Guidance for use of neurofilament light chain as a cerebrospinal fluid and blood biomarker in multiple sclerosis management

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Summary

Neurofilament light chain (NfL) is a long-awaited blood biomarker that can provide clinically useful information about prognosis and therapeutic efficacy in multiple sclerosis (MS). There is now substantial evidence for this biomarker to be used alongside magnetic resonance imaging (MRI) and clinical measures of disease progression as a decision-making tool for the management of patients with MS. Serum NfL (sNfL) has certain advantages over traditional measures of MS disease progression such as MRI because it is relatively noninvasive, inexpensive, and can be repeated frequently to monitor activity and treatment efficacy. sNfL levels can be monitored regularly in patients with MS to determine change from baseline and predict subclinical disease activity, relapse risk, and the development of gadolinium-enhancing (Gd+) lesions. sNfL does not replace MRI, which provides information related to spatial localisation and lesion stage. Laboratory platforms are starting to be made available for clinical application of sNfL in several countries. Further work is needed to resolve issues around comparisons across testing platforms (absolute values) and normalisation (reference ranges) in order to guide interpretation of the results.

Keywords: Multiple sclerosis; Neurofilament light chain; Cerebrospinal fluid biomarkers; Serum biomarkers

Introduction

Neurofilaments are neuron-specific intracellular cytoskeletal proteins consisting of subunits known as neurofilament light-chain (NfL), medium-chain (NfM), heavy-chain (NfH), α-internexin and peripherin.[1] Among the subunits, NfL is currently the most widely studied as a biomarker in neurologic disorders. When neuronal tissues in the central nervous system (CNS) break down due to aging, trauma or disease, neurofilaments are released into the cerebrospinal fluid (CSF) and in small quantities into the blood. The presence of elevated measures of neurofilament proteins has been established as a marker of neuroaxonal injury. Neurofilament levels are significantly elevated in the CSF and blood of patients with neurologic conditions such as MS, traumatic brain injury (TBI), and amyotrophic lateral sclerosis (ALS), compared with those of age-matched controls.[2,3] Among these disorders, MS is one of the most widely studied conditions contributing to our understanding of NfL and neuronal damage.[4–6] In ALS, NfL has been identified as a prognostic indicator of disease progression and survival.[7] As a marker of early disease activity in genetic forms of ALS, NfL and other fluid biomarkers may inform therapeutic decisions in this difficult-to-treat disease.[8]

NfL addresses a major unmet need in management of MS for blood biomarkers to objectively predict disease worsening and capture response to DMT.[9,10] In people with MS, change in NfL from baseline in blood or CSF has been demonstrated to be a meaningful indicator of disease worsening, a predictor of short- and long-term disease prognosis, and a biomarker for

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identifying response to disease-modifying therapy (DMT).[11–15] NfL represents a tissue-specific, objective, and quantitative measure of recent (in the case of sNfL within the past three months) neuronal loss, offering a marker of real-time disease activity. Elevated NfL levels correlate with gadolinium-enhancing (Gd+) lesions, T2 lesion volume, relapse risk, brain atrophy measures, disability progression as determined by Expanded Disability Status Scale (EDSS) and other clinical scales, response to DMT, and other outcome measures.[13,14,16,17] sNfL levels associated with CNS damage can remain elevated for approximately three months from the ictus.[18] In contrast, conventional MRI offers a retrospective view, primarily measuring water change. NfL findings correlate well with both acute Gd + lesions and with T2 burden of disease, and are sensitive enough to detect pathology that may not be readily visible on routine MRI, such as spinal cord deterioration.[19,20] NfL correlates with reductions in retinal neve fiber layer thickness in optical coherence tomography (OCT) studies of patients with MS,[21,22] and correlates with other CSF inflammatory biomarkers such as CHI3L1, CXCL13, and osteopontin.[23,24]

This review summarises the recommendations of a consensus conference among an international panel of expert researchers and clinicians at the forefront of NfL research and application in MS management. The review provides an overview of research studies supporting NfL as a biomarker in MS and presents practical guidance for obtaining NfL samples and interpreting their findings in MS patient care. These recommendations apply primarily to relapsing forms of MS, since NfL measurements are most applicable in relapsing disease. The panel's recommendations for use of NfL in clinical practice are summarised in Table 1.

Methods

Development of this consensus statement originated with the gathering of an international panel of clinicians and scientists with expertise in use of NfL in MS, in partnership with the Consortium of Multiple Sclerosis Centers (CMSC). The speakers and panelists were selected by the co-chairs (MF and SG) based on their significant contributions to the study of this biomarker in neurologic diseases. In September and October 2020, two online consensus meetings were organised by the CMSC to assess what was known about NfL in MS at that time. The co-chairs used a limited Delphi approach to reach a group opinion on the topic of NfL before the meeting using an anonymous survey among the panelists to evaluate what is currently known about NfL as a biomarker in MS and the strength of recommendations for using CSF NfL or sNfL in MS research and clinical practice. Findings from the survey were shared with participants at the meeting, followed by compiled presentations for analysis by the panelists on the following topics: review of neurofilament proteins, NfL assays, CSF vs. serum, considerations for timing of NfL assays, confounders affecting interpretation of neurofilament biomarkers in MS, importance of dynamic change in NfL levels, clinical trials of sNfL for prognosis and treatment in MS, and role of NfL in MS clinical practice. From the discussion, the co-chairs compiled a set of recommendations for review by the panelists on the practical applications of NfL in MS based on current knowledge. The latter used a voting system requiring that all agreed fully with the statements or had minimal reservations with the wording to provide a more carefully considered viewpoint. Subsequently, the authors have periodically reviewed and updated new data and recommendations to reflect ongoing advances in the use of NfL for MS clinical practice.

Prognostic value of NfL in patients with MS

Diagnostic implications

Because NfL is a nonspecific biomarker, elevated levels in CSF and blood can be indicators of neuronal damage from a wide range of causes such as brain injury and degenerative neurologic diseases.[10] For this reason, NfL currently has limited diagnostic value in MS, except in some cases requiring differentiation from other neurologic diseases.[25] NfL levels are usually significantly higher in a person with cerebral ischemia or brain trauma than in a person with MS and active disease.[25] In MS clinical practice, NfL is most valuable as a prognostic indicator for severity of disease, disease progression, and as an indicator of response to therapy. In a prospective observational study from 22 European centers using baseline and 4-year follow-up measures of CSF and sNfL, assessment of sNfL increased diagnostic accuracy in patients with clinically isolated syndrome and early RMS.[26]

Predicting MS relapse

Although NfL is increased during all clinical forms of MS (including clinically isolated syndrome), these values are highest in patients with active relapsing-remitting MS (RRMS), especially during relapse.[13,27] CSF-NfL levels are up to tenfold higher during relapse vs. remission.[28] In studies using either CSF or serum, NfL levels have been shown to be highest in patients with severe relapses.[13,29]

