Section A-Research paper

An Overview about Neurofilament Light Chain in Multiple Sclerosis

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Abstract

Background: Because of the unpredictable course and heterogenous treatment response in multiple sclerosis (MS), there is a need for biomarkers that can reflect disease activity in the clinical follow up of these patients & the Neurofilament light chain (NFL) is considered to be the most promising biomarker in MS patients. The acute and persistent demyelination in MS results in axonal transection and free (NFL) molecules are released in the interstitial space and diffuse into the cerebrospinal fluid (CSF) and into the bloodstream. Serum neurofilament light chain (NFL) is associated with ongoing neuroinflammation and predictive of future neurodegeneration in early MS. Increased levels of serum NFL in early MS stages reflects neuropathological processes driven mainly by ongoing neuroinflammation as indirectly assessed by the accumulation of lesion burden. In addition, serum NFL levels have a stronger association with future development of brain atrophy than with actual or previous brain volume loss. Neurofilament light chain determinations in peripheral blood for detecting axonal damage may represent new possibilities in MS monitoring. The use of plasma for NFL measurement makes this biomarker valuable for clinical studies since sample collection can be performed repeatedly without causing much harm. Most of the reports have been based on results obtained with a commercially-available ELISA that uses two highly-specific, noncompeting monoclonal antibodies to quantify soluble NFL in samples.

Keywords: Neurofilament Light Chain, Multiple Sclerosis

Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating and neurodegenerative disease of the central nervous system, and the leading cause of nontraumatic neurological disability in young adults **(1).**

The first symptoms usually occur during young adulthood, after which patients may experience a heterogeneous relapsing or progressive disease course with a modulating role for age, sex and comorbidities **(2).**

The most common symptoms of MS are fatigue, reduced mobility, bowel and bladder disturbances, diminished cognitive function, pain, sensory loss and depression. The majority of people with MS experience chronic progression that may become incapa-citating and require profound lifestyle **(3).**

MS pathology is characterized by multiple lesions within the central nervous system (CNS). The pathogenesis of brain lesions remains unknown; however, neurodegeneration due to inflammation and immune reaction towards the brain cells is believed to play the central role. Brain plaques in MS are predominantly found within the white matter around the lateral ventricles and optic nerve. Recently, the presence of the brain plaques in the grey matter was demonstrated These lesions could be detected early and correlate with the disease severity Lesions in periventricular locations along the 4th ventricle, midbrain and cerebellar peduncle are also characteristic for MS, though they are less prevalent (**4).**

Inflammation, characterized by the presence of perivascular T- and B-lymphocytes and their dispersion into the parenchyma so this inflammatory process in MS is associated with the formation of different focal lesion types in the white matter of the brain and spinal cord. Cortical lesions, present in the forebrain, the cerebellum, and the hippocampus, have recently been identified as a major substrate of MS pathology as demyelination in the Gray Matter. Diffuse injury in the normal appearing white matter is prominent in the MS brain and spinal cord, in particular in patients in the progressive stage of the disease **(5)**.

The number of people with MS worldwide has increased to 2.8 million in 2020. Globally, females are twice as likely to have MS as males and this is consistent with both prior editions of the Atlas. However, the ratio of women to men is as high as 4:1 in some countries and in others this ratio has doubled since 2013 **(6).**

MS Epidemiology in Egypt:

The mean age of disease onset was 26.6 ± 7.8 years, with the majority being female (2.11:1). Relapsing–remitting MS (RRMS) was the most common type (75.1%). Family history of MS was found in 2.28%. The main presenting symptom was motor weakness (43.9%), which was also the most frequent symptom during the disease course. Higher initial Expanded Disability Status Scale score (EDSS), black holes, and infratentorial lesions on initial magnetic resonance imaging (MRI) were independent factors for disease progression **(6).** The prevalence of MS is rising in Egypt (25/100,000 was recorded from different centers), and patients can typically live with MS for almost 40 years **(7).**

The Intermediate filament (IF) family proteins share a common tripartite structure at a molecular level, which is a conserved central α-helical rod domain flanked by two variable head and tail domains located at the C- and N-terminus, respectively **(8).**

Neurofilaments (NFs) belong to Type IV intermediate filament family (IF), which are specific neuron cytoskeletal components responsible for axonal structure which are important for neuronal electric signal transmissions along the neural axons **(9).**

