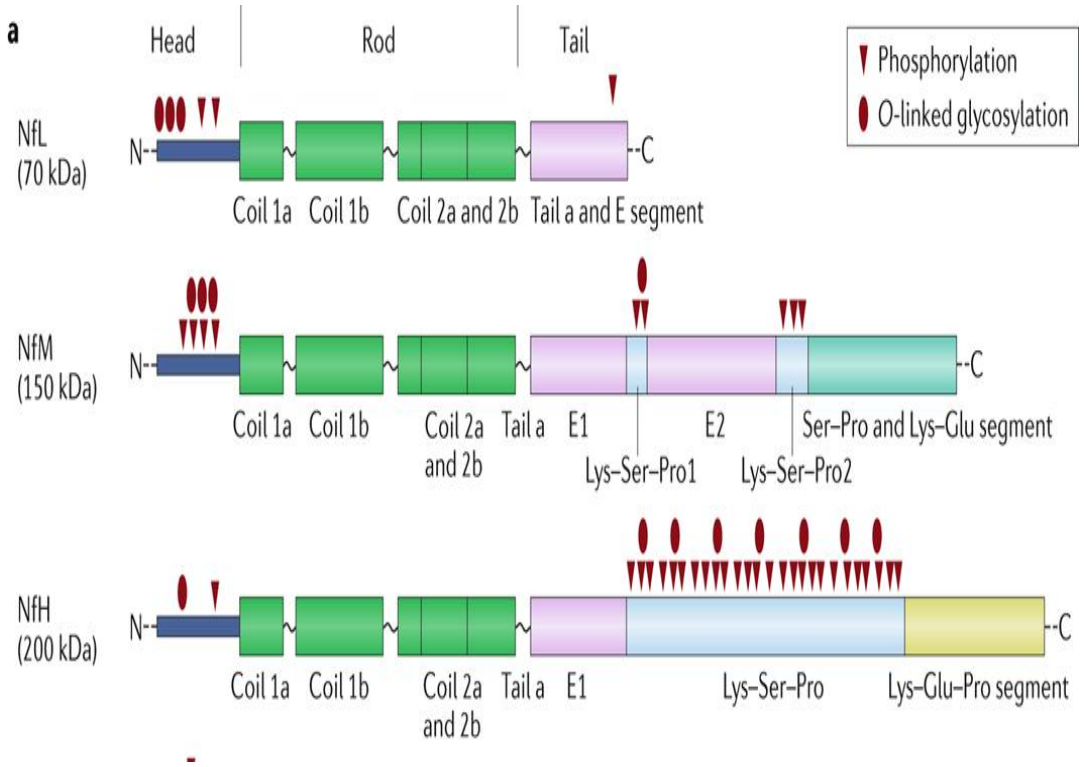

Fig1. Structure of neurofilaments. A domain structure and post-translational modifications of neurofilament subunits. Neurofilament light chain (NfL), neurofilament medium chain (NfM), neurofilament heavy chain (NfH), α-internexin and peripherin are the subunits of neurofilaments in the mature nervous system. (3)

Neurofilament protein expression: In human, genes coding for NFL and NF-M are very closely linked on chromosome 8 (8p21), while NFH gene (NEFHgene) is located on chromosome 22(22q12.2) (2)

They have a central role in specifying axonal conduction properties as their main function corresponds to the growth and maintenance of axonal caliber which in turn determines the conduction velocity. (2) Furthermore, they are the key participants of the intracellular network that supports mechanical stability to the axon, also been implicated in neuronal development and regeneration. (4)

Regarding to the key role in specifying the axonal diameter of large myelinated motor and sensory neurons, the axonal development begins in a post-natal stage parallel with myelination and continues through adulthood. During this development, the axons increase in diameter from

~1-2µm in the early stages to ~14 µm with a corresponding increase in the volume (> 100 fold). (5)

The expansion of axonal size by up to five fold is initiated by localized accumulation of NFs, specifically in the myelinated regions of the axon as direct evidence to this, the radial growth of the axons is correlated to marked up-regulation of the NF mRNA and local accumulation of NFs in axons. (3)

Being the key determinants of axonal diameter, NFs also influence the nerve conduction properties. Conduction velocity is the rate at which action potential is propagated through nerves.It is influenced by three main factors,) axonal diameter 2) myelination and 3) internodal length.The conduction velocity varies directly with respect to axonal diameter. Aberrant phosphorylation of NFs leads to their accumulation in cell bodies and has been observed in the brains of Alzheimer disease patients and those suffering from other neurodegenerative disorders. (3)