Prospective studies are required to better understand meaningful NfL change in individual patients. A recent study in patients treated with mesenchymal stem cells provided an opportunity to determine how well sNfL baseline and longitudinal changes from baseline could predict relapse in patients with active MS.[30] In a group of 58 study participants with MS from Canada and Italy, serial sNfL samples were drawn at three-month intervals over one year along with MRI scans and clinical assessments conducted during these same visits. Higher

Questions	Panel Recommendations
Value of neurofilaments as a biomarker in MS • What is the role of neurofilaments in MS clinical practice? • Is neurofilament light (NfL) the primary biomarker of interest in MS? • What is the role of heavy and medium chain?	• Neurofilament proteins are a marker of neuronal degeneration and can serve as important biomarkers of disease activity in multiple sclerosis (MS). • Elevated NfL levels in blood or cerebrospinal fluid (CSF) are likely a marker of both inflammation and neurodegeneration in MS. The role of inflammation in neurodegenerative processes needs to be further defined. • Neurofilament heavy chain (NfH) may warrant further investigation as a potential biomarker in MS (NfL/NfH ratio also may be informative). • Too little is known about neurofilament medium chain (NfM) in MS to determine its value at this time.
Neurofilament assays • Which assays should be used to measure NfL in patients with MS? • What is known about their sensitivity, specificity, and reproducibility?	• Standard ELISA assays available in most research, hospital and commercial laboratories are appropriate for measuring NfL in CSF only. • The Single-Molecule Array (Simoa) using the Quanterix NF-Light assay is currently the most common method for measuring NfL in blood (serum or plasma). Care should be used when interpreting historical trial data as both lab-developed and commercial (NF-Light) assays have been used and generate different absolute results. • When interpreting NfL findings in research studies the methodology should be clearly identified (Quanterix Simoa NF-Light assay or other emerging assays). • Routine clinical diagnostic laboratory assays capable of accurately measuring blood (serum or plasma) NfL are in development and will soon be available. Results may not be directly comparable to Quanterix NF-Light results. • Emerging assays should be interpreted using method-specific reference intervals (percentiles or Z-scores if available).

Serum vs. CSF
• Is there a role for both CSF and serum?
• If so, when would CSF assays be preferred over serum?
• How often should resampling be done after baseline?
	• Baseline sNfL levels are a valuable contribution to the initial workup in patients with diagnosed or suspected MS and should be interpreted in the context of other clinical information.
	• During periods of perceived clinical quiescence, the panel's recommendation for obtaining sNfL levels are as follows:
	  • Following relapse: At 3- to 6-month follow-up visit
	  • MRI with Gd + lesion: At the 3- to 6-month follow-up
	  • To evaluate the impact of a disease-modifying therapy (DMT) in the absence of clinical or MRI change: Re-baseline prior to starting therapy and re-sample sNfL after 3- to 6-months depending upon the expected response time of the DMT

Confounders/influencing factors that can raise or lower NfL levels • What is the role of age as a confounder of NfL levels? • What diseases, drugs, and other conditions might influence NfL levels and affect interpretation of findings?	• Potential confounding factors should be recognized and controlled for when interpreting NfL in persons with or without MS • Age is an important confounder affecting interpretation of NfL. Mean sNfL levels in a healthy person range are approximately 5 pg/mL to 10 pg/mL between ages 20 and 50 and typically increase at a sharper slope after ages 50 to 60. To counter this phenomenon, sNfL should be calculated based on a Z score/percentile (based on reference database of healthy controls). • An inverse association has been observed between body mass index (BMI) and sNfL levels (that is, people who are heavier tend to have lower sNfL). This may be related to increased blood volume. However, more data are needed. • Increase in sNfL levels in patients with at least moderate (GFR <30) renal insufficiency • No effect of sex on sNfL levels • Race does not appear to influence baseline NfL or changes in NfL values in patients with MS. More data are needed from large databases. • Diabetes is associated with elevated NfL levels related to nerve damage. This should be accounted for when interpreting NfL in patients with MS. No associations have been reported with other comorbidities including hypertension and hyperlipidemia. • Drug therapy or treatments causing neurotoxicity could potentially cause elevations in NfL even if MS disease activity is suppressed by the therapy. • CNS toxicity immediately after autologous hematopoietic bone marrow transplant may be chemotherapy mediated and cause transient increases in MRI atrophy and elevated NfL levels. • Elevated NfL has been associated with other neurologic diseases/conditions. If these conditions occur comorbidly with MS, the effect on NfL should be considered. Follow-up studies are needed to assess whether comorbidities modify longitudinal associations between sNfL and MS outcomes.

Prognostic value of NF in MS
• How often should NfL be measured in people with MS?
• How do NfL levels correlate with other disease characteristics and clinical information in people with MS?
	• NfL has both short-term (within 2 years) and longer-term prognostic value in MS.
	• Higher sNfL level correlates with development of more Gd + lesions and new T2 lesions in the subsequent year
	  • sNfL is increased after a relapse and is associated with increased risk of future relapses
	  • sNfL levels correlate with longer-term outcomes (5 years), including time to Expanded Disability Status Scale (EDSS) > 3.5 and time to clinically definite MS (CDMS)
	  • sNfL levels correlate with brain atrophy measures
	  • Brain atrophy and sNfL together predict time to EDSS 6 over 8 years

(Table 1 continues on next page)

Questions	Panel Recommendations
(Continued from previous page)	

	• Reduced NfL levels can be seen as a result of DMT. Short-term change from treatment is associated with longer-term MRI and clinical outcomes. On a group level, reduction of sNfL 6 months after starting treatment is associated with:
	  • Fewer new T2 lesions at year 2
	  • Less brain atrophy at year 2
	  • Less EDSS change after year 4
	• Persistently high sNfL levels despite treatment are associated with worse MRI outcomes at 4 years.
	• Interpretation of NfL levels is more informative when combined with clinical, MRI, and inflammatory markers, and when corrected for confounding factors such as age, obesity, and diabetes.
	• For sNfL to be more informative in daily clinical practice, we need better cutoff points and to correct for confounders.

Role of NF in research, and goals of ongoing/future research
• How should NF measures and values be standardized?
• How can NFL be combined with other existing and emerging biomarkers?
	• NfL is appropriate for use in all phases of MS clinical trials, and in clinical practice where available.
	• Large-scale studies are under way to assimilate NfL information from databases of persons with MS and healthy controls. These studies will examine the cross-sectional relationship of sNfL levels with demographics and co-morbid conditions, MS clinical characteristics, disability status and imaging measures
	• Coordinated research efforts will help to answer questions such as:
	  • Additional factors associated with sNfL in HC
	  • Relationship of NfL levels to MS clinical measures such as patient-determined disease steps (PDDS), walking speed, manual dexterity, processing speed
	• In addition to prognostic studies, CSF-NfL and sNfL have been adopted as an outcome in many Phase 3 studies of MS DMTs.
	• More studies should be undertaken to determine the biological variation of blood NfL in healthy controls and patients with stable MS. Studies are needed to assess biological variations in sNfL over time frames similar to the recommended monitoring frequency.