Regarding to the neurons of central nervous system (CNS) which are heteropolymers, they are composed of different subunits that are categorized according to their molecular weight in light, medium, and heavy chain types**. (10).**

Neurofilament Light Chain as a Biomarker in Multiple Sclerosis

Because of the unpredictable course and heterogenous treatment response in multiple sclerosis (MS), there is a need for biomarkers that can reflect disease activity in the clinical follow up of these patients & the Neurofilament light chain (NFL) is considered to be the most promising biomarker in MS patients **(11).**

The acute and persistent demyelination in MS results in axonal transection and free (NFL) molecules are released in the interstitial space and diffuse into the cerebrospinal fluid (CSF) and into the bloodstream. **(12).**

Studies confirm that the serum (NFL) levels are higher in patients with multiple sclerosis compared to healthy control**.** Axonal loss and neurodegeneration are main elements of MS pathology, so an objective biomarker to detect and quantify them should be of great value **(13).**

Serum neurofilament light chain (NFL) is associated with ongoing neuroinflammation and predictive of future neurodegeneration in early MS. Increased levels of serum NFL in early MS stages reflects neuropathological processes driven mainly by ongoing neuroinflammation as indirectly assessed by the accumulation of lesion burden. In addition, serum NFL levels have a stronger association with future development of brain atrophy than with actual or previous brain volume loss **(14).**

According to the advancing highly sensitive assay for NFL chain and verifying correlation of NFL chain levels in CSF and those in serum, serum NFL has been proposed a potential biomarker for disease activity in MS **(15).**

Neurofilament light chain determinations in peripheral blood for detecting axonal damage may represent new possibilities in MS monitoring**.** The use of plasma for NFL measurement makes this biomarker valuable for clinical studies since sample collection can be performed repeatedly without causing much harm. The quantity of neurofilament light chain in the serum is rapidly emerging as a convenient and important biomarker in MS, with evidence for its role in monitoring disease activity and treatment response

. Most of the reports have been based on results obtained with a commercially-available ELISA that uses two highly-specific, noncompeting monoclonal antibodies to quantify soluble NFL in samples **(16).**
Several test systems exist to determine neurofilament heavy chain (NFH) and neurofilament light chain (NFL) and a commercially available (ELISA) to detect NFL is advantageous in discriminating patients with MS from healthy control **(13).**
The light or lowest (NFL) runs at 68-70 KiloDaton (KDa), the medium or middle(NFM) runs as about 145-160 KDa and the heavy or highest (NFH) runs at 200-220 KDa **(17).**