The interior of the axon is extensively interconnected structures that maintain mechanical stability to the axons and protects them against external compression. Neurofilaments are the major participants of this cytoskeletal framework. (6)

Neurofilament light chain (NfL) is a major component of neuronal and axonal cytoskeleton proteins, providing structural support in the central and peripheral nervous systems, appropriate expression, assembly, transport and formation of network by these neurofilaments are essential for proper neuronal growth. (7)

Disruptions in any of these aspects could lead to the abnormal accumulation of NfL in neuronal cell. Such accumulated networks are the hallmark of various neurodegenerative disorders including amyotrophic lateral sclerosis , infantile spinal muscular atrophy, and hereditary sensory-motor neuropathies ,several factors can induce abnormal neurofilaments accumulations, among these, NF gene mutation, abnormal post translational modifications, defective NF transport are considered the main factors. (3)

Neurofilament light chain as a biomarker in multiple sclerosis

Due to the heterogeneity of MS, with a big variation in disease activity and prognosis, and with an increasing number of medications with different mechanisms of action, it would be a great advantage to have biomarkers that could facilitate disease activity assessments and individualized treatment. (8)

The biomarker, can be defined as a biological molecule that isfound in blood, other body fluids, or tissues objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. (9)

In MS, a good and reliable biomarker should say something about the stage of the disease, the prognosis and the response to treatment. A biomarker does not, however, need to be disease specific. increased NF-L level appears to reflect ongoing neuronal damage, irrespective of the underlying pathology, making it a potentially interesting biomarker . As NFL is a neuronal specific protein, the finding of these proteins in serum reflects leakage or diffusion through the blood-brain barrier . It can also reach the blood through CSF drainage into venous blood.**(9)**

A recent study demonstrated that NFL can be released into the csf during several neurodegenerative diseases. Its levels were elevated in different neurological disorders such as cerebral infarction, amyothropic lateral sclerosis, late onset Alzehimer disease, vascular dementia and MS. Monitoring NFL levels in CSF would require repeated lumbar punctures for CSF sampling which would cause more discomfort for the patients and would be more time consuming for neurologists than using a biomarker that could be monitored by repeated blood sampling **(10)**.

As regards MS diagnosis and NfL, without considering any additional clinical context, sNfL alone is insufficient for a diagnosis of MS or for differentiating it from other neuroinflammatory disorders with neuroaxonal damage and elevated sNfL levels. **(11,12)**

Lesions in MRI and oligoclonal bands in the CSF are established factors for a diagnosis of MS. Elevated NfL values in the CSF was an additional predictor of future relapses as in the study of **Van der Vurst et al., (13)** on 88 adult and 65 paediatric patients with a first attack of demyelination, similar findings were concluded by **Dalla Costa et al., (14)** who studied serum NfL on 222 CIS patients .

In a cohort study of more than 800 patients from the German Clinical Competence Network for Multiple Sclerosis, the role of sNfL levels in early cases of MS could have a diagnostic value. The inclusion of sNfL levels as an additional parameter into the current 2017 version of the McDonald criteria may increase the sensitivity and specificity of differentiating patients with CIS and RRMS.**(15)**

Regarding to MS activity, a relapse is considered to be a classic sign of disease activity, but absence of relapses does not mean that there is no disease activity. New hyper intense T2 lesions, Gadolinium-enhancing lesions and/or hypo intense T1 lesions in follow-up MRIs are also well-established signs of disease activity, with or without clinical symptoms. **(15)**

Sustained Expanded Disability Status Scale (EDSS) progression may also be a sign of disease activity. The no evidence of disease activity (NEDA) concept can be useful when discussing disease activity. The NEDA-3 denotes a situation where a patient has no relapses, no sustained EDSS progression and no MRI activity. **(16)**

A spectrum of clinical and MRI parameters which linked to inflammatory processes and disease activity have demonstrated correlations to sNfL level as in the study on 814 RRMS and CIS patients by **Bittner et al., (17)**that clarified that sNfL levels correlated with concurrent relapses, the presence of gadolinium-enhancing lesions, the occurrence and the number of new T2-weighted lesions .

Srpova et al., (18)as wellmeasured sNfl in 172 patients in the early stages of MS and found its levels correlated with the presence of T1- hypo intense lesions and T1 lesion volume. They also concluded that increased levels of sNfL in early MS stages reflects neuropathological processes driven mainly by ongoing neuroinflammation as indirectly assessed by the accumulation of lesion burden.