Table 1: **Neurofilament consensus statement in MS: Summary of panel recommendations.**

baseline sNfL levels were shown to predict future relapse (Log-rank p = 0.0068), MRI lesions (p = 0.0096), composite-relapse associated worsening (p = 0.01), and progression independent of relapse activity (PIRA; p = 0.0096). In the cross-sectional analysis, a two-fold difference in baseline sNfL (e.g., from 10 to 20 pg/mL) was associated with a 2.3-fold increased risk of relapse during follow-up (95% confidence interval 1.65–3.17). Looking at the change in sNfL value from baseline, a two-fold increase was associated with an additional 1.46 times increased risk of relapse (1.07–2.00). The impact of longitudinal increases in sNfL on the risk of relapse were most pronounced for patients with lower baseline values of sNfL (<10 pg/mL: HR = 1.54, 1.06–2.24). The associations remained significant after adjusting for potential confounders such as age, sex, disease subtype, disease duration, and EDSS.[30] In a prospective study by Uphaus and colleagues, increased sNfL levels at baseline correlated with relapse-free disability progression and conversion to secondary progressive MS (SPMS) at 6-year follow-up in the Neurofilamentandlongtermoutcome in MS (NaloMS) cohort.[31] In the multivariable logistic regression model, increased sNfL levels at baseline (OR 1.02, 95% confidence interval (CI) 1.01–1.04, p = 0.012) were an independent risk factor for relapse-free progression and predicted individual progression risk with an accuracy of 82% (NaloMS).[31]

Predicting development of Gd+ and T2 lesions

Higher sNfL levels correlate a patient's risk for developing Gd + lesions and new T2 lesions on MRI in the following year.[15,20] Studies by the Swiss Multiple Sclerosis Cohort Study Group established strong associations between CSF-NfL and sNfL (p < 0.001), showing that patients with Gd + lesions in the brain, spinal cord, or both had higher sNfL levels than those without enhancing lesions.[13] Another study by the Swiss group in conjunction with Harvard University measured sNfL levels before and after relapse or formation of Gd + lesions.[19] Elevations of sNfL averaging 32% were observed in the three months after the appearance of Gd + lesions (p < 0.0001), relative to samples taken during remission. In the presence of Gd + lesions or just prior to their development, sNfL elevations averaged 32.3% higher (p < 0.002) compared with remission values. In this study, significant elevations in sNfL after a clinical relapse occurred only when associated with a Gd + lesion.[19] The authors concluded that sNfL levels peak within a three-month window around the appearance of Gd + lesions, suggesting that NfL captures an aspect of the disease pathology that escapes routine MRI.[19]

UK researchers prospectively evaluated the relationships of CSF NfL and MRI activity at baseline and one-year follow-up.[32] Whilst baseline CSF NfL was not found to correlate well with current or future MRI activity or

lesion location, activity on baseline MRI activity explained around 53% % (R^2 0.53) of the variation in the follow-up CSF NfL levels when the data were adjusted for type of MS and disease duration. This suggests that focal inflammatory activity as demonstrated by MRI activity contributes to the majority of increase in CSF NfL that follows.

Predicting disease worsening and clinical change in MS

High NfL levels at disease onset correlate with disease worsening on the EDSS in clinically stable patients and those experiencing active relapse.[29,33] Investigators from the Karolinska institute analysed data from 4385 people with MS from two prospective studies followed over a median five-year time period and found that those with high plasma NfL levels (>80th percentile of control levels) were 40%–70% more likely to experience worsening disability due to MS in the following year.[34]

Serum samples from 259 patients with MS (189 relapsing and 70 progressive) and 259 healthy controls were analysed as part of an ongoing cohort study.[14] Clinical assessment, serum sampling, and MRI were performed annually over a median follow-up period of 6.5 years. In this analysis of 2183 samples, sNfL level above the 90th percentile of healthy controls was an independent predictor of EDSS worsening in the subsequent year. sNfL levels above the 90th percentile were associated with increased odds of EDSS worsening at the next visit, compared to levels below the 90th percentile (estimated odds ratio (OR) = 2.577, 95% CI = 1.553–4.278, $p < 0.001$, n = 677 observations). The probability of EDSS worsening gradually increased with higher sNfL percentile categories. sNfL also correlated with concurrent and future clinical and MRI measures of disease activity and disease severity. Gd+ and new/enlarging lesions were independently associated with increased sNfL.[14]

Predicting disease progression independent of relapse activity (PIRA)

The Swiss MS Cohort looked at sNfL as a predictor of confirmed EDSS progression independent of relapse activity (PIRA).[35] The analysis included 4000 serum samples from 800 patients (all MS subtypes) who had at least three prospective follow-up visits and no relapses during a median of 4.7 years follow-up. sNfL levels increased with age (1.7% per year) and baseline EDSS (7.6% per EDSS step). The 153 patients who experienced PIRA (19.0%) had 11.6% higher sNfL levels compared with stable or non-PIRA patients.[35]

Correlation with brain atrophy

sNfL levels have been shown to correlate well with measures of brain atrophy in MS. Brain atrophy and sNfL together have been shown to predict how quickly a patient will reach the disability milestone of EDSS 6.0 over an 8-year observation period.[17] In the Swiss study described above, higher sNfL percentiles at baseline were associated with a greater risk of more pronounced brain and cervical spinal volume loss over time.[14] Brain atrophy changes on MRI represent tissue damage that has already occurred, while NfL is highly predictive of future brain atrophy and thus could be used to support a change in therapy that may prevent loss of brain tissue.[14]

Some data suggest that increased sNfL may predict longitudinal changes in cognitive function. In patients with SPMS from the MS-STAT trial (median age 51, median EDSS 6.0), investigators showed that increased sNfL levels from baseline over 12 and 24 months was associated with faster cognitive decline, independent of T2 lesion volume.[36]

Evaluating the effects of DMT

While higher NfL levels are found during active inflammation and in patients with more severe MS, decreased NfL levels have been consistently documented following initiation of DMT. Short-term changes observed with the onset of treatment have correlated with longer-term MRI and clinical outcomes on a group level.[12,20] Six months after starting treatment with a second-line or high-efficacy DMT, reduced levels of sNfL have been associated with:[20]

- Fewer new T2 lesions at year 2
- Less evidence of brain atrophy at year 2
- Less advancement in EDSS scores after year 4
- Response to therapy

The most significant drops in NfL values are typically seen in treatment-naïve patients started on a new therapy.[16,37] With subsequent monitoring of stabilised patients on therapy, changes in NfL levels may become more subtle. A growing number of MS treatment trials have begun to include NfL as a biomarker for DMT efficacy (Table 2).[20]

Recommendations for measuring NfL in patients with MS

Assays for NfL in CSF and blood

Measurement of NfL in the CSF can be achieved using standard enzyme-linked immunoassays (ELISA).[46] Traditional ELISA assays measure total fluorescence and are not sensitive enough to identify the pg/mL quantities of NfL present in blood. The single-molecule array or "Simoa" assay is currently the predominant method for measuring NfL levels in blood (serum or plasma). Simoa is a bead-based immunoassay in which dye-encoded magnetic beads are coated with the capture antibody. NfL-specific monoclonal antibodies are used for detection and lack cross-reactivity for other neurofilament types (NfM, NfH) or glial fibrillary acidic protein (GFAP).[47] The Simoa assay is sensitive enough to

