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Abstract

In the management of neurological diseases, the identification and quantification of axonal damage could allow for the improvement of diagnostic accuracy and prognostic assessment. Neurofilament light chain (NfL) is a neuronal cytoplasmic protein highly expressed in large caliber myelinated axons. Its levels increase in cerebrospinal fluid (CSF) and blood proportionally to the degree of axonal damage in a variety of neurological disorders, including inflammatory, neurodegenerative, traumatic and cerebrovascular diseases. New immunoassays able to detect biomarkers at ultra-low levels have allowed for the measurement of NfL in blood, thus making it possible to easily and repeatedly measure NfL for monitoring diseases' courses. Evidence that both CSF and blood NfL may serve as diagnostic, prognostic and monitoring biomarkers in neurological diseases is progressively increasing, and NfL is one of the most promising biomarkers to be used in clinical and research setting in the next future. Here we review most important results on CSF and blood NfL and we discuss its potential applications and future directions.

Neurofilament light chain as a biomarker in neurological disorders

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Introduction

In the management of neurological diseases, there is a compelling need for reliable biomarkers that can improve the accuracy of differential diagnosis and of prognostic assessment as well as predict the response to treatments. This applies to central nervous system (CNS) disorders of all causes, including inflammatory, neurodegenerative, traumatic and vascular diseases. Another application for biomarkers in neurological diseases could be to identify or rule out the presence of neurodegenerative processes, which would be useful for subsequent clinical management.

In CNS and peripheral nervous system diseases associated with axonal injury or degeneration, the concentration of neurofilament light chain (NfL) has been found to increase in cerebrospinal fluid (CSF) and blood.[1][S1] Over the last two decades, an increasing number of studies have shown that NfL levels in the CSF and blood are altered in CNS diseases and are correlated with the disease characteristics. Furthermore, as a quantitative measure of the ongoing axonal injury, the increase in NfL levels could have a prognostic value in a variety of neurological diseases. Since it is feasible to measure NfL concentration in the blood, it may be a promising biomarker for monitoring the disease course in CNS disorders and, ideally, for evaluating patients' response to treatments.

In this paper, we provide a brief overview of the structure, function, and mechanisms of release of NfL, and the methods by which NfL concentration can be measured. We then review its potential diagnostic and prognostic value in a variety of CNS diseases, as well as its usefulness in monitoring response to treatment, and we discuss how NfL could be applied in clinical practice.

Structure, function and measurement of NfL

NfL is a subunit of neurofilaments (Nfs), which are cylindrical proteins exclusively located in the neuronal cytoplasm (Figure 1).[S2] Nfs confer structural stability to neurons and are present in dendrites and neuronal soma, as well as in axons, where their expression is particularly high. Since Nfs enable the radial growth of axons, larger myelinated axons abundantly express Nfs and NfL.[S2] Under normal conditions, low levels of NfL are constantly released from axons, probably in an age-dependent manner, with higher levels of NfL being released at older ages (Panel 1).[2] However, in response to CNS axonal damage because of inflammatory, neurodegenerative, traumatic or vascular injury, the release of NfL sharply increases. The NfL that is released reaches the interstitial fluid, which communicates freely with the CSF, and the blood, where its concentration is roughly 40-fold lower than it is in the CSF.[2][S2] Among Nfs subunits, neurofilament heavy chain (NfH) extensively undergoes post-translational phosphorylation (pNfH), which influences the dynamics of Nfs transport along axons and, therefore, axonal stability.[S2] Although less investigated than NfL, an increase of pNfH in CSF may act as a biomarker of axonal injury, especially in amyotrophic lateral sclerosis (ALS), for which pNfH is particularly specific.[3] Nevertheless, since NfL is the backbone of Nfs, it is the most abundant subunit and it is also the most soluble one, which makes NfL the most reliably measurable Nfs subunit in biofluids.[S3] In CSF, NfL can be measured by sandwich enzyme-linked immunosorbent assays (ELISA) technology.[S4] However, the sensitivity of ELISA for measuring blood NfL concentration is not sufficient.[S5]

Electrochemiluminescence (ECL) assay technology is a more sensitive alternative than ELISA, but it is not sufficient for detecting the lowest concentrations of NfL in blood.[S6] Recently, single molecule array (Simoa) technology has been used for the quantification of blood NfL even in samples from young healthy controls.[2] This ultrasensitive technique has made it

possible to detect longitudinal changes of blood NfL at the group-level, but also at the individual-level when its increase exceeds the analytical variation, which is still around 6% (Panel 2).[2]

Potential diagnostic value of NfL

The concentration of NfL in CSF is higher in patients with neurological diseases than in healthy controls (HCs) (Figure 2), and recently, similar findings have been reported for blood NfL too. The role of NfL as a biomarker has been largely reported in multiple sclerosis (MS), Alzheimer's disease (AD), frontotemporal dementia (FTD), ALS, atypical parkinsonian disorders (APDs) and traumatic brain injury (TBI). At a lesser extent, NfL has been studied in Creutzfeldt-Jakob disease (CJD) and neurological complications of HIV infection, where it reaches very high concentration in the CSF (Figure 2), in Huntington's disease (HD) and in normal pressure hydrocephalus (NPH).

Since NfL is a sensitive but unspecific marker of axonal injury, its potential diagnostic value does not lie in the ability to discriminate between neurological diseases characterized by a similar degree of axonal loss, but rather, between CNS diseases with a different degree of large myelinated axon damage and/or with a different progression rate or disease intensity, or between neurodegenerative and non-neurodegenerative diseases. For these reasons, the potential diagnostic role of NfL in the clinical setting should be complemented with other neurological assessments, as well as more disease-specific biomarkers and brain imaging findings.

NfL in the diagnostic work-up of multiple sclerosis

The CSF NfL concentration is increased both in MS and in its first clinical presentation, that is, clinically isolated syndrome (CIS).[4] In both these conditions, CSF NfL can be used to identify patients from controls without neurological diseases with high accuracy (area under the curve [AUC] = 0.83 for CIS versus controls; AUC = 0.90 for MS versus controls; no further details available).[4] Similar findings have been reported for serum NfL as well.[2] The timing of NfL measurement could influence its concentration, especially in relation to the time point of the last acute inflammatory episode. Indeed, CSF and serum NfL tend to be higher in relapsing-remitting MS (RRMS) patients with a recent relapse (no longer than 60 days before) than in clinically stable RRMS patients.[5] It is plausible that CSF NfL remains high for 2–3 months after a relapse and then drops to lower levels.[S7] Therefore, CSF NfL could have the highest diagnostic accuracy within three months from the last relapse. This probably applies to blood NfL as well, whose concentration seems to follow the same dynamics as CSF NfL.[2]

When considering the potential diagnostic applications of NfL in MS, it should be noted that the ability of CSF and blood NfL to discriminate MS from MS mimics has been reported in only a few studies, which have shown conflicting results.[S8,S9] For instance, while one study showed that the CSF NfL concentration was higher in neuromyelitis optica than in MS (no information is available on the diagnostic accuracy),[S10] this was not found to be true for serum NfL.[6] Furthermore, both CSF and serum NfL have been found to be increased in patients with white matter hyperintensity due to cerebral small vessel disease, which is one of the most common differential diagnoses of MS.[S11,S12]

The lack of disease specificity and anatomical characterization of NfL indicates that its CSF and blood measurement cannot replace magnetic resonance imaging (MRI) in the diagnosis of MS and CIS and in the exclusion of MS mimics. Nevertheless, NfL measurement during the diagnostic work-up of CIS and MS patients may still be useful for predicting disease prognosis,

as discussed above and in the section on NfL in the monitoring and prognostic evaluation of MS.