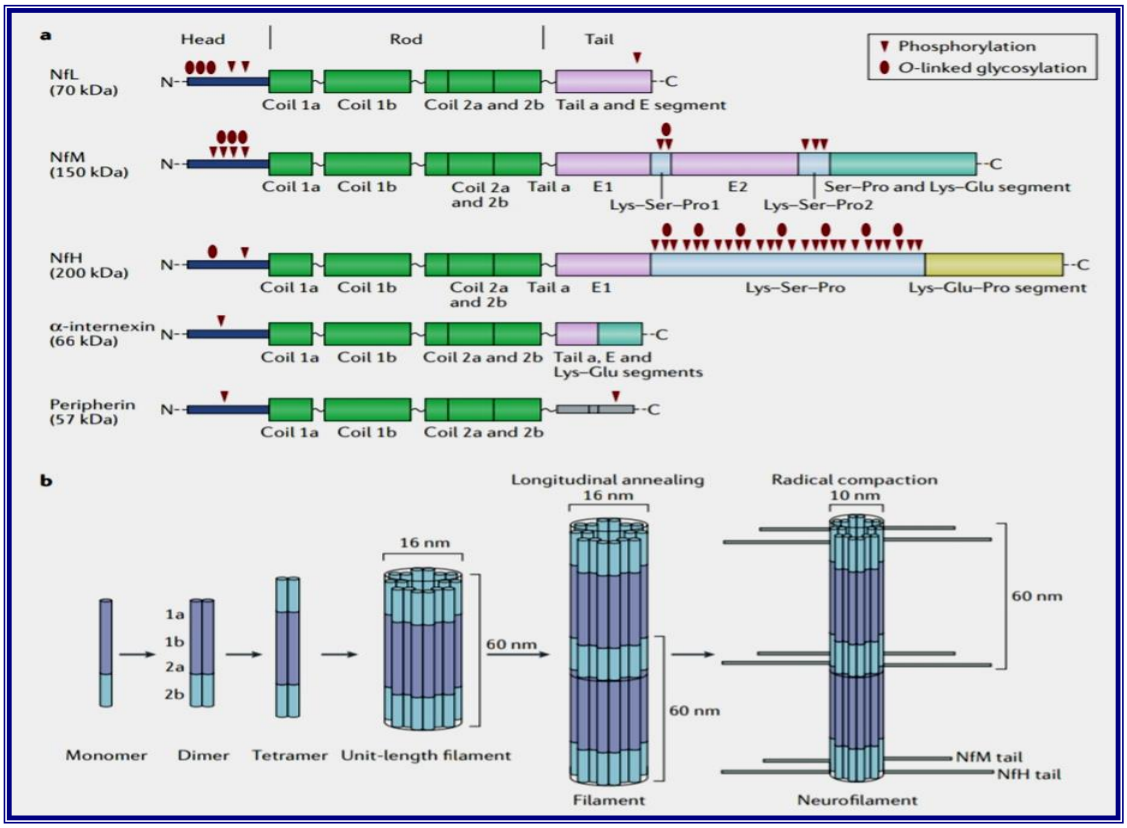

Fig. (1): Neurofilament structure **(18)**

Neurofilaments structure:

At a molecular level, IF proteins share a common tripartite structure which consist of a conserved central α-helical rod domain flanked by two variable head and tail domains.Neurofilaments (NFs), the intermediate filaments of mature neurons, are among the most abundant proteins in brain and expand the calibers of large myelinated axons and unlike the intermediate filaments of other cell types, central nervous system (CNS) NFs are hetero-polymers and differ from most other intermediate filament proteins in being regulated by phosphorylation at many sites by multiple protein kinases **(19).**

Neurofilaments function:

Neurofilaments expand the calibers of large myelinated axons to support nerve conduction and support dendrites of large motoneurons **(19)** also determine axonal caliber, which controls signal conduction and regulate transportation of synaptic vesicles **(8).**

Neurofilaments translational modification:

Neurofilaments modification includes phosphorylation and glycosylation, which contributes to NF morphology and function **(20).**

Phosphorylation: Phosphorylation of the neurofilaments head domain is mediated by the second messenger dependent kinases protein kinase A (PKA) and C (PKC) **(19),** as known to modulate their interaction with fodrin which is an important protein of the sub-axolemmal cytoskeleton **(20).**

Glycosylation: Neurofilaments are modified by O-linked N-acetyl glucosamine or by post-translational serine and acetyl glucosamine residues, which regulate protein stability, subcellular localization & protein-protein interaction **(20).**

Neurofilaments assembly: Developing neurons express a series of IF proteins, sequentially, at distinct stages of mammalian cell differentiation. This correlates with altered morphologies during the neuronal development, including axon outgrowth, guidance and conductivity **(21).**

So, abnormal phosphorylated NFs in the cell bodies have been proposed to be the common feature of neurodegenerative diseases, such as amyotrophic lateral sclerosis and Alzheimer disease **(20).**

Neurofilaments transportation: Neurofilaments transportation is accelerated at nodes of Ranvier, where axons are locally constricted. These constrictions are accompanied by sharp decreases in neurofilament number, no decreases in microtubule number, and increases in the packing density of these polymers, which collectively bring nodal neurofilaments closer to their microtubule tracks **(22).**

Neurofilaments in ageing: Normal ageing is associated with the neurodegenerative processes that can be detected with assessment of various markers, such as volumetric loss of brain tissue and levels of fluid biomarkers including neurofilaments. The advantage of an easy-to-access body fluid biomarker, such as neurofilaments in the blood, is that it provides real-time information about neuroaxonal damage in the CNS at low cost and with the ability to repeat **(18)**

Neurofilaments aggregation and its role in neurodegeneration: Neurofilaments light chain (NFL) and heavy chain (NFH) are encoded by two independent genes, located on chromosome 8p21 and 22q12.2, respectively **(20).**

They are known to be resistant to proteolysis, so they were less likely to be degraded in blood than the other NFs. So, because of its stability and testability so, most studies focus on its potential value as a biomarker in neurodegenerative disease **(20).**

The mechanism by which neurofilaments aggregate is still unknown, but hyper-phosphorylation is considered one of the main triggers for their aggregation **(8).**