DMTs studied/source/trial name	Study population	Design	Findings
2nd-line DMTs Novakova et al. *Mult Scler Relat Disord.* 2020;46: 102463[38]	90 RRMS 47 PMS with and without ongoing disease activity (relapse or Gd + lesions)	sNfL and other blood biomarkers measured at baseline, before initiating or switching DMT, and at 12 and 27 months	• Before DMT, all patients with disease activity had increased sNfL • Among those without disease activity, 39% of RRMS and 50% of PMS had elevated NfL or CXCL13 • After 12 months of DMT: Reduced CXCL13 and sNfL in 80%–90% of patients with disease activity
Alemtuzumab Akgun et al. *Neurol Neuroimmunol Neuroinflamm.* 2019; 6(3):e555.[39]	15 patients after immune reconstitution with alemtuzumab	Monthly sNfL was correlated with EDSS, MRI, and relapse activity over 102-month period	• After treatment, sNfL levels decreased within 6 months • In patients with NEDA-3, sNfL declined and persisted at a low steady-state level of <8 pg/mL • sNfL peaks during follow-up correlated with clinical or MRI disease activity.
Fingolimod, natalizumab, IFN, GA Yaldizli et al. Mult Scler. 2018; 24(Suppl. 2):97–98.[40] (Swiss MS Cohort Study)	237 patients with RRMS taking established DMTs: fingolimod (n = 182) natalizumab (n = 27) IFNB or GA (n = 28)	All patients were on continuous DMT 3–24 months prior to baseline serum sampling, and during median 2-year follow-up. sNfL measured yearly	• Higher sNfL level on treatment was associated with 2–6 times higher relapse rates and 2 to 5 times more T2 white matter lesions in the subsequent 12–24 months • Patients with sNfL levels above the 99th percentile had an additional 0.95% yearly brain volume loss vs. those with sNfL below 99th percentile • Study suggests that sNfL can be used to predict suboptimal response in patients on DMT
Fingolimod, IFNB Kuhle et al. *Neurology.* Mar 5 2019; 92(10):e1007-e1015.[20] (FREEDOMS, TRANSFORMS trials)	589 patients with RRMS from phase 3 fingolimod trials, 35 healthy controls	NfL in blood samples from trial subjects with MS and healthy controls were compared with clinical and MRI outcomes	• Baseline NfL levels in patients with MS were higher than in healthy controls and correlated significantly with T2 lesion load and number of Gd + T1 lesions • Baseline NfL levels, treatment type, and number of new or enlarging T2 lesions predicted NfL levels at the end of study • High baseline NfL levels were associated with T2 lesion load, relapses, brain volume loss, and risk of confirmed disability worsening • Fingolimod significantly reduced NfL levels at 6 months vs. placebo and IFNB groups, difference was sustained throughout studies
Fingolimod Haring et al. *Neurol Neuroimmunol Neuroinflamm.* Sep 2020; 7(5):e856.[41] (FREEDOMS, TRANSFORMS, LONGTERMS trials)	Fingolimod 0.5 mg (NfL set), n = 301 FREEDOMS full analysis set, n = 1272; TRANSFORMS full analysis set, n = 1280	Patients were classified as having high NfL (≥30 pg/mL, n = 110) or low NfL (<30 pg/mL, n = 164) based on baseline plasma NfL value or geometric mean	• High baseline NfL predicted risk of earlier progression to EDSS ≥ 4 • Patients with high NfL levels at 6 and 12 months had a 2-fold higher risk of 6-month disability progression, compared with those with low levels at both time points. • High baseline NfL predicted higher brain volume loss over 120 months
Siponimod Kuhle et al. *Neurology.* 2018; 90(15 Supplement):S8.006.[42] (EXPAND trial)	1452 SPMS 378 PPMS	Baseline sNfL levels categorized as: • Low (<30 pg/mL) • Medium (30–60 pg/mL) • High (>60 pg/mL) Mean baseline sNfL was higher for patients with SPMS (32.1 pg/mL) vs. PPMS (22.0 pg/mL)	• Gd+ and T2 lesion volumes at baseline correlated with baseline CSF-NfL, sNfL, and brain atrophy • Elevated NfL (≥30 pg/mL) was shown to increase the risk of disability progression by 32% • More pronounced decrease in sNfL during siponimod treatment occurred in patients who had relapses in the prior year • Patients with higher NfL had more brain atrophy at 12 and 24 months (with or without prior relapse) • NfL can be used to predict brain volume loss in patients with or without prior relapses, suggesting its value as a biomarker of neurodegenerative changes

(Table 2 continues on next page)

DMTs studied/source/trial name	Study population	Design	Findings
(Continued from previous page)			
Natalizumab Kapoor et al. Presented at ECTRIMS 2019. Abstract P1740.[43] (ASCEND trial)	748 patients with SPMS randomized to natalizumab (n = 379) or placebo (n = 365). Enrolled patients were natalizumab-naïve, had SPMS for ≥2 years, EDSS between 3.0 and 6.5, and disability progression unrelated to relapses in the previous year.	Patients with and without relapses were categorized according to presence or absence of Gd + lesions at baseline	• Baseline sNfL levels were associated with age, number of Gd + lesions, T2 lesion volume, timed 25-foot walk, 9-hole peg test, and brain atrophy over 96 weeks • At week 96, sNfL levels were significantly higher in patients with progression compared to those without progression during the study • sNfL levels at week 48 and week 96 were significantly lower in natalizumab-treated patients vs. placebo • sNfL change was less pronounced in patients without evidence of acute inflammatory activity, but the degree of dynamic change was thought to be clinically meaningful in this group
Alemtuzumab, IFN Kuhle et al. *Mult Scler.* Aug 11, 2021.[44] (CARE-MS 1 study)	Patients with RRMS, subgroup with highly active disease. Alemtuzumab (n = 354) compared with IFNB-1a (n = 159).	sNfL levels were measured over 7 years in alemtuzumab group and 2 years in IFNB group	• Median sNfL levels at baseline were similar in both treatment groups but decreased more with alemtuzumab group up to year 2 • Alemtuzumab-treated patients had sNfL at or below the healthy control median at year 2 • Alemtuzumab was superior to IFNB in reducing sNfL. These levels remaining stable in alemtuzumab-treated patients through year 7
Peginterferon beta 1a Calabresi et al. *Mult Scler.* Dec 14, 2020.[45] (ADVANCE trial)	Baseline sNfL was measured in 859 patients (393 placebo, 466 peginterferon). sNfL was assayed at 3-month intervals from baseline to 2 years (n = 511) and then every 6 months until year 4 (n = 282). sNfL was assayed 1 year from baseline in an additional 813 patients.	Post hoc analysis of longitudinal data and samples.	• Baseline sNfL predicted 4-year brain atrophy and development of new T2 lesions • Patients receiving peginterferon beta-1a whose sNfL decreased to <16 pg/mL after 12 months had greater improvement in clinical and MRI outcomes compared with those whose sNfL remained elevated (≥16 pg/mL) • Mean sNfL levels were shown to decrease in patients on peginterferon but increased in those receiving placebo (−9.5% vs. 6.8%; p < 0.01)

DMT = disease modifying therapy. RRMS = relapsing-remitting MS. PMS = progressive MS. sNfL = serum neurofilament light. EDSS = expanded disability status scale. NEDA = no evidence of disease activity. IFNB-1a = interferon beta 1a. GA = glatiramer acetate. Gd+ = gadolinium enhancing. PPMS = primary progressive MS. SPMS = secondary progressive MS.