NfL in the diagnostic work-up of Alzheimer's disease and frontotemporal dementia

In AD patients, CSF and blood NfL are higher than in HCs.[7] AD patients can be differentiated from HCs with good accuracy in the case of CSF NfL (AUC up to 0.77, 95% CI 0.64-0.89).[8] Similarly, blood NfL showed excellent accuracy (AUC = 0.87; no further details available).[7] In addition, NfL changes in blood appear to precede the first clinical manifestations of AD by about 16 years, as demonstrated by longitudinal studies on AD mutation carriers.[S13] In this same population, moreover, a peak in the rate of increase of blood NfL has been observed near with the onset of symptoms, thus suggesting that NfL marks onset and intensity of neurodegeneration in AD.[S13,S14]

As a marker of ongoing neuronal damage in AD, one might wonder what the benefit of CSF NfL over CSF total tau (t-tau) can be, even in the context of the recently proposed biological definition of the disease.[S15] To this regard, while CSF t-tau values seem to reflect amyloid-dependent neurodegeneration or increased tau secretion from amyloid-affected neurons, CSF NfL might be a measure of both amyloid-dependent and -independent neuronal loss,[9] which is particularly relevant if considering the contribution of different proteinopathies, vascular disease and neuroinflammation (the so-called mixed pathology) in AD pathophysiology.[S16] CSF NfL is also increased in FTD patients as compared to cognitively normal controls (AUC = 0.93, 95% CI 0.90-0.97),[10] and a similar difference has been reported for serum NfL (84% sensitivity and 96% specificity).[11]

In terms of the potential clinical applications of CSF or serum NfL, the differences between AD or FTD patients and HCs imply that this biomarker may help in the differential diagnosis between neurodegenerative dementias and non-neurodegenerative disease-mimics (i.e.

depression).[1] For instance, it could be difficult to distinguish between the behavioural variant of FTD (bvFTD) and psychiatric disorders in cases where neuroimaging does not reveal frontotemporal atrophy or hypometabolism. In such cases, CSF NfL can help in distinguishing FTD from psychiatric diseases with excellent accuracy (AUC = 0.93, 95% CI 0.85-1.00, $p < 0.001$).[S17] Although this finding needs to be confirmed with further investigations, it implies that NfL could be used to rule out neurodegenerative diseases in patients with psychiatric disturbances.

In addition, it would be interesting to investigate whether CSF and blood NfL can be used to identify patients with neurodegenerative diseases among individuals with subjective memory complaints; this could guide clinicians to further proceed with the diagnostic work-up. In this sense, CSF or blood NfL measurement may be useful as a first-line test, i.e. as a screening test, for AD and other neurodegenerative diseases. While NfL changes in CSF might be more sensitive in identifying a neurodegenerative process in its earliest stages, blood NfL measurement would be more feasible as a screening test, due to its lower invasiveness.

A recent study on a population of cognitively healthy individuals has shown that higher CSF NfL values are associated with a three-fold higher risk of mild cognitive impairment (MCI) over a median follow-up of 3.8 years (hazard ratio = 3.13, 95% CI 1.36-7.18 for the top quartile of CSF NfL vs. the bottom quartile; $p = 0.01$).[12] Interestingly, CSF t-tau, phosphorylated tau (p-tau) and neurogranin were not found to have a similar potential as predictors of MCI.[12] NfL might additionally be useful for better discrimination between AD and FTD. Indeed, in AD (including early-onset forms), the increase in CSF NfL is less pronounced than in FTD, [S18] and it discriminates between the two disorders with good accuracy (AUC = 0.80, 82% sensitivity, 70% specificity).[13] These results have also been recently replicated in patients with autopsy-confirmed AD and FTD, thus strengthening the evidence on the potential utility of NfL for the differential diagnosis between these two disorders.[1]

Within the FTD spectrum, primary progressive aphasia (PPA) shows the highest CSF NfL values in comparison with AD.[S18] With regard to the differentiation between PPA and AD, CSF NfL performs better than CSF amyloid beta 1-42 (A β 42), and t-tau/A β 42 ratio (AUC = 0.84, 95% CI 0.76-0.93 for NfL vs. 0.65, 95% CI 0.50-0.80 for A β 42, 0.67, 95% CI 0.54-0.80 for t-tau/A β 42 ratio).[14] CSF NfL could also serve as a biomarker for the differential diagnosis between non-fluent and semantic variant PPAs (nfvPPA and svPPA) and logopenic variant PPA (lvPPA), since it is higher in nfvPPA/svPPA compared to lvPPA (AUC = 0.87, 95% CI 0.79 – 0.96, $p < 0.0001$).[15] Serum NfL also is higher in nfvPPA/svPPA vs lvPPA, although in such comparison its accuracy is lower than CSF NfL (AUC = 0.77, 95% CI 0.65-0.89, $p < 0.001$).[15]

Since NfL seems to lack disease specificity, it cannot be used alone to discriminate between AD and FTD in a clinical setting. However, the addition of NfL to other fluid biomarkers can increase the sensitivity and diagnostic accuracy of the measurements. For instance, while CSF A β 42 and p-tau were found to be useful for discriminating between early-onset AD and FTD with an AUC of 0.89 (75% sensitivity, 94% specificity), by adding CSF NfL an increase in AUC to 0.92 (86% sensitivity, 100% specificity) was obtained.[13]

Another application of NfL in this field might be the differential diagnosis between rapidly progressive dementias and prion diseases, since in these latter NfL hugely increases in the CSF and blood, much more than in AD and other forms of dementia.[16,17] CSF NfL seems to accurately distinguish prion diseases from atypical or rapidly progressive neurodegenerative dementias (AUC = 0.84 ± 0.04 , 85.5% sensitivity, 75% specificity) and from atypical or rapidly progressive AD (AUC = 0.95 ± 0.02 , 86.4% sensitivity, 91.9% specificity), with the highest accuracy obtained when NfL is combined with CSF p-tau (AUC for the NfL/p-tau ratio = 0.99 ± 0.007 , 92.9% sensitivity, 97.3% specificity).[18]

NfL in the diagnostic work-up of amyotrophic lateral sclerosis

CSF NfL is higher in patients with ALS compared to healthy and neurological controls,[S5] as well as to patients with other motor neuron diseases (MNDs) (i.e. primary lateral sclerosis, spinal muscular atrophy and Kennedy disease),[19] and ALS mimics (i.e. chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, and cervical myeloradiculopathy).[3] CSF NfL exhibits the highest accuracy (AUC = 0.99, sensitivity 97%, specificity 95%, $p < 0.0001$) in distinguishing ALS patients from HCs,[20] but its accuracy in distinguishing ALS patients in the early symptomatic phase (onset within six months) from other neurological diseases (AUC = 0.95, 95% CI 0.91-0.99), and ALS mimics (AUC = 0.94, 95% CI 0.94-1.00) is still high.[19] These results are highly relevant, since they provide evidence that NfL may have diagnostic utility even during the first clinical assessment of patients with suspected MND. Moreover, in ALS, CSF and serum NfL have shown to be strongly correlated ($r = 0.78$, $p < 0.0001$).[20] The same correlation was found to be weaker in HCs ($r = 0.57$, $p < 0.01$), thus leading to hypothesize that ongoing axonal injury with higher CSF NfL in ALS compared to HCs may be associated with a more rapid redistribution of NfL through the blood-brain barrier from CSF to blood.[20]