There are numbers of studies have confirmed that high sNfL levels have predictive value for future MRI-based brain atrophy over the next 2–5 years.**(16)**

Conventional MRI without Gadolinium enhancing lesions can be viewed as showing some of the things that have happened in the brain in the past, while NFL can be said to measure, in real time, the ongoing axonal damage status. This has inspired the concept stated that peripheral blood NFL levels being "the neurologist´s C- reactive protein . **(19)**

Over a relatively short period, high sNfL levels were associated with an increased risk for relapses and/or EDSS deterioration over the next 1–3 years. In a case control study of patients with relapsing and progressing MS (n = 259 each), sNfL levels above the 90th percentile could predict EDSS worsening in the subsequent year . **(20)**

In a more recent study, the levels of sNfL were increased 6 years before the clinical MS onset, indicating that MS may have a prodromal phase lasting several years and that neuroaxonal damage occurs already during this phase as concluded by the nested case-control study by **Bjornevik et al .,(21).**

In a 5-year longitudinal study of more than 1200 Swiss MS patients, high age-adjusted NfL was associated with increased risk of relapse and new MRI activity in the following year. Even in patients who met criteria for "no evidence of disease activity", indicating that NfL is capable of predicting subclinical disease activity otherwise not captured . **(12)**

The clinical course of multiple sclerosis (MS) is highlyvariable, ranging from rapidly reversible episodes ofimpairment to severe disability within months after diseaseonset. Focal inflammation, chronic diffuse neuronal damage, and failure of repair or compensation all contribute tothe development of permanent disability.

Biomarkersreflecting tissue damage and allowing the monitoring of sub-clinical disease activity are highly desirable for assessment oftherapeutic response and prediction of disability in bothclinical studies and management of individual patients.

Together with the medium and heavy subunits,neurofilament light chain (NfL) represents one of the clinical course of multiple sclerosis (MS) is highlyvariable, ranging from rapidly reversible episodes ofimpairment to severe disability within months after diseaseonset. Focal inflammation, chronic diffuse neuronal dam-age, and failure of repair or compensation all contribute tothe development of permanent disability. **(22).**

Disanto et al., (23)investigated serum NfL levels in 100 patients with CIS their age ranged from 25 to 45 years with about two thirds of cases were females and found that higher sNfL independently could predict faster conversion to clinically definite MS.

Bhan et al., (24) studied CSF NFL levels in 44 RRMS patients (30 females and 14 males) with mean age 41 years concluded that its levels predicted the transition from RRMS to secondary SPMSafter 5-years follow-up.

Regarding to the Relationship between serum NfL and cognitive impairment in MS, In one larger study using BICAMS (Brief International Cognitive Assessment for Multiple Sclerosis), cognitively impaired in MS patients had higher sNfL levels and a greater longitudinal sNfL increase compared with non-cognitively impaired patients.The CSF NfL levels is higher in MS patients with cognitive impairment and especially in those with impaired information processing speed and verbal fluency, assessed by the Brief Repeatable Battery of Neuropsychological Tests. **(25)**

In most recent study illustrated thatlarge lesions in the frontal lobe would likely result in a very significant elevation in sNfL with minimal appreciable disability, conversely a small lesion affecting key brainstem structures may result in a smaller sNfL rise but significant long-term disability. So, axon density in different brain and spine regions could be important determinants of the quantitively rise in sNfL in response to a given lesion. **(26)**

Neurofilament light chain (NFL) and treatment response in Multiple sclerosis regarding DMTs:

An important treatment goal in modern MS treatment regimens is to suppress disease activity as much as possible, sometimes expressed as “treating to NEDA”.(No Evidence of Disease Activity) . Defining and evaluating disease activity is of major importance for decisions on therapy initiation, therapy continuation and therapy change. In clinical practice, the initial NEDA-3 concept (no relapses, no sustained EDSS progression and no focal MRI activity) is still dominating. **(19)**

No Evidence of Disease Activity has been criticized for not necessarily picking up diffuse low grade disease activity and neurodegeneration. Increased brain volume loss and abnormal levels of some body fluid biomarkers are considered by many to be signs of disease activity as well, and including such parameters in the NEDA concept would expand it to NEDA-4 or NEDA-5 which could include **(no relapse, no focal MRI activity, no EDSS progression, no increased in brain atrophy and no elevation of NFL)** and make it a more comprehensive disease activity evaluation tool **(20)**

Expanded NEDA concept would better reflect disease activity in MS, initial findings in the study done by **Håkanssonet al., (27)** strengthened the opinion that NFL level is a clinically useful biomarker in CIS and RRMS and that NFL could be included in the expanding NEDA concept .Independent of the type of disease-modifying treatment, the majority of investigators have detected an inverse correlation between NF-L levels and treatment. Lower levels of NF-L were found in treated patients compared to treatment-naïve individuals, and it has also been shown that NFL levels fall in follow-up studies of disease-modifying treatment vs. no treatment **(22)**