***Table 2*: Studies in MS using NfL outcome measures to demonstrate treatment effect.**

detect a single NfL molecule bound to a single bead, providing an analytical sensitivity in sub-picograms per milliliter (pg/mL) range.[46] The lower limit of quantitation is approximately 0.1 pg/mL. The dynamic range of the assay is quite broad, about 1800 pg/mL, allowing for a high dynamic range from a small volume of fluid. The Quanterix NF-Light assay is authorised for NfL in the U.S. and other countries. Future platforms may utilise different capture antibodies, thus their numeric results may differ from those of the Quanterix NF-Light assay. The panel therefore recommends using a consistent NfL assay methodology when monitoring individual patients.

Obtaining CSF and serum samples for monitoring

NfL is significantly more concentrated in CSF than in serum (up to 100 times), making CSF-NfL a more robust biomarker.[6,48] However, the invasiveness of lumbar puncture limits the practicality of using CSF-NfL for the purposes of routine monitoring in MS. The panel recommended obtaining baseline CSF and blood NfL measurements in patients with suspected or confirmed MS who are undergoing diagnostic lumbar puncture. If additional lumbar punctures are indicated at other time points, repeat CSF-NfL levels may be assessed. For routine follow-up, the panel recommended that subsequent NfL monitoring be done using serum (sNfL).

During periods of perceived clinical quiescence, the panel's recommendations for obtaining updated baseline sNfL levels were as follows:

- Following relapse: obtain sNfL at the 3- to 6-month follow-up visit[28]
- If MRI results show Gd + lesions: obtain sNfL at the 3- to 6-month follow-up visit[19,30]

- In the presence of new or enlarging lesions on MRI: re-measure NfL
- To evaluate the effectiveness of a DMT in patients with no evident clinical or MRI changes: repeat sNfL analysis at 3- to 6-month intervals in accordance with patient follow-up protocols.

Potential confounding factors affecting NfL interpretation

Age

Age is an important confounder affecting interpretation of NfL findings, particularly in people aged 50 to 60 or older. Mean sNfL levels of approximately 10 pg/mL in a healthy person at age 20 will rise steadily about 2.2% annually and then increase at an even steeper slope around between ages 50 to 60.[15,49] Studies of CSF-NfL conducted at Sahlgrenska Academy in Gothenburg, Sweden suggested that neuronal degeneration in a person over age 59 is five times higher than in those under age 30.[50] In serum, the differential may be lower. The use of Z-scores/percentiles relative to a reference database of control subjects can help to counter the phenomenon of expected NfL increase in older populations.

Body weight

Obesity is associated with decreased brain density and is a known risk factor for neurodegeneration, so it would seem logical that NfL levels might be elevated in obese persons with or without MS. However, multiple studies have demonstrated that blood NfL actually tends to be lower among obese individuals compared with that of leaner subjects.[51,52] This may be due to higher blood volume documented in obese individuals. It is of note that body weight appears not a confounder when NfL is measured in CSF. Therefore, if body weight appears to be a consideration in interpreting a given NfL serum sample, it may be helpful to refer to the baseline CSF sample or obtain a follow-up CSF measurement if indicated. In serum, the use of age-specific and BMI-specific Z scores or percentiles eliminates these factors.[15] Further research is needed to determine whether and how to adjust for this confounder in patients with MS and other neurologic diseases.[52]

Sex and race

Neither race nor sex appear to influence baseline NfL or change from baseline, based on the data available thus far. More information is needed from large normative databases and in studies specific to MS to determine whether change in NfL differs across race/ethnicity groups.[6]

Other diseases

Peripheral nerve cell damage may be reflected in elevated NfL levels in people with diabetes.[53,54] Elevated sNfL levels have been observed in patients with diabetes, but the panel noted that the impact of these variations on people with MS and comorbid diabetes is unclear. Other factors such as hypertension, hyperlipidemia, and renal function have been investigated, but the clinical relevance of potential associations is unclear.[55,56]

Drug treatment

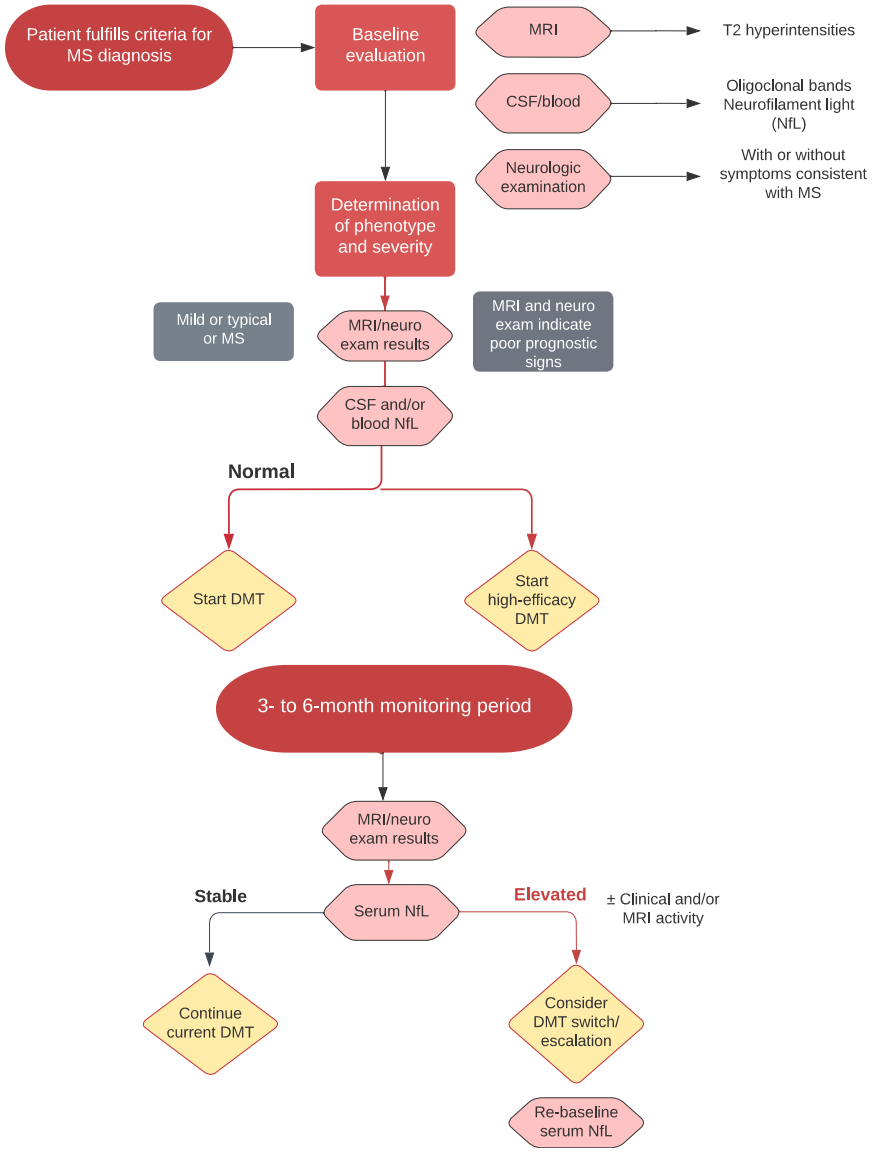
Any drug treatment that causes neurotoxicity could potentially cause transient NfL elevations, even if the therapy suppresses MS disease activity. In patients who are undergoing autologous hematopoietic bone marrow transplant, chemotherapy-mediated CNS toxicity may contribute to transient increases in brain atrophy on MRI and elevated NfL levels immediately after the procedure.[57]