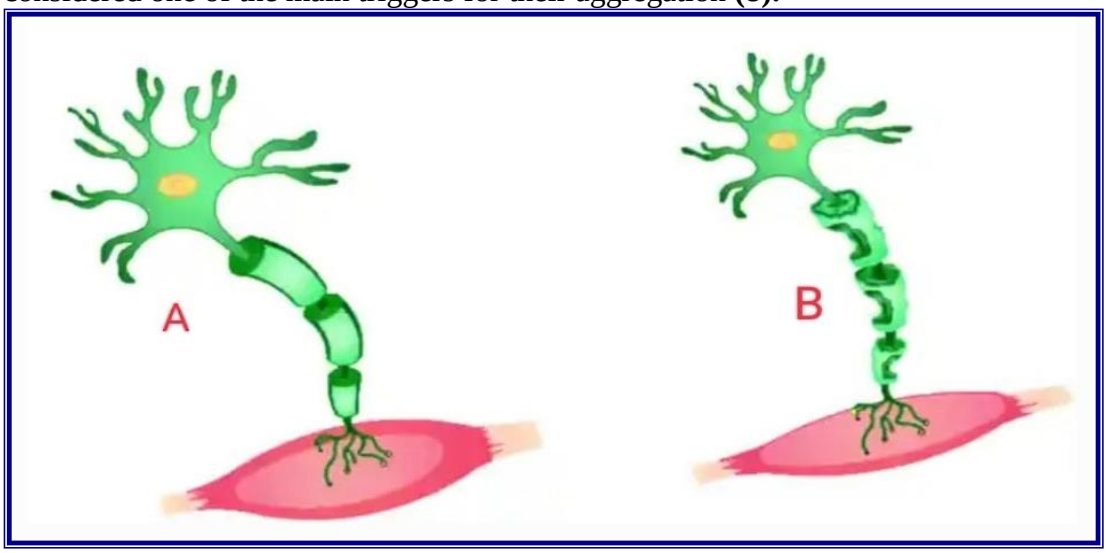

Fig. (2): Show normal axon (A) and the sclerosed damaged axon (B) **(23)**.

All types of Neurofilaments as abio marker in neurological disease:

CSF and blood levels of neurofilament proteins have been measured in various neurological diseases, and evidence has accumulated that they can be clinically useful biomarkers in many of these diseases **(18)**

Relevance of neurofilaments to neurological disorders:

Many good evidence supports their diagnostic and prognostic value and/or their use for monitoring treatment responses. The disorders reviewed as follows:

Amyotrophic lateral sclerosis (ALS), Parkinson's Disease, Alzheimer's Disease, Frontotemporal Dementia, Stroke, Huntington's Disease, Bipolar Disorder, Charcot–Marie–Tooth and traumatic brain injury **(18)**

Assays to detect soluble neurofilaments:

The detection of neurofilaments biomarkers has also advanced, moving toward more clinically relevant applications. First-generation immunoassays were only semi-quantitative in nature which were able to show only the presence of neurofilament isoforms in the blood and CSF. Second-generation is Enzyme Linked Immunosorbent Assay (ELISA) technologies created the first trusted quantitative assays that enabled evaluation of the diagnostic and prognostic value of NFL and NFH determinations in the CSF and serum of patients **(23).**

Neurofilament Light Chain Protein

Neurofilament light chain (NFL) is a neuronal cytoplasmic protein highly expressed in large calibre myelinated axons**.** Especially axons of neurons **(18).**

They are Considered as a marker of neuro-axonal damage which can be measured in both cerebrospinal fluid and serum so, allows for repeated assessment **(18)**

Association of Neurofilament light chain with age and gender:

Normal aging is associated with neuronal loss so, calculation of serum (NFL) levels in the different decades for the examined population were quite similar between the 4th and 5th decades then increased in a nonlinear manner and show that the increase of serum (NFL) in the age group above 60 years was paralleled by a substantial rise in variability of this marker. This suggests that contribution of subclinical brain tissue damage beyond the "normal" process of aging. So, this is the evidence that serum (NFL) was associated with subclinical morphologic brain **(18)**