Given the high correlation between CSF and serum NfL in ALS, blood NfL has shown an excellent accuracy (AUC = 0.99; 95% CI 0.97-1.00) for differentiating between early symptomatic ALS and ALS mimics.[19] Recently, a serum NfL cut-off value of 62 pg/mL was found to have a sensitivity of 85.5% (95% CI 78-91.2%) and a specificity of 81.8% (95% CI 74.9-87.4%) in distinguishing ALS from other neurological disorders.[21]

Of note, in asymptomatic ALS mutations carriers, no difference has been found in CSF NfL values compared to HCs, while a sharp increase of CSF NfL was described in symptomatic ALS mutations carriers, thus suggesting that, in these patients, NfL could also serve as a marker of disease-onset.[22] In these patients, when longitudinally assessing serum NfL, elevated

levels were found in asymptomatic ALS mutations carriers who later developed ALS as far back as 11.6 months before phenoconversion. In addition, serum NfL levels continued to increase in the first six months after symptom onset. On the contrary, in ALS patients serum NfL were found to be substantially stable over a median time of one year.[23] These results suggest that neurodegeneration in ALS probably begins almost one year before the appearance of clinical manifestations and that serum NfL might be used as a biomarker for the early identification of neurodegeneration, with hopefully positive implications for patient selection in clinical trials on neuroprotective therapies in ALS.

Among the subunits of Nfs, pNfH has shown to be present in increased concentrations in the CSF of ALS patients, and it has shown excellent accuracy in differentiating between early symptomatic ALS and ALS mimics (AUC = 0.98, 95% CI 0.95-1.00).[19] So far, very few data are available on the serum pNfH concentrations in ALS.[S19] The current diagnostic criteria for ALS are based on the extent of upper (UMN) and lower motor neuron (LMN) involvement.[S20] Since both CSF NfL and pNfH are significantly correlated with the number of regions with both UMN and LMN involvement,[3] their use may enable early diagnosis of ALS.

In conclusion, CSF and serum NfL have shown excellent diagnostic accuracy for ALS, even in the early phases of the disease. These promising results call for assay standardization and validation, as discussed further below, before NfL could be used in the clinical practice.

NfL in the diagnostic work-up of parkinsonian and movement disorders

In Parkinson's disease (PD) patients, it seems that the NfL levels in CSF do not increase, as it has repeatedly been reported that the levels are similar to those in HCs.[24] On the contrary, CSF NfL is reportedly increased in patients with progressive supranuclear palsy (PSP), multiple system atrophy (MSA), and corticobasal syndrome (CBS) as compared to HCs and

to PD patients as well.[24] Among APDs patients, CSF and blood NfL are higher in PSP than in MSA patients.[S4,S47] Further, CSF NfL can be used to differentiate between PD and APDs with high diagnostic accuracy (AUC = 0.82, 75% sensitivity, 83% specificity for PSP vs. PD, AUC = 0.94, 80% sensitivity, 96.9% specificity for MSA vs. PD),[25,26] and blood NfL exhibits similar diagnostic performance.[27] Finally, patients with DLB and PDD seem to have lower CSF NfL values than MSA, PSP and CBS patients,[28] as well as patients with other neurodegenerative dementias, e.g. FTD and late-onset AD.[29] While CSF NfL alone is not adequate for distinguishing between DLB and AD (AUC = 0.53, 33% sensitivity, 82% specificity),[13] the addition of CSF A β 42, p-tau and α -synuclein improves the diagnostic accuracy (AUC = 0.90, 95% CI 0.85-0.96, 90% sensitivity, 81% specificity).[28]

Finally, a few studies have investigated NfL as a biomarker in NPH, where it correlates with the degree of motor impairment (correlation coefficient with gait disturbance = 0.4, $p \leq 0.01$),[S21] and in HD patients. In these latter, elevated CSF and blood NfL concentrations have been described,[S22,S23] especially in patients with disease manifestation compared to asymptomatic patients who are carriers of CAG expansion.[S23]

In conclusion, either CSF or blood NfL could be useful for the differential diagnosis of PD and APDs. Since evidence for the diagnostic value of NfL can be found only in studies performed on patients with an established diagnosis, CSF or blood NfL would be more appropriate as a supplementary measurement to help movement disorder specialists in the differential diagnosis between PD and APDs.

NfL in the diagnostic work-up of traumatic brain injury

CSF and blood NfL concentrations are found to be increased after TBI. Studies on TBI provide a good understanding of the dynamics of NfL from the brain to the periphery after acute axonal damage. In the first two weeks following severe TBI, NfL sharply increases in both CSF and

blood as compared to patients with other neurological diseases and HCs.[30] Within one year of severe TBI, the blood NfL level normalizes, but no information is available about its levels between the acute phase and after one year.[30]

Studies on mild TBI mainly focus on athletes engaged in contact sports. Boxers have higher CSF and serum NfL concentrations than non-boxers, especially after a bout with ≥ 15 hits.[31] CSF NfL does not peak immediately after a bout, but it peaks after 15 days and normalizes after 3–9 months.[32] In contrast, soccer headings in amateur players do not seem to result in an increase in the CSF NfL values, according to measurements obtained 7–10 days after a heading training session.[33] Similar to TBI, in a few studies traumatic spinal cord injury (TSCI) has been associated to an increase of NfL values in both CSF,[34] and blood.[5]

Based on the findings so far, it seems that further studies are required to define the dynamics of blood NfL after a head trauma and, therefore, the best timing for its measurement. It is also not clear whether NfL measurement would be beneficial for the comprehensive management of TBI.[S24] A potential clinical utility of this biomarker would be to help clinicians in deciding whether a patient with TBI has to undergo a head CT or MRI. In one study, it has been shown that blood NfL can be used to accurately identify patients with abnormal head CT findings after a head trauma (AUC = 0.84, 95% CI 0.77-0.92).[35] Further investigations in which different diagnostic modalities (i.e. blood NfL, EEG, and head CT or MRI scan) are compared are therefore recommended.

Association of NfL with disease characteristics and its potential prognostic value

There would be two prognostic uses of NfL: as a baseline measure at disease onset or diagnosis, and as a longitudinal and repetitive measure. Its repeated measurement may be applicable to patient monitoring in clinical practice as well as in clinical trials. The ability of NfL to reflect

the degree of axonal damage makes it a reliable marker of disease intensity and/or activity across a range of CNS diseases.[S2] The potential correlation of both CSF and blood NfL with specific disease characteristics has been widely investigated (Table 1). Furthermore, the potential value of baseline and/or longitudinal measurements of CSF and blood NfL in predicting the course of different neurological diseases, i.e. MS, AD, FTD, ALS, APDs and TBI, has also been verified.

NfL levels in both CSF and blood have been shown to be additional independent prognostic factors in a variety of neurological disorders, thus confirming their potential to contribute to existing prognostic factors.

NfL in the monitoring and prognostic evaluation of multiple sclerosis

MS monitoring is nowadays largely dependent on serial MRI, but this is limited by several factors, including the high frequency of gadolinium administration and difficulties in precisely registering serial MRI scans. In addition, it is difficult to image the spinal cord longitudinally. Given this situation, a CSF or blood test may provide an alternative or complementary option for monitoring MS disease activity over time.