Varhaug et al., (28)stated thatserum NF-L levels fall after initiation of interferon-beta and plasma NF-L levels fell by 34% after 12 months of fingolimod treatment (shifted from interferon or glatiramer acetate)

The effect of dimethyl-fumarate (DMF) on serum NfL levels was assessed in a cohort of 104 previously treatment-naïve RRMS patients (their age ranged from 33 – 47 years and about two thirds of the cases were females) . After one year of treatment, CSF and serum NfL levels had all been reduced to levels comparable to that measured at baseline before treatment. **(29)**

Several studies evaluated NfL in patients receiving natalizumab (NAT), an already significant decrease in CSF and serum NfL concentrations was demonstrated after just 12 months of treatment with NAT in RRMS patients **(30)**

Serum NfL levels during Natalizumab treatment also correlated with Progressive multifocal Leucoencephalopathy (PML) risk and furthermore showed a significant increase in the case of PML onset. **(31)**

In a more recent study evaluated the effectiveness of cladribine, and reported significantly reduced CSF NfL levels at follow-up in a subpopulation of patients with elevated baseline NfL levels **(32)**

Fingolimod significantly decreased NfL levels in the CSF after one year and in serum after 6 months in RRMS patients. The effect of this drug seems to be quite rapid, since a significant decline is visible already after 6 months of treatment with subsequent stabilization of NfL levels. **(33)**

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References:

[1] Zhao Y, Arceneaux L, Culicchia F and Lukiw W (2021): Neurofilament Light (NF-L) chain protein from a highly polymerized structural component of the neuronal cytoskeleton to a neurodegenerative disease biomarker in the periphery. HSOA j. alzheimer'sneurodegener. dis., 7(2):1-16.

[2] Perrot , Rodolphe, and Joel Eyer (2013) :"Intermediate filaments in neurodegenerative diseases." Neurodegenerative diseases. InTech, Rijeka, Croatia : 457-489.

[3] Yuan A, Hassinger L, Rao M, Julien JP, Miller C and Nixon R (2015): Dissociation of axonal neurofilament content from its transport rate. PLoS One, 10(7), e0133848

[4] Wang H, Wu M, Zhan C, Ma E, Yang M, Yang X, and Li Y (2012): Neurofilament proteins in axonal regeneration and neurodegenerative diseases. Neural Regen. Res., 7(8), 620-632.

[5] Tymanskyj SR, Scales TM and Gordon-Weeks PR (2012): MAP1B enhances microtubule assembly rates and axon extension rates in developing neurons. Mol. Cell. Neurosci, 49(2), 110-119.

[6] Laser-Azogui A, Kornreich M, Malka-Gibor E and Beck R (2015): Neurofilament assembly and function during neuronal development. Curr. Opin. Chem. Biol., 32, 92-101.

[7] Ghasemi M and Brown RH. (2018): Genetics of amyotrophic lateral sclerosis. Cold Spring Harb. perspect. med., 8(5), a024125.

[8] Paul A, Comabella M and Gandhi R (2019): Biomarkers in multiple sclerosis. Cold Spring Harbor perspectives in medicine, 9(3), a029058-78.

[9] Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D and Ghezzi P (2015): Clinical relevance of biomarkers of oxidative stress. ARS, 23(14), 1144-1170.

[10] Hyun J, Kim Y, Kim G, Kim S and Kim H (2020): Longitudinal analysis of serum neurofilament light chain: a potential therapeutic monitoring biomarker for multiple sclerosis. MultScler J, 26(6), 659-667.

[11] Biernacki T, Kokas, Z, Sandi D, Füvesi J, Fricska-Nagy Z, Faragó P and Vécsei, L (2022): Emerging biomarkers of multiple sclerosis in the blood and the CSF: a focus on neurofilaments and therapeutic considerations. Int. J. Mol. Sci.". 23(6), 3383-3389.

[12] Gärtner J, Chitnis T, Arnold D L, Banwell B and et al (2018): Trial of fingolimod versus interferon beta-1a in pediatric multiple sclerosis. NEJM, 379(11), 1017-1027.

[13] Van der Vuurst de Vries R, Wong Y, Mescheriakova J, van Pelt E, Runia T, Jafari N and Hintzen R (2019): High neurofilament levels are associated with clinically definite multiple sclerosis in children and adults with clinically isolated syndrome. MultScler J, 25(7), 958-967.

[14] Dalla Costa G, Martinelli V, Sangalli F, et al. (2019): Prognostic value of serum neurofilaments in patients with clinically isolated syndromes. Neurol; 92(7): 733 –741.