Recommendations for using NfL in clinical decision-making in MS

The panel's recommendations for application of NfL in various clinical scenarios are outlined in Fig. 1. The first evaluation of NfL in patients with MS would be to inform on prognosis, using CSF or serum initially (in patients without overt clinical and MRI disease activity) and serum for subsequent follow-up. After a baseline value is established for the individual patient (best established within three months from any acute attack/relapse), changes from baseline can help to inform clinical decisions. For example, if NfL levels have not decreased (or are found to increase) within six months of initiating DMT, consideration should be made for changing the therapy or escalating to a higher-efficacy DMT.[45] In contrast, a low or normal-range NfL would support the decision to keep the patient on the current therapy. It has been shown that an individual's sNfL value can vary by up to 40% over time, thus changes that are at least greater than 40% from baseline may represent an informative change.[58] Information from clinical and MRI assessments should be factored into the decision about whether to escalate therapy or to monitor patients more frequently.

Using reference values for sNfL

The panel members agreed that absolute NfL levels (pg/mL) must be interpreted with caution because of potential confounders such as patient age and body mass index (BMI).[13–15,59] Z scores and percentiles (considered interchangeable) drawn from control populations have been validated in controlled trials as a way to express the deviation from "normal," thereby adjusting for relevant confounders such as age and body mass index (BMI).[15] Z scores represent the degree of deviation from the mean in a control population, while percentiles express the proportion of the general population expected to have lower sNfL values, after adjusting for variables such as age and BMI.[15] Some Z-score reference values



web 4C/FPO

Fig. 1: **Algorithm for use of serum and CSF NfL in clinical decision-making for patients with multiple sclerosis.** The panel recommends that evaluation of NfL be used in conjunction with other measures of MS severity and prognosis, including MRI, other imaging biomarkers, and findings of neurologic examination. If a patient shows clinical worsening and/or MRI changes either while on therapy, elevations in sNfL levels may signal the need to perform further study or consider a change in therapy. For a patient who appears to be clinically stable but has elevations in sNfL, this may warrant closer monitoring and/or escalation of therapy.

used in MS were derived from MS PATHS, a database that currently encompasses more than 7000 participants from seven U.S. and three European Union cities.[60] MS PATHS captures data from electronic medical records and includes data about absence of treatment and medication switches. An ongoing study by Calabresi and colleagues using this database has examined the cross-sectional relationship of sNfL levels with demographics, comorbidities, clinical characteristics, disability status, imaging measures, and clinical outcomes in patients with MS.[61] Preliminary data reported in April 2021 showed that patients with MS and elevated sNfL had higher T2 lesion volume, lower brain volume measures, and worse clinical outcomes in walking speed and manual dexterity, compared with patients whose sNfL levels were not elevated.[61]

Benkert and colleagues retrospectively obtained 10,133 blood samples from 5390 control subjects from four cohorts from the U.S. and Europe.[15] This investigation employed a statistical model (GAMLSS) to

describe the non-linear association between sNfL and two relevant confounders, age and BMI. From this model, Z scores and percentiles were derived which allow to better quantify deviations from expected levels in healthy controls facilitating the identification of pathological values (e.g., in people with MS disease activity).[15] As observed in other large population-based studies, sNfL concentrations rose steadily with age and at a steeper curve after the approximate age of 50. The same researchers examined 7769 samples from 1313 participants in the Swiss Multiple Sclerosis Cohort and used Z scores and percentiles. In patients with MS, higher sNfL percentiles and Z scores predicted an increased risk for future acute and chronic disease activity over a median follow-up period of 5–6 years. People with MS whose Z scores were above 1.5 had an increased risk of future clinical or MRI disease activity (odds ratio 3.15, 95% CI 2.35–4.23; $p < 0.0001$), including many whose MS appeared to be stable with no evidence of disease activity (2.66, 1.08–6.55; $p = 0.034$). These findings were validated in the Swedish MS Registry.[15] Effects of treatment with MS DMTs were evident at the group level. Treatment with monoclonal antibody therapies was associated with a decrease in sNfL Z scores to levels comparable to that of control subjects without MS. This apparent treatment effect was seen to a lesser degree in patients treated with oral therapies, while those treated with injectable DMTs showed a more transient and less-pronounced reduction in sNfL Z scores.[15]

Z-scores/percentiles have been shown to reflect the deviation of a patient's sNfL value from the mean value documented from same-age healthy controls. In a population of patients with MS who exhibited no evidence of disease activity (NEDA-3), higher Z scores were shown to predict EDSS worsening or relapse in the following year.[15] Thus a high sNfL Z score in a patient with MS likely signals a need to consider escalation of therapy to prevent subclinical and clinical disease activity. Whether Z score vs. significant individual absolute sNfL level changes are better for monitoring treatment response will require more study.

New NfL assays are on the horizon may or may not directly compare with the absolute NfLvalues produced by the Simoa NF-Light assay.[62] Each will need assay-specific reference intervals, percentiles and/or z-scores. Ideally, reference interval studies will need to be sufficiently large and encompass the full range of ages and comorbidities known to confound NfL results. The Clinical Laboratory Standards Institute (CLSI) has recommendations for the minimum number of reference subjects that should be included in each reference group (e.g., age, BMI, or other stratification).[63] Alternatively, the reference intervals, percentiles and/or z-scores can be transferred from a previously established method following recommendations from CLSI.

Search strategy and selection criteria

Pre-clinical and clinical studies for this consensus were contributed by the committee members and by searches of PubMed for relevant articles using the search terms "neurofilaments", "neurofilament light chain".