Overall, it has been found a trend towards a reduction of serum NfL values over time in CIS and RRMS patients, which was significant relative to baseline at month 6 ($p = 0.008$), 12 ($p = 0.001$) and 24 ($p = 0.007$).[36] Since in that study patients had active disease at baseline, such reduction could be interpreted as a possible regression to the mean. Also, these patients were started on a DMT after the baseline, and this could have contributed to the decrease over time of serum NfL.[36]

CSF and serum NfL have been tested as indicators of disease activity (defined by a clinical relapse occurred within 3 months before sampling or by the presence of Gd⁺ lesions in MRI scans performed within 6 weeks before sampling). CSF NfL shows good accuracy (AUC =

0.77, 95% CI 0.71–0.84, 67% sensitivity, 75% specificity) and serum NfL shows sufficient accuracy (AUC = 0.63, 95% CI 0.59–0.74, 45% sensitivity, 80% specificity) in detecting patients with disease activity. Serum NfL accuracy is improved when it is used as an indicator of new Gd+ lesions (AUC = 0.85, sensitivity 84%, specificity 66%).[S9]

It has been proposed that blood NfL should be integrated with the current measures to determine the ‘no evidence of disease activity’ (NEDA) status.[37] Indeed, NfL measurement may provide more information on the degree of ongoing axonal damage in normal-appearing white matter, which is not accurately reflected by standard MRI and relapse rate.[38] The relatively low accuracy of serum NfL in detecting classic disease activity markers (i.e. relapses or Gd+ lesions) means that serial NfL measurement cannot be used alone as a substitute for clinical and MRI monitoring, but rather, it can be used as a supplementary measure for detecting axonal damage. With regard to the potential prognostic applications of NfL, it could be used for the identification of patients with pre-clinical MS (i.e. radiologically isolated syndrome or RIS) or with CIS who are likely to develop MS. It has been found that a higher CSF NfL concentration is an independent risk factor for the development of MS in RIS patients, although it has minor relevance (hazard ratio = 1.03, 95% CI 1.01-1.05 p = 0.003) in comparison to other prognostic markers such as CSF IgG oligoclonal bands (OCB) (hazard ratio = 8.9, 95% CI 1.04-75.6 p = 0.046).[39] The ability of CSF NfL to predict conversion to MS in CIS patients is controversial. While some authors have reported higher CSF NfL values at the baseline in CIS patients who were later diagnosed with MS,[S8] some others have reported contrasting findings.[40] Moreover, even in studies where CSF NfL was found to be an independent risk factor for clinically defined MS development, it was not as relevant as CSF IgG OCB and MRI T2 lesions, with a hazard ratio increase of (i) 1.005, 95% CI 1.000–1.011 (p = 0.040), for every 100 ng/L increase in CSF NfL, (ii) 2.6, 95% CI 1.009-6.683 (p = 0.048), in case of CSF IgG OCB evidence and (iii) 11.5, 95% CI 1.4-91.9 (p = 0.022) for ≥ 4 lesions

on MRI.[41] Similar to CSF NfL, serum NfL also does not show a clear correlation with a higher risk of subsequent MS development in CIS patients.[40]

However, CSF NfL at the time of CIS onset seems to correlate with the number of new T2 lesions (correlation coefficient = 0.59, $p = 0.003$ at year 5) and Gd+ lesions (correlation coefficient = 0.46, $p = 0.004$ at year 1) over the follow-up, and with the percentage of brain volume change within five years (correlation coefficient = -0.89, $p < 0.0001$).[41] Similar results have been obtained for serum NfL in RRMS patients, as it was correlated with a higher number of Gd+ lesions (10-fold higher NfL was associated with 2.9-fold [95% CI 2.2-3.8, $p = 0.001$] more Gd+ lesions over time) and with a decrease in brain volume (regression coefficient = -0.85, 95% CI -0.04 to -1.66, $p = 0.05$ at month 12).[36] These findings have been recently confirmed in a study in which higher serum NfL values were independently associated with a reduction in both brain volume (regression coefficient = -0.29, 95% CI -0.545 -0.042, $p = 0.023$) and spinal cord volume (regression coefficient = -0.488, 95% CI -0.783 -0.192, $p = 0.001$) over five years.[42]

Also, longitudinal NfL changes have shown a similar prognostic value compared to baseline measurement. For instance, a 10-fold increase in serum NfL over 24 months is associated with a 4.7-fold (95% CI 3.3-6.9, $p < 0.001$) increase in new Gd+ lesions over the same period.[36]

Another potential prognostic application of NfL could be for the prediction of disability. CSF and serum NfL at the baseline are independent predictors of Expanded Disability Status Scale (EDSS) scores and Multiple Sclerosis Severity Score (MSSS) at follow-up.[2][S7] Longitudinal changes of serum NfL also correlate with EDSS changes over time (a 10-fold increase in serum NfL over 24 months being associated with an EDSS score increase of 0.53 [95% CI 0.14-0.91, $p = 0.001$] over the same period).[36]

In optic neuritis, baseline CSF NfL seems to positively correlate with MSSS assessed after a median time of 13 years (correlation coefficient = 0.41, $p = 0.018$).[43] Further, CSF NfL was

shown to be an independent risk factor for conversion into the secondary progressive phenotype. Indeed, in a retrospective study with a 14-year median follow-up time, it was found that in patients with higher baseline CSF NfL concentrations (> 386 ng/L), conversion from RRMS to secondary progressive MS (SPMS) was more likely than it was in patients with low or intermediated CSF NfL values (< 60 ng/L, $p = 0.01$; $60-386$ ng/L, $p = 0.03$, respectively).[44] With regard to the prognostic value of blood NfL, the timing of longitudinal measurements is an important issue. Indeed, within two months after CIS, serum NfL does not seem to be dependent from the interval between CIS onset and blood sampling.[40] On the contrary, six months after optic neuritis, CSF NfL shows a median decrease of 45% of its baseline values, but it is retained at higher levels in subjects with poorer visual outcomes.[S25] Therefore, while the first assessment of NfL can be performed at any time within two months after the first clinical event without expecting any significant variations in its levels, a second measurement six months later could have a more specific prognostic value.

NfL in the prognostic evaluation of Alzheimer's disease and frontotemporal dementia

In prodromal AD, CSF and blood NfL values can predict longitudinal changes in cognition and in MRI measures of brain atrophy (regression coefficient for plasma NfL and Trail-Making Test part B score = 0.28, $p < 0.01$).[7] Specifically, plasma NfL correlates with lower Mini-Mental State Examination (MMSE) scores (regression coefficient for plasma NfL = -0.1, $p < 0.01$) and higher scores for Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) at the follow-up (regression coefficient for plasma NfL = 0.1, $p < 0.001$).[7] Moreover, higher plasma NfL concentrations have been associated with faster lateral ventricle enlargement (regression coefficient = 0.032, $p < 0.001$); hippocampal atrophy (regression coefficient = -0.019, $p < 0.001$); and decrease in entorhinal, inferior temporal, middle temporal,

and fusiform cortical thickness (regression coefficient = -0.049, $p < 0.001$) over four years of follow-up.[7]

Another potential application of NfL could be in the monitoring of subjects with genetic risk factors for AD. Indeed, it has been demonstrated that serum NfL correlates with the estimated years to symptom onset in autosomal dominant AD mutations carriers (correlation coefficient = 0.75, $p < 0.001$ for serum NfL).[45] This finding points to the possibility of evaluating the effects of drugs in subjects with pre-clinical AD in clinical trials.