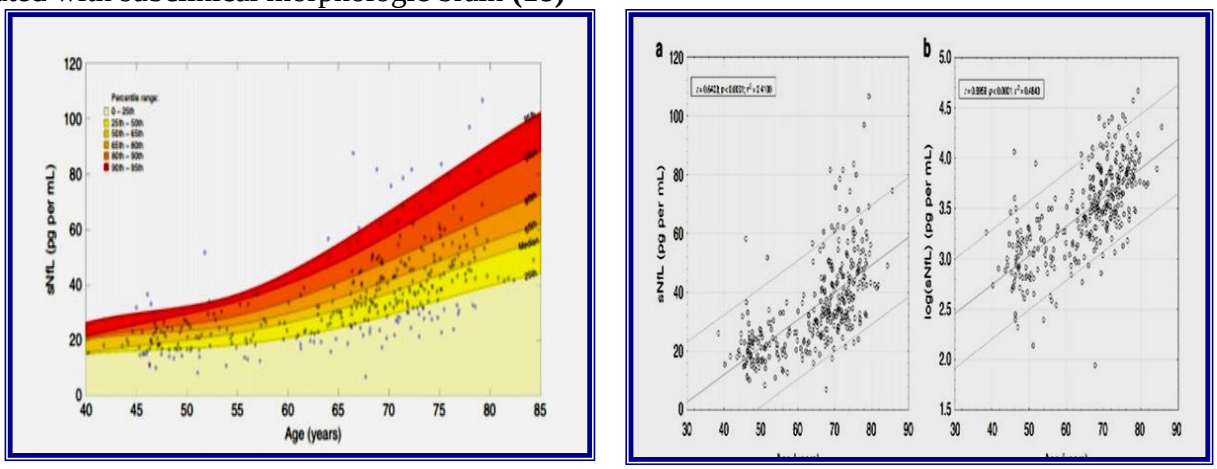

Fig. (3): Show relation between NFL & age **(18)**

Clinical significance of Neurofilament Light chain protein:

Body fluid biomarkers have the potential to be more pathologically specific than imaging biomarkers, as they may reflect ongoing pathology over the entire CNS, and may be more responsive to the effects of treatment **(24).**

A correlation between (NFL) levels in the serum or plasma & levels in the CSF has been demonstrated for various neurologic diseases and suggests that measures of ongoing neuroaxonal injury can be obtained from blood (NFL) levels without the need to obtain CSF by lumbar puncture **(24).**

That is why the interest in NFL research shifted toward blood as an ideal biomarker easily measurable, accurate, quantitative& reproducible **(24).**

As Neuro filament light chain is a highly sensitive marker of neuronal injury, irrespective of the cause of that injury **(24),** the management of neurological diseases such as identification and quantification of axonal damage could be allowed for the improvement of diagnostic and prognostic accuracy assessment according to the degree of axonal damage in a variety of neurological disorders, including inflammatory, neurodegenerative, traumatic and cerebrovascular disease **(25).**

Evaluation of serum Neurofilament light chain in pre symptomatic MS patients:

The levels of serum neurofilament light chain were increased six years before the clinical MS onset which indicate that MS have a prodromal phase lasting several years and that neuroaxonal damage already occurs during this phase while most patients experience their first clinical symptoms of the disease around age of thirty, the underlying pathophysiologic processes often occur long before these symptoms become apparent.

So, serum NFL serves as a biomarker from very early stages of MS, namely in patients with clinically isolated syndrome (CIS) or even in presymptomatic stages of the disease **(26).**

Evaluation of neurofilament light chain in Multiple Sclerosis Diagnosis:

Baseline serum neurofilament light chain (NFL) levels is higher in MS patients as a whole compared to controls **(27).**

There are elevated peripheral blood concentrations of NFL in patients with MS. Therefore, NFL chain in blood sample can be used to discriminate the MS patients from healthy people. Furthermore, several studies suggest that the NFL in MS patients could predict disease activity and would decrease after treatment. There is evidence that NFL levels could be used as a useful prognostic biomarker to monitor disease progression, disease activity, and treatment efficacy **(13).**

Baseline serum neurofilament light chain levels predict brain atrophy progression:

As neurofilament levels reflect ongoing neuro-axonal damage, patients with higher serum NFL levels would suffer from increased brain atrophy latterly. Over the next 6–37 months, brain parenchyma decreased more rapidly in patients with higher baseline serum NFL. Serum NFL levels at baseline were significantly associated with the percentage of brain volume change over 2 years: an increase in serum NFL by 10 pg/ml was associated with an average additional reduction in brain volume of 0.17% **(28).**

Increased baseline serum NFL levels were concurrently associated with significantly greater whole brain atrophy and, more specifically, greater deep gray and cortical pathology **(12).**