Conclusion

Although other biomarkers such as glial fibrillary acidic protein (GFAP) are under investigation and may be more sensitive than sNfL in detecting disease progression,[58] sNfL is emerging as the first bona fide blood-based biomarker for relapsing forms of MS. Its applications are being studied in other neurodegenerative conditions as well as in head injury and even cognitive change following cardiac surgery.[6,64] Most major clinical trials in MS now include sNfL as an outcome measure, and an increasing number of therapeutic trials in MS have used change in sNfL as the primary outcome in place of MRI. While MRI testing may be cost-prohibitive or have limited availability, sNfL analysis offers a more practical and cost-effective way to assess disease status. Laboratories capable of analysing sNfL samples have been expanding internationally. Most provide interpretation that is biochemically comparable across platforms, although it is advisable to use the same assay platform to measure NfL change within an individual patient.

Future perspectives

Recommendations for the use of NfL as a biomarker in MS will evolve with expanded use and reporting of research results, including longitudinal population-wide databases of normative NfL levels and prospective measurements in large clinical studies. To better support management decisions in MS, there is a need for robust reference ranges based on normative data from control subjects, potentially using Z-scores or percentiles. There is also a need to identify disease-specific cutoffs to assist MS clinicians with therapeutic decision-making. These cutoff levels should guide clinicians on the degree of abnormality or change in sNfL that would suggest worsening MS, and the degree of change that would warrant consideration for therapeutic switch. Most of the prognostic value in NfL has been shown in patients with relapsing disease.[15,45,65] More work is needed to evaluate the potential applications in progressive forms of MS.[66]

Outstanding questions

Normative databases based on age and other identified population-specific confounders will help to inform on actual levels of NfL needed for evaluation of neurological conditions as a whole. Such databases will likely

need to be disease-specific and one can be compiled uniquely for patients with MS so as to calculate specific cut-off values to aid decision-making. NfL will likely be combined with other pending biomarkers, such as serum glial fibrillary acidic protein (GFAP) to better inform on disease progression.

Contributors

MSF and SG conducted the literature search, wrote the initial draft of the manuscript, wrote revisions to the manuscript, and addressed reviewers' comments. RAB, PAC, MK, JK, JL, and TO conceptualized and wrote the draft of the manuscript, provided revisions, and responded to reviewers' comments (Note: Faculty panelists listed in the Appendix did not contribute to nor review any aspect of the manuscript.).

Declaration of interests

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Appendix A. Supplementary data

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References

1 Liu Q, Xie F, Siedlak SL, et al. Neurofilament proteins in neurodegenerative diseases. *Cell Mol Life Sci*. 2004;61(24):3057–3075.
2 Varhaug KN, Torkildsen O, Myhr KM, Vedeler CA. Neurofilament light chain as a biomarker in multiple sclerosis. *Front Neurol*. 2019;10:338.
3 Olsson B, Portelius E, Cullen NC, et al. Association of cerebrospinal fluid neurofilament light protein levels with cognition in patients with dementia, motor neuron disease, and movement disorders. *JAMA Neurol*. 2019;76(3):318–325.
4 Kuhle J, Plattner K, Bestwick JP, et al. A comparative study of CSF neurofilament light and heavy chain protein in MS. *Mult Scler*. 2013;19(12):1597–1603.
5 Martinez-Morillo E, Childs C, Garcia BP, et al. Neurofilament medium polypeptide (NFM) protein concentration is increased in CSF and serum samples from patients with brain injury. *Clin Chem Lab Med*. 2015;53(10):1575–1584.
6 Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 2018;14(10):577–589.
7 Zhou YN, Chen YH, Dong SQ, et al. Role of blood neurofilaments in the prognosis of amyotrophic lateral sclerosis: a meta-analysis. *Front Neurol*. 2021;12:712245.
8 Benatar M, Wuu J, Andersen PM, et al. Design of a randomized, placebo-controlled, phase 3 trial of tofersen initiated in clinically presymptomatic SOD1 variant carriers: the ATLAS study. *Neurotherapeutics*. 2022;19(4):1248–1258.
9 Khalil M. Are neurofilaments valuable biomarkers for long-term disease prognostication in MS? *Mult Scler*. 2018;24(10):1270–1271.
10 Thebault S, Booth RA, Freedman MS. Blood neurofilament light chain: the neurologist's troponin? *Biomedicines*. 2020;8(11).
11 Anderson V, Bentley E, Loveless S, et al. Serum neurofilament-light concentration and real-world outcome in MS. *J Neurol Sci*. 2020;417:117079.
12 Delcoigne B, Manouchehrinia A, Barro C, et al. Blood neurofilament light levels segregate treatment effects in multiple sclerosis. *Neurology*. 2020;94(11):e1201–e1212.
13 Disanto G, Barro C, Benkert P, et al. Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis. *Ann Neurol*. 2017;81(6):857–870.
14 Barro C, Benkert P, Disanto G, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. *Brain*. 2018;141(8):2382–2391.
15 Benkert P, Meier S, Schaedelin S, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol*. 2022;21(3):246–257.
16 Kapoor R, Smith KE, Allegretta M, et al. Serum neurofilament light as a biomarker in progressive multiple sclerosis. *Neurology*. 2020;95(10):436–444.
17 Kuhle J, Plavina T, Barro C, et al. Neurofilament light levels are associated with long-term outcomes in multiple sclerosis. *Mult Scler*. 2020;26(13):1691–1699.
18 Barro C, Chitnis T, Weiner HL. Blood neurofilament light: a critical review of its application to neurologic disease. *Ann Clin Transl Neurol*. 2020;7(12):2508–2523.
19 Rosso M, Gonzalez CT, Healy BC, et al. Temporal association of sNfL and gad-enhancing lesions in multiple sclerosis. *Ann Clin Transl Neurol*. 2020;7(6):945–955.
20 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–e1015.
21 Tavazzi E, Jakimovski D, Kuhle J, et al. Serum neurofilament light chain and optical coherence tomography measures in MS: a longitudinal study. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(4):e737.