In FTD patients, higher baseline CSF NfL levels are independently correlated with a worse prognosis and shorter survival. For instance, while the five-year survival of FTD patients with a baseline CSF NfL < 1989 pg/mL is 73%, it decreases to 36% when the baseline CSF NfL is > 3675 pg/mL (estimated hazard ratio 1.7, 95% CI 1.3-2.1, $p < 0.001$).[46] Such a prognostic effect is superior to that of CSF p-tau/t-tau (estimated hazard ratio 0.7, 95% CI 0.56–0.86, $p = 0.001$).[46] In addition, the baseline serum NfL levels seem to correlate with the rate of frontal and parietal lobe atrophy over the year following serum sampling (correlation coefficient = 0.53, $p = 0.003$ and 0.38, $p = 0.04$, respectively).[11] CSF NfL has shown a positive correlation with the magnitude of annual MMSE score loss in FTD patients (correlation coefficient = 0.5, $p = 0.003$).[1] In other studies, CSF and serum NfL did not show a significant association with the progression of cognitive impairment.[11,47] However, changes over time could have been less detectable in patients with low executive function scores already at the baseline.[11]

Finally, since serum NfL correlates with functional impairment and brain atrophy in FTD at different disease stages,[11] a potential application would be the identification of patients who might currently have possible bvFTD and are likely to develop probable bvFTD, i.e. those patients with clinical hallmarks of bvFTD who later show a functional decline and frontotemporal abnormalities in neuroimaging. Serum NfL might help distinguishing such patients from patients who have possible bvFTD but are not likely to show clinical progression

or changes in neuroimaging findings over time (the so-called ‘benign bvFTD phenocopy syndrome’).[S26]

NfL in the prognostic evaluation of amyotrophic lateral sclerosis

According to cross-sectional studies, baseline CSF NfL is lower in ALS patients with slower progression who are referred to neurologists after one year from symptom onset compared to those with faster progression seeking medical attention earlier.[48] These findings do not demonstrate that CSF NfL decreases over time in ALS, a dynamic that may not be consistent with the pathophysiological model of the disease, in which neurodegeneration has a focal onset and then spreads within the CNS.[S27] Moreover, blood-based longitudinal studies have shown little or no change in NfL over time in ALS patients.[49]

NfL has shown to be an independent prognostic marker for ALS. In fact, as mentioned earlier, the CSF and serum NfL concentrations are associated with the number of regions with both UMN and LMN involvement (Table 1).[3,50] Also, CSF NfL is predictive of the time to the generalization of motor symptoms (hazard ratio = 7.9, 95% CI 2.9–21.4, $p < 0.0001$, over about one year of follow-up).[3] Accordingly, higher CSF and blood values are associated with a more rapid progression and shorter overall survival.[3,49,51] For instance, patients within the highest tertile of CSF NfL reach a mortality hazard ratio of up to 31.82 (95% CI 3.8 – 269.7, $p = 0.002$), up to 3.82 (95% CI 2 – 7.4, $p < 0.001$) for blood NfL.[20]

NfL in the prognostic evaluation of parkinsonian and movement disorders

The prognostic value of NfL has been assessed in PD and PSP, but there are no data on its prognostic value in MSA and CBS. In the case of PD, the baseline CSF NfL values associate with the mean change per year in the Dementia Rating Scale scores (correlation coefficient = -0.25, $p = 0.03$).[1] Also, they predict the risk of conversion into PDD in the following 5–9

years (hazard ratio for CSF NfL >1100 pg/mL = up to 2.6, 95% CI 1.1-5.9, $p = 0.03$), but the prediction model performs better with the addition of other biomarkers, such as CSF A β 42 (hazard ratio for CSF NfL/A β 42 ratio >1 = up to 6.7, 95% CI 1.5-30.5, $p = 0.01$).[25] In PSP, higher baseline CSF and blood NfL values seem to correlate with faster worsening of motor and cognitive symptoms. For instance, patients with baseline plasma NfL levels ≥ 36.7 pg/mL were found to have more severe worsening of the PSP rating scale score (mean increase in score = 36.5%, 95% CI 28.8-44.3%) over one year than patients with baseline plasma NfL levels <36.7 pg/mL (mean increase in score = 28.9%, 95% CI 22-35.9%).[52] The combination of NfL with other biomarkers (i.e. the CSF NfL/p-tau ratio) further improved the ability to predict the annual change in the PSP rating scale scores ($p = 0.003$).[53] In addition, the longitudinal one-year change in CSF NfL is inversely correlated with the changes in superior cerebellar peduncle volumes over one year (correlation coefficient = -0.45, $p = 0.04$).[S28] Finally, among movement disorders, blood NfL might have a prognostic value in HD patients, since it correlates with the degree of motor and cognitive impairment and it predicts diffuse and regional brain atrophy, as well as worse outcomes at follow-up.[S23,S29]

NfL in the prognostic evaluation of traumatic brain injury

TBI is a risk factor for both short-term (e.g. post-concussion syndrome and post-traumatic epilepsy) and long-term neurological *sequelae* (e.g. AD and chronic traumatic encephalopathy) but, so far, no reliable prognostic marker for TBI has been discovered. [S24] CSF and serum NfL have been proven to be good prognostic markers that are able to predict the clinical and neuroradiological outcomes.[30,54]

In patients with mild TBI, serum NfL values measured at 1 and 36 h after the trauma can be used to differentiate between patients with rapidly resolving symptoms and patients with prolonged post-concussion syndrome (AUC = 0.82, 95% CI 0.6-1 at 1 h; AUC = 0.83, 95% CI

0.6-1 at 36 h). [S24] Moreover, boxers with high CSF NfL concentrations after a 14-day rest exhibit worse cognitive performance on tests for assessing information processing speed.[S30] In ice hockey players, the baseline CSF NfL seems to be correlated with the number of previous incidents of mild TBI and tends to be higher in players with a history of prolonged post-concussive syndrome.[S31] In addition, one hour after sport-related TBI, serum NfL was found to be highly accurate for distinguishing between athletes who, after a concussion, returned to play within 10 days and athletes who returned after a longer delay (AUC = 0.82, $p < 0.0001$).[55] Serum NfL (measured six days after sport-related TBI) shows even higher accuracy in identifying ice hockey players who resign due to prolonged post-concussion syndrome (AUC = 0.89, $p = 0.005$).[55] Of interest, serum NfL has shown a potential prognostic value in TSCI as well, where its values 24 hours after the trauma have shown a good correlation with the motor outcome 3-12 months later ($r = -0.72$, $p < 0.001$).[5]

NfL as a marker of response to therapy in neurological diseases

NfL measurement in biological fluids has been proposed for monitoring the therapeutic effect of drugs aimed at reducing axonal damage. In this respect, MS represents the ideal pathological condition, since several disease-modifying therapies (DMTs) targeting immune-mediated CNS injury are available. CSF NfL is decreased in RRMS and SPMS patients after 6 and 12 months of treatment with natalizumab,[56,57] as well as in patients who switch to natalizumab from less effective treatments.[S32] A decrease in CSF NfL has been also observed in patients treated with alemtuzumab, cyclophosphamide, fingolimod, mitoxantrone, and rituximab.[58–60] In addition, in a randomized clinical trial, RRMS patients treated with fingolimod showed a significant decrease in CSF NfL values after 12-month treatment compared to placebo. [S33] Similar results have been reported for the blood NfL values in patients treated with fingolimod for 12 months, [S34] as well as in patients treated with other drugs (interferon beta, glatiramer

acetate, natalizumab and rituximab).[2] Thus, blood NfL could be explored as potential indicator of the treatment effects of DMTs.

Repeated lumbar punctures represent a significant obstacle to treatment monitoring, and therefore, blood NfL measurement may be a promising alternative to overcome this limitation. Since CSF NfL can detect axonal damage that has occurred in the last three months,[S7] it can be hypothesized that blood NfL measurement every three months might be useful to profile the ongoing axonal injury in MS patients. Whether this could influence clinical management and decision-making should be further investigated by means of longitudinal studies at the individual level on blood NfL versus MRI measures.