Brain atrophy is present early in the disease course and associated with disability progression**.** So, serum NFL concentrations were associated with short- and long-term clinical disability and brain atrophy and considered as a prognostic marker of worse outcomes regarding brain atrophy and disability progression **(29).**

Increased levels of serum NFL in early MS stages reflects neuropathological processes driven mainly by ongoing neuroinflammation as indirectly assessed by the accumulation of lesion burden. In addition, serum NFL levels have a stronger association with future development of brain atrophy than with actual or previous brain volume loss **(14).**

Serum neurofilament light chain and cognitive imparmient:

Cognitive impairment is a common clinical hallmark present in roughly 45–60% of patients with multiple sclerosis and can be found early in the disease. Demonstrated as a result of accumulating neurodegenerative pathology. The decline of cognitive performance has been reliably correlated with thalamic pathology, hippocampal changes and cortical lesions seen on double-inversion recover (DIR) imaging **(12).**

Evaluation of serum neurofilament light chain in disease activity:

The assessment of disease activity is usually based on the appearance of new hyperintense, gadolinium-enhancing lesions on MRI and frequency of clinical relapses **(16).**

The significant association of serum NFL with relevant clinical and neuroimaging outcomes in MS were confirmed and extended, supporting the potential of serum NFL as an objective surrogate of ongoing MS disease activity**.** The serum NFL levels are higher in patients with clinical relapse than in patients in clinical remission **(16).**

The serum NFL levels increased at the clinically and sub-clinically (radiologically) active status, and all serum NFL levels from clinically and radiologically inactive status were lower than those from active status. So, changes of serum NFL levels well-reflected the clinical and radiological disease activity in MS patients

Relapse is more strongly associated with elevated serum NFL levels than the development of progression **(30).**

Association between serum neurofilament light chain and gadolinium-enhancing lesions:

As elevations of serum NFL levels reflect neuro-axonal damage, mediated both by acute inflammatory damage in an acute relapse and chronic neurodegenerative processes (**11).** Gadolinium-enhancing lesions provide a direct correlate of CNS inflammation serum NFL levels were significantly higher within 90 days after a gadolinium-enhancing Gd+ lesion compared to remission samples. Blood NFL levels are associated with clinical and MRI-related measures of disease activity and neuroaxonal damage and have prognostic

value. At baseline and after 2 years of follow-up, serum NFL concentrations correlated with measures of MRI disease activity (Gd+ lesions) and MS disease burden (T2 lesion volume) **(31).**

Evaluation of therapeutic response:

There is increasing evidence that NFL levels are reduced after effective MS treatment The reduction of NFL with the treatment seems to differ according to the efficacy of the drug used reduction correlated with reduction in relapse rates and MRI measures **(16).** Lower levels of NFL were found in treated patients compared to treatment-naïve individuals (**11).**

Fingolimod vs placebo Following 12 months of treatment, median changes from baseline in NFL levels were lower than zero in the fingolimod groups correlated with an improvement in relapse and MRI outcomes **(31).**

Neurofilament light chain levels reduced after initiation of IFNB-1a treatment (**11).** Before alemtuzumab (ATZ), serum NFL was significantly increased in correlation with previous relapse/MRI activity. After ATZ, NFL decreased quickly within the first 6 months**,** but patients who started alemtuzumab displayed the highest reduction in peripheral NFL concentration and lowest on-treatment peripheral NFL concentrations, while those starting teriflunomide had the smallest decrease and highest on-treatment levels, but also starting from lower values. Both on-treatment peripheral NFL and decrease in peripheral NFL concentrations were highly dependent on baseline concentrations **(32).**

Natalizumab treatment reduced NFL levels to similar values obtained in the CSF of healthy controls. This significant reduction occurred regardless of previous disease-modifying treatments or previous activity of the disease. Significant reduction in NFL levels was reported in 43 MS patients receiving fingolimod for 4-12 months, and those previously treated with first-line drugs **(16).**

So, early indications are that serum NFL levels respond to effective disease-modifying therapies and that this response will predict a better long-term outcome. As patients with MS who received early treatment, the prognostic power of serum NFL for relapse activity and long-term disability progression was limited. So, there is now clear evidence that NFL is a good biomarker for treatment response in MS, especially for high efficacy drugs. This is probably due to the better prevention of brain damage in these treatments, underlining the role of NFL as a marker of neuronal and axonal damage **(11).**

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