**Neurofilament light chain as a biomarker in neurological disorders**

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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 calibre 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 ultralow 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 research setting in the future. Here we review the most important results on CSF and blood NfL and discuss its potential applications and future directions.

**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. 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). 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. 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). 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. 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. 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. 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.

In CSF, NFL can be measured by sandwich ELISA technology. However, the sensitivity of ELISA for measuring blood NFL concentration is not sufficient. 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.
single-molecule array (Simoa) technology has been used for the quantification of blood NfL even in samples from young healthy controls (HC). 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).

**POTENTIAL DIAGNOSTIC VALUE OF NfL**

The concentration of NfL in CSF is higher in patients with neurological diseases than in 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 (APD) and traumatic brain injury (TBI). At a lesser extent, NfL has been studied in Creutzfeldt-Jakob disease 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 characterised 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 workup of MS**

The CSF NfL concentration is increased both in MS and in its first clinical presentation, that is, clinically isolated syndrome (CIS). 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]=0.80 for CIS vs controls; AUC=0.90 for MS vs controls; no further details available). Similar findings have been reported for serum NfL as well. 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 especially in relation to the time point of the last acute inflammatory episode. Indeed, CSF and serum NfL tend to be higher in patients with relapsing-remitting MS (RRMS) with a recent relapse (no longer than 60 days before) than in patients with clinically stable RRMS. It is plausible that CSF NfL remains high for 2–3 months after a relapse and then drops to lower levels. Therefore, CSF NfL could have the highest diagnostic accuracy within 3 months from the last relapse. This probably applies to blood NfL as well, whose concentration seems to follow the same dynamics as CSF NfL.

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. For instance, while one study showed that the CSF NfL concentration was higher in neuromyelitis optica than in MS (no information is available for serum NfL)
**Neurodegeneration**

![Graph showing the increase of cerebrospinal fluid neurofilament light chain in a variety of neurological diseases associated with axonal damage.](image)

**Figure 2** The increase of cerebrospinal fluid neurofilament light chain (NfL) with respect to healthy controls (HC) in a variety of central nervous system (CNS) diseases. Columns represent mean fold increases and SEM of CSF NfL in neurological diseases versus 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 SEM values have been calculated based on the values of CSF NfL reported in papers in which patients with CNS diseases were compared with age-matched HCs. For this figure, studies published within January 2019 were selected. The specific reference list is reported in the online supplementary material. 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; 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.

available on the diagnostic accuracy), [S10] this was not found to be true for serum NfL. 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 characterisation of NfL indicates that its CSF and blood measurement cannot replace MRI in the diagnosis of MS and CIS and in the exclusion of MS mimics. Nevertheless, NfL measurement during the diagnostic workup of patients with CIS and MS 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 workup of AD and FTD**

In patients with AD, CSF and blood NfL are higher than in HCs. Patients with AD can be differentiated from HCs with good accuracy in the case of CSF NfL (AUC up to 0.77, 95% CI 0.64 to 0.89). Similarly, blood NfL showed excellent accuracy (AUC=0.87; no further details available). 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, 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 patients with FTD as compared with cognitively normal controls (AUC=0.93, 95% CI 0.90 to 0.97), 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 patients with AD or FTD and HCs imply that this biomarker may help in the differential diagnosis between neurodegenerative dementias and non-neurodegenerative disease mimics (ie, 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 to 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 workup. In this sense, CSF or blood NfL measurement may be useful as a first-line test, that is, 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 threefold higher risk of mild cognitive impairment (MCI) over a median follow-up of 3.8 years (HR=3.13, 95% CI 1.36 to 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 (Aβ42), and t-tau/Aβ42 ratio (AUC=0.84, 95% CI 0.76 to 0.93 for NfL vs 0.65, 95% CI 0.50 to 0.80 for Aβ42, 0.67, 95% CI 0.54 to 0.80 for t-tau/Aβ42 ratio).14 CSF NfL could also serve as a biomarker for the differential diagnosis between non-fluent and fluent variant PAPas (nvPPA and svPPA) and logopenic variant PPA (lvPPA), since it is higher in nfvPPA/svPPA compared with lvPPA (AUC=0.87, 95% CI 0.79 to 0.96, p<0.0001).15 Serum NfL also is higher in nfvPPA/svPPA versus lvPPA, although in such comparison its accuracy is lower than CSF NfL (AUC=0.77, 95% CI 0.65 to 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 Aβ42 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, 85.3% 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 workup of ALS
CSF NfL is higher in patients with ALS compared with healthy and neurological controls, [S5] as well as to patients with other motor neuron diseases (MND) (ie, primary lateral sclerosis, spinal muscular atrophy and Kennedy disease)18 and ALS mimics (ie, 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 patients with ALS from HCs,20 but its accuracy in distinguishing patients with ALS in the early symptomatic phase (onset within 6 months) from other neurological diseases (AUC=0.95, 95% CI 0.91 to 0.99), and ALS mimics (AUC=0.94, 95% CI 0.94 to 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 hypothesis that ongoing axonal injury with higher CSF NfL in ALS compared with 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 to 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% to 91.2%) and a specificity of 81.8% (95% CI 74.9% to 87.4%) in distinguishing ALS from other neurological disorders.21