Conclusions and future directions

Over the last two decades, CSF and blood NfL have been shown to be reliable biomarkers of axonal damage across a variety of neurological disorders. Even though NfL changes in biofluids are not specific to any particular CNS disease, this biomarker may have diagnostic value and significant potential in terms of prognostic assessment and disease monitoring.

With respect to its diagnostic potential, NfL might be useful for the diagnosis of ALS and for the early identification of presymptomatic ALS mutations carriers who are about to become symptomatic. In addition, NfL might serve for identifying a neurodegenerative process in patients with psychiatric manifestations and, hopefully, in individuals with subjective memory complaints. In these cases, once a neurodegenerative disorder is suspected, NfL, together with other disease-specific biomarkers (e.g. CSF AD core biomarkers) might be especially beneficial for the differential diagnosis between FTD and AD and between prion diseases and rapidly progressive neurodegenerative dementias. Finally, NfL could help clinicians in the differential diagnosis between APDs and PD, in cases with overlapping clinical manifestations.

Even though in MS and in TBI NfL *per se* does not have any specific diagnostic value, it might still be useful to determine its CSF or serum concentrations during the diagnostic work-up and disease monitoring, since they provide clinicians with an overview of the severity of the ongoing axonal damage, which has important prognostic implications.

As a prognostic marker indeed, NfL may have potential as a predictor of disease activity in MS patients, thus potentially guiding clinicians in the choice of the best DMT, but also as a predictor of cognitive worsening in AD, FTD, and PD and of motor worsening in ALS and APDs patients.

Although there may be many potential contexts of use of NfL, before it can be applied as a biomarker in the clinical setting, there are some steps that need to be undertaken in order to assess the analytical validity and the clinical validity and utility of NfL. One of the limitations to its use is the lack of standard reference materials and methods for NfL measurement both in CSF and blood. Standardization efforts and round robin studies will allow for reliable comparison of results from different laboratories (Panel 3). In addition, normal values across age groups need to be established if NfL is to be used at the individual patient level (Panel 1). Thus, studies on large populations of healthy individuals are required to generate normative data.

Blood NfL measurement represents an important opportunity to verify the effects of different therapeutic interventions on axonal integrity, especially in research settings and in clinical trials. Indeed, NfL could be used as an outcome measure, particularly in both proof-of-concept and dose-finding studies (e.g. phase IIA and IIB studies), where the drug biological activity and the optimal dose for biological activity have to be demonstrated. Studies that focus on the association between longitudinal changes in blood NfL and relevant clinical and radiological measures in neurological diseases are therefore encouraged.

Tables

Table 1. Association of CSF and blood NfL with clinical/paraclinical characteristics in multiple sclerosis, Alzheimer’s disease, frontotemporal dementia, amyotrophic lateral sclerosis, Parkinson’s disease and atypical parkinsonian disorders from cross-sectional studies.

Disease	Biofluid	Clinical features	Other fluid biomarkers	Imaging findings
Multiple sclerosis	CSF	<ul style="list-style-type: none"> • ↑ during relapses [S8,S9] • = in RRMS and PMS [S35,S36] • positive correlation with EDSS (r = 0.2, 95% CI 0.2–0.3, p<0.001) [S9] • positive correlation with MSSS (r = 0.3, 95% CI 0.3–0.4, p<0.001) [S9] 	<ul style="list-style-type: none"> • ↑ in OCB+ patients [S8] 	<ul style="list-style-type: none"> • positive correlation with number of T2 lesions (r = 0.6, p<0.0001) [41] • positive correlation with volume of T2 lesions (r = 0.6, p<0.0001) [41] • positive correlation with number of Gd+ lesions (r = 0.5, p<0.001) [S8]
	Blood	<ul style="list-style-type: none"> • ↑ during relapses [2][S9] • ↑ in PMS vs RRMS [2] • positive correlation with EDSS (r = 0.4, 95% CI 0.3–0.5, p<0.001) [S9] • positive correlation with MSSS (r = 0.4, 95% CI 0.3–0.5, p<0.001) [S9] 	<ul style="list-style-type: none"> • ↑ in OCB+ patients [2] 	<ul style="list-style-type: none"> • positive correlation with number of T2 lesions (β = 2.5, p<0.001) [2] • positive correlation with number of Gd+ lesions (β = 2.1, p<0.001) [2]
Alzheimer’s disease	CSF	<ul style="list-style-type: none"> • ↑ in early and late onset AD [S37] • AD-dem > prodromal AD [S38] • negative correlation in AD-dem patients with MMSE (β = -0.03, p=0.006) [S37] • positive correlation with ADAS-cog (β = 0.006, p=0.008) [S37] 	<ul style="list-style-type: none"> • negative correlation with CSF Aβ42 (β = -0.1, p=0.01) [S37] • positive correlation with CSF t-tau (β = 0.2, p<0.01) [S37] • positive correlation with CSF p-tau (β = 0.1, p=0.02) [S37] 	<ul style="list-style-type: none"> • positive correlation with load of white matter lesions (β = 0.5, p<0.001) [S37]
	Blood	<ul style="list-style-type: none"> • AD-dem > prodromal AD [7] • negative correlation with MMSE (β = -0.07, p<0.01) [7] • positive correlation with ADAS-cog (β = 0.1, p<0.01) [7] • positive correlation with TMT-B (β = 0.08, p=0.02) [7] 	<ul style="list-style-type: none"> • negative correlation in MCI patients with CSF Aβ42 (β = -0.2, p<0.01) [7] • positive correlation in MCI patients with CSF t-tau (β = 0.2, p=0.01) [7] 	<ul style="list-style-type: none"> • positive correlation with lateral ventricles volumes (β = 0.1, p<0.001) [7] • negative correlation with hippocampal volume (β = -0.1, p<0.001) [7] • negative correlation with AD-cortex thickness (β = -0.2, p<0.001) [7]
Frontotemporal dementia	CSF	<ul style="list-style-type: none"> • FTLT-TDP > FTLT-Tau,[S39] not confirmed [S40] • C9orf72 and GRN > MAPT mutations’ carriers [46][S40,S41] • = in bvFTD and PPA [S42] • conflicting results on correlation with MMSE [10][S41] • negative correlation with executive function’s tests scores (r=-0.4, p=0.04;r=-0.5, p= 0.02) [S43] 	<ul style="list-style-type: none"> • positive correlation with CSF t-tau (r = 0.5, p<0.001) [46] • positive correlation with CSF p-tau (r = 0.1, p=0.02) [46] • negative correlation with CSF p-tau/t-tau ratio (r = -0.6, p<0.001) [46] 	<ul style="list-style-type: none"> • negative correlation with volume and density of grey matter (r = -0.4, p<0.05) [S43] • negative correlation with frontal lobe volume (r = -0.7, p<0.001),[S41] not confirmed [10]
	Blood	<ul style="list-style-type: none"> • svPPA > other FTD phenotypes [11] • no correlation with MMSE [S41] • negative correlation with executive function’s tests scores (r=-0.32, p=0.03; r=-0.35, p=0.03) [11] 	<ul style="list-style-type: none"> • N/A 	<ul style="list-style-type: none"> • no correlation with brain volumetric measures [11]
Amyotrophic lateral sclerosis	CSF	<ul style="list-style-type: none"> • = in any ALS diagnostic category [19] • = in bulbar and spinal forms • = in ALS and primary lateral sclerosis [S5] • ↑ ALS vs flail arm or flail leg syndrome or progressive muscular atrophy • = in sporadic and familial ALS [S44] 	<ul style="list-style-type: none"> • positive correlation with CSF pNfH (r = 0.9) [S5] 	<ul style="list-style-type: none"> • negative correlation with fractional anisotropy of corticospinal tract,[S45] not confirmed [S5]