22 Lin TY, Vitkova V, Asseyer S, et al. Increased serum neurofilament light and thin ganglion cell-inner plexiform layer are additive risk factors for disease activity in early multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm*. 2021;8(5):e1051.
23 Modvig S, Degn M, Horwitz H, et al. Relationship between cerebrospinal fluid biomarkers for inflammation, demyelination and neurodegeneration in acute optic neuritis. *PLoS One*. 2013;8(10):e77163.
24 Romme Christensen J, Börnsen L, Khademi M, et al. CSF inflammation and axonal damage are increased and correlate in progressive multiple sclerosis. *Mult Scler*. 2013;19(7):877–884.
25 Bridel C, van Wieringen WN, Zetterberg H, et al. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. *JAMA Neurol*. 2019;76(9):1035–1048.
26 Bittner S, Steffen F, Uphaus T, et al. Clinical implications of serum neurofilament in newly diagnosed MS patients: a longitudinal multicentre cohort study. *EBioMedicine*. 2020;56:102807.
27 Disanto G, Adiutori R, Dobson R, et al. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. *J Neurol Neurosurg Psychiatry*. 2016;87(2):126–129.
28 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):1720–1725.
29 Rosenstein I, Axelsson M, Novakova L, Blennow K, Zetterberg H, Lycke J. Exploring CSF neurofilament light as a biomarker for MS in clinical practice; a retrospective registry-based study. *Mult Scler*. 2022;28(6):872–884.
30 Thebault S, Reaume M, Marrie RA, et al. High or increasing serum NfL is predictive of impending multiple sclerosis relapses. *Mult Scler Relat Disord*. 2022;59:103535.
31 Uphaus T, Steffen F, Muthuraman M, et al. NfL predicts relapse-free progression in a longitudinal multiple sclerosis cohort study. *EBioMedicine*. 2021;72:103590.
32 Adams A, Tilden W, Bestwick J, et al. The relationship of cerebrospinal fluid neurofilament levels with magnetic resonance imaging lesion location and disease activity in multiple sclerosis. *Eur J Neurol*. 2022;29(9):2754–2760.
33 Norgren N, Sundström P, Svenningsson A, Rosengren L, Stigbrand T, Gunnarsson M. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. *Neurology*. 2004;63(9):1586–1590.
34 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–e2467.
35 Lorscheider J, Benkert P, Yaldizli O, et al. Serum neurofilament light chain captures and predicts confirmed progression independent of relapses (PIRA) in multiple sclerosis. *MS Virtual*. 2020, Sept 11–13, P0154. https://doi.org/10.13140/RG.2.2.10281.42080.
36 Williams T, Tur C, Eshaghi A, et al. Serum neurofilament light and MRI predictors of cognitive decline in patients with secondary progressive multiple sclerosis: analysis from the MS-STAT randomised controlled trial. *Mult Scler*. 2022;28(12):1913–1926.
37 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):1324–1330.
38 Novakova L, Axelsson M, Malmeström C, et al. NFL and CXCL13 may reveal disease activity in clinically and radiologically stable MS. *Mult Scler Relat Disord*. 2020;46:102463.
39 Akgun K, Kretschmann N, Haase R, et al. Profiling individual clinical responses by high-frequency serum neurofilament assessment in MS. *Neurol Neuroimmunol Neuroinflamm*. 2019;6(3):e555.
40 Ö Yaldizli, Barro C, Michalak M, et al. Value of serum neurofilament light chain levels as a biomarker of suboptimal treatment response in MS clinical practice. *Mult Scler*. 2018;24(Suppl. 2):97–98.
41 Häring DA, Kropshofer H, Kappos L, et al. Long-term prognostic value of longitudinal measurements of blood neurofilament levels. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(5):e856.
42 Leppert D, Kropshofer H, Haring DA, et al. Blood neurofilament light in progressive multiple sclerosis: post hoc analysis of 2 randomized controlled trials. *Neurology*. 2022;98(21):e2120–e2131.
43 Kapoor R, Sellebjerg F, Hartung H-P, et al. Natalizumab reduced serum levels of neurofilament light chain in secondary progressive multiple sclerosis patients from the Phase 3 ASCEND study. Presented at ECTRIMS 2019. 2019:Abstract P1740.
44 Kuhle J, Daizadeh N, Benkert P, et al. Sustained reduction of serum neurofilament light chain over 7 years by alemtuzumab in early relapsing-remitting MS. *Mult Scler*. 2021;28(4):573–582.
45 Calabresi PA, Arnold DL, Sangurdekar D, et al. Temporal profile of serum neurofilament light in multiple sclerosis: implications for patient monitoring. *Mult Scler*. 2020;27(10):1497–1505.
46 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):1655–1661.
47 Hendricks R, Baker D, Brumm J, et al. Establishment of neurofilament light chain Simoa assay in cerebrospinal fluid and blood. *Bioanalysis*. 2019;11(15):1405–1418.
48 Novakova L, Axelsson M, Khademi M, et al. Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis. *Mult Scler*. 2017;23(1):62–71.
49 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.
50 Lycke J, Zetterberg H. The role of blood and CSF biomarkers in the evaluation of new treatments against multiple sclerosis. *Expert Rev Clin Immunol*. 2017;13(12):1143–1153.
51 Rebelos E, Rissanen E, Bucci M, et al. Circulating neurofilament is linked with morbid obesity, renal function, and brain density. *Sci Rep*. 2022;12(1):7841.
52 Manouchehrinia A, Piehl F, Hillert J, et al. Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. *Ann Clin Transl Neurol*. 2020;7(1):139–143.
53 Altmann P, De Simoni D, Kaider A, et al. Increased serum neurofilament light chain concentration indicates poor outcome in Guillain-Barré syndrome. *J Neuroinflammation*. 2020;17(1):86.
54 Polymeris AA, Coslovksy M, Aeschbacher S, et al. Serum neurofilament light in atrial fibrillation: clinical, neuroimaging and cognitive correlates. *Brain Commun*. 2020;2(2):fcaa166.
55 Fitzgerald KC, Sotirchos ES, Smith MD, et al. Contributors to serum NfL levels in people without neurologic disease. *Ann Neurol*. 2022;92(4):688–698.
56 Koini M, Pirpamer L, Hofer E, et al. Factors influencing serum neurofilament light chain levels in normal aging. *Aging*. 2021;13(24):25729–25738.
57 Petzold A, Mondria T, Kuhle J, et al. Evidence for acute neurotoxicity after chemotherapy. *Ann Neurol*. 2010;68(6):806–815.
58 Hviid CVB, Madsen AT, Winther-Larsen A. Biological variation of serum neurofilament light chain. *Clin Chem Lab Med*. 2022;60(4):569–575.
59 Curtis AE, Smith TA, Ziganshin BA, Elefteriades JA. The mystery of the Z-score. *Aorta*. 2016;4(4):124–130.
60 Mowry EM, Bermel RA, Williams JR, et al. Harnessing real-world data to inform decision-making: multiple sclerosis partners advancing technology and Health solutions (MS PATHS). *Front Neurol*. 2020;11:632.
61 Sotirchos E, Fitzgerald K, Smith M, et al. Associations of serum neurofilament light chain with clinico-radiological characteristics in the MSPATHS Network: a cross-sectional evaluation. In: *Presented at: 2021 American Academy of neurology virtual annual meeting*. 2021.
62 Petzold A. The 2022 lady estelle wolfson lectureship on neurofilaments. *J Neurochem*. 2022;163(3):179–219.
63 Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory. Approved guideline, 3rd ed. CLSI document C28-A3c. ISBN 1-56238-682-4. 2010.
64 Alifier M, Olsson B, Andreasson U, et al. Cardiac surgery is associated with biomarker evidence of neuronal damage. *J Alzheimers Dis*. 2020;74(4):1211–1220.
65 Ziemssen T, Arnold DL, Alvarez E, et al. Prognostic value of serum neurofilament light chain for disease activity and worsening in patients with relapsing multiple sclerosis: results from the phase 3 ASCLEPIOS I and II trials. *Front Immunol*. 2022;13:852563.
66 Comabella M, Sastre-Garriga J, Carbonell-Mirabent P, et al. Serum neurofilament light chain levels predict long-term disability progression in patients with progressive multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2022;93(7):732–740. https://doi.org/10.1136/jnnp-2022-329020.