Of note, in asymptomatic ALS mutation carriers, no difference has been found in CSF NfL values compared with HCs, while a sharp increase of CSF NfL was described in symptomatic ALS mutation 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 mutation carriers who later developed ALS as far back as 11.6 months before phenocconversion. In addition, serum NfL levels continued to increase in the first 6 months after symptom onset. On the contrary, in patients with ALS serum NfL were found to be substantially stable over a median time of 1 year.23 These results suggest that neurodegeneration in ALS probably begins almost 1 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 NfL, pNfH has shown to be present in increased concentrations in the CSF of patients with ALS, and it has shown excellent accuracy in differentiating between early symptomatic ALS and ALS mimics (AUC=0.98, 95% CI 0.95 to 1.00). 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, 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 standardisation and validation, as discussed further below, before NfL could be used in the clinical practice.

NfL in the diagnostic workup of parkinsonian and movement disorders

In patients with Parkinson’s disease (PD), 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. [S4, S47] Further, CSF NfL can be used to differentiate between PD and APDs with high diagnostic accuracy (AUC=0.94, 80% sensitivity, 96.9% specificity for MSA vs PD). The addition of CSF Aβ42, p-tau and α-synuclein improves the diagnostic accuracy (AUC=0.90, 95% CI 0.85 to 0.96, 90% sensitivity, 81% specificity). [S8]

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≤0.01), and in patients with HD. In these latter, elevated CSF and blood NfL concentrations have been described, especially in patients with disease manifestation compared with asymptomatic patients who are carriers of cytosine-adenine-guanine (CAG) triplet repeats. [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 workup of TBI

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 2 weeks following severe TBI, NfL sharply increases in both CSF and blood as compared with patients with other neurological diseases and HCs. Within 1 year of severe TBI, the blood NfL level normalises, but no information is available about its levels between the acute phase and after 1 year. [S10]

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. CSF NfL does not peak immediately after a bout, but it peaks after 15 days and normalises after 3–9 months. 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. Similar to TBI, in a few studies traumatic spinal cord injury (TSCI) has been associated to an increase of NfL values in both CSF and blood. [S5]

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 to 0.92). Further investigations in which different diagnostic modalities (ie, blood NfL, electroencephalography and head CT or MRI) 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, that is, 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 MS