		<ul style="list-style-type: none"> • positive correlation with UMN score ($r = 0.5, p=0.02$) [S45] • \uparrow in ALS with 2 or 3 regions with both UMN and LMN involvement vs ALS patients with only 1 involved region [3] 		
	Blood	<ul style="list-style-type: none"> • = in any ALS diagnostic category ALS [19] • = in sporadic and familial ALS [15] • positive correlation with UMN score ($r = 0.3, p=0.03$) [S45] • \uparrow in ALS with 2 or 3 regions with both UMN and LMN involvement vs ALS patients with only 1 involved region [50] • \uparrow in ALS with 3 regions with UMN involvement vs ALS patients with only 1 region [50] • Stable with increasing number of regions with LMN involvement [50] 	<ul style="list-style-type: none"> • N/A 	<ul style="list-style-type: none"> • N/A
Parkinson's disease	CSF	<ul style="list-style-type: none"> • stable over time [25] • positive correlation with H&Y stage ($r = 0.3, p=0.02$) [28] • negative correlation with MoCA scores ($r=-0.3, p=0.004$) and DRS scores ($r=-0.24, p=0.03$) [1] 	<ul style="list-style-type: none"> • positive correlation with CSF total α-synuclein ($r = 0.4, p=0.02$) [S46] • negative correlation with CSF Aβ42 ($r = -0.3, p=0.05$) [S46] 	<ul style="list-style-type: none"> • N/A
	Blood	<ul style="list-style-type: none"> • positive correlation with L-DOPA equivalent daily dose ($\beta = 0.2, p=0.03$) [S47] • positive correlation with H&Y stage ($\beta = 0.2, p=0.04$) [S47] • positive correlation with UPDRS-III ($\beta = 0.2, p<0.001$) [S47] 	<ul style="list-style-type: none"> • negative correlation with CSF Aβ42 ($r=-0.2, p=0.001$) [S47] • negative correlations with t-tau ($r=0.2, p=0.02$) [S47] • no correlation with p-tau [S47] 	<ul style="list-style-type: none"> • no correlation with load of white matter lesions [S47]
Atypical parkinsonian disorders	CSF	<ul style="list-style-type: none"> • positive correlation with H&Y stage ($\beta = 0.4, p=0.008$) in PSP [28] • 18% increase over time in PSP [53] 	<ul style="list-style-type: none"> • N/A 	<ul style="list-style-type: none"> • negative correlation between one year change in NfL levels and the change in superior cerebellar peduncle's volume ($r = -0.5, p=0.05$) in PSP [53]
	Blood	<ul style="list-style-type: none"> • 13% increase over time in PSP [53] 	<ul style="list-style-type: none"> • negative correlation with CSF Aβ42 ($r=-0.2, p=0.001$) [S47] • negative correlations with t-tau ($r=0.2, p=0.02$) [S47] • no correlation with p-tau [S47] 	<ul style="list-style-type: none"> • no correlation with load of white matter lesions [S47]

Legend. β : regression coefficient. **ADAS-cog**: Alzheimer Disease Assessment Scale-cognitive subscale **AD-cortex**: entorhinal, inferior temporal, middle temporal and fusiform cortex **AD-dem**: dementia due to Alzheimer's disease. **bvFTD**: behavioural variant of frontotemporal dementia. **C9orf72**: chromosome 9 open reading frame 72. **DRS**: Dementia Rating Scale. **EDSS**: Expanded Disability Status Scale. **FTLD-TDP**: frontotemporal lobar degeneration with TAR DNA binding protein 43 inclusions. **FTLD-tau**: frontotemporal lobar degeneration with tau-positive inclusions. **Gd+**: gadolinium-enhancing lesions. **GRN**: progranulin. **H&Y**: Hoehn and Yahr stage. **LMN**: lower motor neuron. **MAPT**: microtubule-associated protein tau. **MMSE**: Mini-Mental State Examination. **MoCA**: Montreal Cognitive Assessment. **MSSS**: Multiple Sclerosis Severity Scale. **OCB**: cerebrospinal fluid IgG oligoclonal bands. **PMS**: progressive multiple sclerosis (both primary and secondary progressive). **pNfH**: phosphorylated neurofilament heavy chain. **PPA**: primary progressive aphasia. **r**: correlation coefficient. **RRMS**: relapsing-remitting multiple sclerosis. **svPPA**: semantic variant of primary progressive aphasia. **TMT-B**: Trail-Making Test part B. **UMN**: upper motor neuron. **UPDRS**: unified Parkinson's disease rating scale.

Panels

Panel 1. NfL and ageing

- CSF NfL has a clear positive association with age ($r = 0.77$, $p < 0.0001$), showing a 2-fold increase in 50-year-old and a 6-fold increase in 80-year-old subjects vs 20-year-old subjects.[S48] Such a relationship is partly explained by axonal structural alterations and metabolic changes taking place along ageing.
- Therefore, upper normal values of CSF NfL are age-dependent. It has been proposed, for instance, an upper normal value of 387 pg/mL if age is 20 years, which raises up to 2417 pg/mL if age is 80 years.[S48]
- Blood NfL also correlates with age (regression coefficient = 1.022, 95% CI 1.018–1.026, $p < 0.001$), showing an estimated yearly increase of 2.2%, with percentile values almost doubling from age 30 to age 70.[2]
- Similar to CSF NfL, upper normal values of blood NfL are age-dependent. For instance, in healthy individuals aged 30, blood NfL 95th percentile corresponds to 27.9 pg/mL, which raises up to 65.1 pg/mL in individuals aged 70.[2]
- Accordingly, when testing potential clinical applications of either CSF or blood NfL, the effects of ageing should be taken into account.
- Multi-centre studies on large populations of healthy individuals performed in qualified laboratories are needed in order to define CSF and blood NfL reference values according to age groups.

Panel 2. Overview of the available assays for NfL measurement

Assays to measure NfL in CSF

- *Enzyme-linked immunosorbent assays (ELISA)*. A sandwich ELISA technique, based on the binding of specific monoclonal antibodies to NfL, is commercially available since 2003 and the vast majority of the studies carried out on CSF NfL have used this assay.[S4] Although this ELISA shows high precision, further standardization is needed.[S49,S50] In 2018, a new ELISA for CSF NfL has been developed and applied in a variety of neurological diseases, confirming the validity of CSF NfL as a biomarker.[S36] ELISA is mainly restricted to CSF because of its limited sensitivity to measure the small concentrations of NfL in blood.

Assays to measure NfL in blood

- *Electrochemiluminescence (ECL)*. ECL technique relies on the binding of specific monoclonal antibodies to NfL within electron-enriched wells, with the subsequent generation of an electrochemiluminescent signal. In 2013, ECL has been introduced for NfL measurement in blood, with improved analytical sensitivity, although some healthy control samples were still not measurable due to their low concentrations of the biomarker.[5][S6]

Single-molecule array (Simoa). Simoa technology is based on single-molecule arrays and simultaneous counting of singulated capture microbeads.[S51] Simoa kits are commercially available and results on plasma/serum NfL have been published from 2016 on. This technique has sharply increased the sensitivity for NfL measurement in blood and has allowed a reliable quantification in blood samples from young healthy controls.[2][S52] A strong correlation has been consistently found between blood NfL and CSF NfL, thus suggesting that blood NfL measurements with this technique may

become a valid alternative to CSF analysis.[2,7][S9,S53] However, it would be ideal to further improve the assay precision in order to use it to detect small within-subject changes of blood NfL. The current assay version, indeed, has an analytical variation ranging from 5.6-6.9%.[2] Therefore, it can detect group-level changes that are quite small and intra-individual changes exceeding its analytical variation.