[15] Zipp F, Oh J, Fragoso Y, Waubant E (2019): implementing the 2017 McDonald criteria for the diagnosis of multiple sclerosis. Nat Rev Neurol.; 15(8):441–445.

[16] Hegen H, Bsteh G, Berger T (2018): 'No evidence of disease activity' - is it an appropriate surrogate in multiple sclerosis? Eur .J .Neurol, 25:1107-e1101.

[17] Bittner S, Oh J, Havrdová EK, Tintoré M and Zipp F (2020): The potential of serum neurofilament as biomarker for multiple sclerosis. Brain, 144(10), 2954-2963.

[18] Srpova B, Uher T, Hrnciarova T, Barro C, Andelova M, Michalak Z and Horakova D (2021): Serum neurofilament light chain reflects inflammation-driven neurodegeneration and predicts delayed brain volume loss in early stage of multiple sclerosis. MultScler J , 27(1), 52-60.

[19] Giovannoni G (2018): Peripheral blood neurofilament light chain levels: the neurologist's C-reactive protein? Brain, 141(8), 2235-2237.

[20] Barro C, Chitnis T and Weiner H (2020): Blood neurofilament light: a critical review of its application to neurologic disease. Ann Clin Transl Neurol, 7(12), 2508-2523.

[21] Bjornevik K, Munger KL, Cortese M, Barro C, Healy BC, Niebuhr, DW and Ascherio A (2020): Serum neurofilament light chain levels in patients with presymptomatic multiple sclerosis. JAMA Neurol., 77(1), 58-64.

[22] Lu G , Beadnall H, Barton J, Hardy, T, Wang C, & Barnett M (2018): The evolution of "No Evidence of Disease Activity" in multiple sclerosis. Mult. Scler. Relat. Disord., 20, 231-238.

[23] Disanto G, Zecca C, MacLachlan S, Sacco R, Handunnetthi L, Meier UC and Gobbi C (2018): Prodromal symptoms of multiple sclerosis in primary care. Ann. Neurol., 83(6), 1162-1173.

[24] Bhan A, Jacobsen C, Myhr K , Dalen I, Lode K and Farbu E (2018): Neurofilaments and 10-year follow-up in multiple sclerosis. MultScler. 24(10):1301–1307

[25] Gaetani L, Salvadori N, Lisetti V, et al. (2019): Cerebrospinal fluid neurofilament light chain tracks cognitive impairment in multiple sclerosis. J Neurol. 2019; 266(9):2157–2163.

[26] Thebault S, Booth RA and Freedman MS (2020): Blood neurofilament light chain: the neurologist's troponin? Biomedicines, 8(11), 523-534.

[27] Håkansson I, Johansson L, Dahle C, Vrethem M and Ernerudh J (2019): Fatigue scores correlate with other self-assessment data, but not with clinical and biomarker parameters, in CIS and RRMS. Mult. Scler. Relat. Disord, 36, 101424.

[28] Varhaug K, Barro C, Bjørnevik K, Myhr KM, Torkildsen Ø, Wergeland S and Vedeler C (2018): Neurofilament light chain predicts disease activity in relapsing-remitting MS. Neurology-, 5(1).

[29] Walo-Delgado PE, Sainz de la Maza S, Villarrubia N, Monreal E, Medina S, Espiño M and Villar LM (2021): Low serum neurofilament light chain values identify optimal responders to dimethyl fumarate in multiple sclerosis treatment. Scientific Reports, 11(1), 1-8.

[30] De Flon P, Laurell K, Sundström P, Blennow K , Söderström L, Zetterberg H and Svenningsson A (2019): Comparison of plasma and cerebrospinal fluid neurofilament light in a multiple sclerosis trial. Acta Neurol. Scand, 139(5), 462-468.

[31] Loonstra FC, Verberk IM, Wijburg MT, Wattjes MP, Teunissen C E, van Oosten BW and van Kempen ZL (2019): Serum neurofilaments as candidate biomarkers of natalizumab associated progressive multifocal leukoencephalopathy. Ann.Neurol, 86(2), 322-324.

[32] Oreja-Guevara C, Brownlee W, Celius EG, Centonze D, Giovannoni G, Hodgkinson S and Yamout B (2022): Expert opinion on the long-term use of cladribine tablets for multiple sclerosis: Systematic literature review of real-world evidence. Mult. Scler. Relat. Disord, (69),104459-67.

[33] Kuhle J, Kropshofer H, Haering DA, Kundu U, Meinert R, Barro C and Kappos L (2019): Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. J. Neurol., 92(10), e1007-e1015.