MS monitoring is nowadays largely dependent on serial MRI, but this is limited by several factors, including the high frequency of gadolinium (Gd) 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 patients with CIS and RRMS, which was significant relative to baseline at months 6 (p=0.008), 12 (p=0.001) and 24 (p=0.007). 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 disease-modifying therapy (DMT) after the...
### 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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Biofluid</th>
<th>Clinical features</th>
<th>Other fluid biomarkers</th>
<th>Imaging findings</th>
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</thead>
<tbody>
<tr>
<td><strong>Multiple sclerosis</strong></td>
<td>CSF</td>
<td>► ↑ During relapses [58, 59]</td>
<td>► ↑ In OCB+ patients [58]</td>
<td>► Positive correlation with number of T2 lesions (r=0.6, p&lt;0.0001) 46</td>
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<td></td>
<td></td>
<td>= In RRMS and PMS [535, 536]</td>
<td></td>
<td>► Positive correlation with volume of T2 lesions (r=0.6, p&lt;0.0001) 46</td>
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<td></td>
<td></td>
<td>► Positive correlation with EDSS (r=0.2, 95% CI 0.2 to 0.3, p&lt;0.001) [59]</td>
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<td>► Positive correlation with number of Gd+ lesions (r=0.5, p&lt;0.001) [58]</td>
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<td></td>
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<td>► Positive correlation with MSSS (r=0.3, 95% CI 0.3 to 0.4, p&lt;0.001) [59]</td>
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<td></td>
<td>Blood</td>
<td>► ↑ During relapses 2 [59]</td>
<td>► ↑ In OCB+ patients 2</td>
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<td>= In PMS versus RRMS 2</td>
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<td></td>
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<td>► Positive correlation with EDSS (r=0.4, 95% CI 0.3 to 0.5, p&lt;0.001) [59]</td>
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<td>► Positive correlation with MSSS (r=0.4, 95% CI 0.3 to 0.5, p&lt;0.001) [59]</td>
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<tr>
<td><strong>Alzheimer’s disease</strong></td>
<td>CSF</td>
<td>► ↑ In early and late-onset AD [337]</td>
<td>► Negative correlation with CSF Aβ [β=−0.1, p&lt;0.01] [337]</td>
<td>► Positive correlation with load of white matter lesions (β=0.5, p&lt;0.001) [337]</td>
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<td>= AD-dem &gt;prodromal AD 7</td>
<td>► Positive correlation with CSF t-tau [β=0.2, p&lt;0.01] [337]</td>
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<td>► Negative correlation in patients with AD-dem with MMSE (β=−0.03, p=0.006) [337]</td>
<td>► Positive correlation with CSF p-tau [β=0.1, p=0.02] [337]</td>
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<td>► Positive correlation with ADAS-cog (β=0.006, p=0.008) [337]</td>
<td>► Negative correlation with executive function’ test scores (r=−0.4, p=0.04; r=−0.5, p=0.02) [337]</td>
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<td></td>
<td>Blood</td>
<td>= AD-dem &gt;prodromal AD 7</td>
<td>► Negative correlation in patients with MCI with CSF Aβ [β=−0.2, p&lt;0.01] 7</td>
<td>► Positive correlation with lateral ventricle volumes (β=0.1, p&lt;0.001) 7</td>
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<td>► Negative correlation with MMSE (β=−0.07, p&lt;0.01) 7</td>
<td>► Positive correlation in patients with MCI with CSF t-tau (β=0.2, p=0.01) 7</td>
<td>► Negative correlation with hippocampal volume (β=−0.1, p&lt;0.001) 7</td>
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<td>► Positive correlation with ADAS-cog (β=0.1, p&lt;0.01) 7</td>
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<td>► Negative correlation with AD-cortex thickness (β=−0.2, p&lt;0.001) 7</td>
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<td>► Positive correlation with TMT-B (β=0.08, p=0.02) 7</td>
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<tr>
<td><strong>Frontotemporal dementia</strong></td>
<td>CSF</td>
<td>► FTLD-TDP &gt;FTLD tau,[539] not confirmed [540]</td>
<td>► Positive correlation with CSF t-tau (r=0.5, p&lt;0.001) 46</td>
<td>► Negative correlation with volume and density of grey matter (r=−0.4, p&lt;0.05) [543]</td>
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<td>= C9orf72 and GRN &gt;MAPT mutations’ carriers 46 [540, 541]</td>
<td>► Positive correlation with CSF p-tau (r=0.1, p=0.02) 46</td>
<td>► Negative correlation with frontal lobe volume (r=−0.7, p&lt;0.001), [541] not confirmed 10</td>
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<td></td>
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<td>= In bvFTD and PPA [542]</td>
<td>► Negative correlation with CSF t-pau/t-tau ratio (r=−0.6, p&lt;0.001) 46</td>
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<td>= Conflicting results on correlation with MMSE 16 [541]</td>
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<td>► Negative correlation with executive function’s test scores (r=−0.4, p=0.04; r=−0.5, p=0.02) [543]</td>
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<td></td>
<td>Blood</td>
<td>► svPPA &gt;other FTD phenotypes 16</td>
<td>► NA</td>
<td>► No correlation with brain volumetric measures 16</td>
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<td>= No correlation with MMSE [541]</td>
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<td>► Negative correlation with executive function’s test scores (r=−0.32, p=0.02; r=−0.35, p=0.03) 11</td>
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Table 1 Continued