Panel 3. Unanswered questions and future directions

- Certified reference methods and materials for global assay standardization have to be developed to allow external calibration of the assays. This would increase the comparability of studies.
- Multicentre and round robin studies (i.e. interlaboratory testing of the same samples with the same analytical methods) have to be performed in order to validate the available assays and to standardize the preanalytical and analytical procedures.
- It would be ideal to further improve the analytical precision of the assay for measuring blood NfL in order to use it at the individual level to longitudinally monitor small within-subjects changes.
- Data on NfL at the individual level have to be obtained in different neurological diseases, in order to clarify how to interpret NfL changes in the single patient.
- The range of normal values in different age categories have to be defined for both CSF and blood NfL with multicentre studies on healthy individuals.
- In order to use NfL as an outcome measure in clinical trials, the correlations between NfL and currently used clinical outcomes has to be thoroughly investigated. Once verified, NfL may be used as a surrogate outcome in phase II clinical trials.
- Data on the correlation between NfL and clinical outcomes in different neurological disorders, followed-up for a long period of time, are highly needed.

Search strategy and selection criteria.

References for this Review were identified by searches of PubMed between 1990 and January 31st 2019, and references from relevant articles. The following search terms were used: “neurofilament light”, “neurofilament light chain”, “neurofilament light protein”, “NfL”; “cerebrospinal fluid”, “CSF”, “serum”, “plasma”, “blood”; “demyelinating diseases”, “multiple sclerosis”, “MS”, “clinically isolated syndrome”, “CIS”, “radiologically isolated syndrome”, “RIS”, “optic neuritis”, “myelitis”; “amyotrophic lateral sclerosis”, “ALS”, “motor neuron diseases”; “frontotemporal dementia”, “frontotemporal lobar degeneration”, “FTD”, “FTLD”, “primary progressive aphasia”, “PPA”, “Alzheimer’s disease”, “AD”, “mild cognitive impairment”, “MCI”, “dementia”, “Parkinson’s disease”, “PD”, “Parkinson’s disease dementia”, “PDD”, “dementia with Lewy body”, “DLB”, “progressive supranuclear palsy”, “PSP”, “multiple system atrophy”, “MSA”, “corticobasal syndrome”, “CBS”, “Huntington’s disease”, “HD”, “normal pressure hydrocephalus”, “NPH”, “HIV”, “AIDS”, “AIDS dementia complex”, “ADC”, “antiretroviral therapy”, “ART”, “Stroke”, “transient ischemic attack”, “hemorrhage”, “subarachnoid hemorrhage”, “SAH”, “intracerebral hemorrhage”, “cardiac arrest”, “resuscitation”, “autoimmune encephalitis”, “paraneoplastic encephalitis”. There were no language restrictions. The final reference list was generated based on relevance to the topics covered in this Review. Additional references can be found in the online supplementary file.

Figure legends

Figure 1. Overview of the structure of neurofilaments and neurofilament light chain.

Large calibre myelinated axons abundantly express neurofilaments (Nfs). Nfs are cylindrical structures of 10 nm calibre and they are exclusively located in neurons. They confer structural stability to the axons, enable the radial growth of the myelinated axons and expand their calibre thus allowing a higher conduction velocity. Nfs are classified as intermediate filaments (IFs), i.e. as filaments with an intermediate diameter (10 nm) between actin (6 nm) and myosin (15 nm). In the central nervous system (CNS), Nfs are made of neurofilament light chain (NfL), neurofilament middle chain (NfM), neurofilament heavy chain (NfH) and α -internexin (α -int). All of these subunits have a conserved α -helical rod domain with a variable amino-terminal and carboxy-terminal region. The length of these latter confers a different molecular weight. NfH has the highest molecular weight and presents, in its tail, a glutamic-acid-rich segment (E segment), multiple lysine-serine-proline (KSP) repeats that are phosphorylated and a lysine-glutamic acid-proline (KEP) segment. NfM has a shorter tail with two glutamic-acid-rich segments (E1 and E2 segments), two lysine-serine-proline (KSP) repeats segments and a serine-proline (SP) and lysine-glutamic acid (KE) segment. The tail of NfL is made of the glutamic-acid-rich segment (E segment). Finally, α -int has, in its tail, a glutamic-acid-rich segment (E segment) and a lysine-glutamic acid (KE) segment.

Figure 2. The increase of cerebrospinal fluid neurofilament light chain in a variety of neurological diseases associated with axonal damage.

The figure shows the increase of cerebrospinal fluid (CSF) neurofilament light chain (NfL) with respect to healthy controls (HCs) in a variety of central nervous system (CNS) diseases. Columns represent mean fold increases and standard error of mean of CSF NfL in neurological diseases vs HCs. Columns in red illustrate CNS diseases with mean fold-increase of CSF NfL ≥ 10 , columns in blue CNS diseases with mean fold-increase between 2 and 10 and columns in grey CNS diseases with mean fold-increase < 2 . Mean and standard error of mean values have been calculated based on the values of CSF NfL reported in papers in which patients with CNS diseases were compared to age-matched HCs. For this Figure, studies published within January 2019 were selected. The specific reference list is reported in the supplementary material.

Legend: AD: Alzheimer's disease (it includes both prodromal AD and dementia due to AD). ALS: amyotrophic lateral sclerosis. CBD: corticobasal degeneration. CJD: Creutzfeldt-Jakob disease. CSF: cerebrospinal fluid. DLB: dementia with Lewy bodies. FTD: frontotemporal dementia. HAD: HIV-associated dementia. Mild TBI: mild traumatic brain injury. MS: multiple sclerosis (it includes clinically isolated syndrome, relapsing-remitting multiple sclerosis, primary progressive multiple sclerosis and secondary progressive multiple sclerosis). MSA: multiple system atrophy. NfL: neurofilament light chain. NPH: normal pressure hydrocephalus. PD: Parkinson's disease. PDD: Parkinson's disease dementia. PSP: progressive supranuclear palsy.

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Declaration of interests

LG received travel grants from Biogen-Idec, Biogen, Novartis, Teva, Genzyme and Almirall to attend national and international conferences. KB has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. PC received/receive research support from Bayer Schering, Biogen-Dompé, Boehringer Ingelheim, Eisai, Lundbeck, Merck-Serono, Novartis, Sanofi-Aventis, Sigma-Tau, and UCB Pharma. MDF participated to advisory boards of Biogen Idec, Teva and Bayer, received travel grants from Bayer Schering, Biogen-Dompé, Biogen-Idec, Merck-Serono, Novartis and Sanofi-Aventis to attend national and international conferences and speaker and writing honoraria from Biogen Idec, Novartis and Sanofi-Genzyme. HZ has served at advisory boards for Eli Lilly, Roche Diagnostics and Pharmasum Therapeutics and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. LP reports no conflict of interest.

Contributors' statement

LG, LP, MDF and HZ made the literature search and drafted the manuscript. LG, MDF and LP prepared the figures and the tables. KB, PC, MDF, LP and HZ reviewed the manuscript. All the authors read and approved the final version of the manuscript.