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<td>◁ β regression coefficient; AD, Alzheimer’s disease; ADAS-cog, Alzheimer’s Disease Assessment Scale-cognitive subscale; AD-cortex, entorhinal, inferior temporal, middle temporal and fusiform cortex; AD-dem, dementia due to Alzheimer’s disease; ALS, amyotrophic lateral sclerosis; CSF, cerebrospinal fluid; C9orf72, chromosome 9 open reading frame 72; DRS, Dementia Rating Scale; EDSS, Expanded Disability Status Scale; FTD, frontotemporal dementia; FTLD-TDP, frontotemporal lobar degeneration with TAR DNA binding protein 43 inclusions; FTLD-tau, frontotemporal lobar degeneration with tau-positive inclusions; GRN, progranulin; Gd+, gadolinium-enhancing lesions; H&amp;Y, Hoehn and Yahr stage; L-DOPA, levodopa; LMN, lower motor neuron; MAAPT, microtubule-associated protein tau; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MSSS, Multiple Sclerosis Severity Scale; MoCA, Montreal Cognitive Assessment; NA, not applicable; OCB, cerebrospinal fluid IgG oligoclonal bands; PMS, progressive multiple sclerosis (both primary and secondary progressive); PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; RRMS, relapsing-remitting multiple sclerosis; TMT-B, Trail-Making Test part B; UMN, upper motor neuron; UPDRS, Unified Parkinson’s Disease Rating Scale; bvFTD, behavioural variant of frontotemporal dementia; pNfH, phosphorylated neurofilament heavy chain; r, correlation coefficient; svPPA, semantic variant of primary progressive aphasia.</td>
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<td>◁ 13% increase over time in PSP 28</td>
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<td>◁ Negative correlations with t-tau (r=0.2, p=0.02) 57</td>
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of serum NfL in detecting classic disease activity markers (ie, 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 preclinical MS (ie, 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 patients with RIS, although it has minor relevance (HR=1.03, 95% CI 1.01 to 1.05, p=0.003) in comparison to other prognostic markers such as CSF IgG oligoclonal bands (OCB) (HR=8.9, 95% CI 1.04 to 75.6, p=0.046).

The ability of CSF NfL to predict conversion to MS in patients with CIS is controversial. While some authors have reported higher CSF NfL values at the baseline in patients with CIS who were later diagnosed with MS, [8] some others have reported contrasting findings. 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 an HR increase of (1) 1.005, 95% CI 1.000 to 1.011 (p=0.040), for every 100 ng/L increase in CSF NfL; (2) 2.6, 95% CI 1.09 to 6.683 (p=0.048), in case of CSF IgG OCB evidence; and (3) 11.5, 95% CI 1.4 to 91.9 (p=0.022) for ≥4 lesions on MRI. Similar to CSF NfL, serum NfL also does not show a clear correlation with a higher risk of subsequent MS development in patients with CIS.

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 5 years (correlation coefficient=−0.89, p<0.0001). Similar results have been obtained for serum NfL in patients with RRMS, 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 to 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). 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 to 0.042, p=0.023) and spinal cord volume (regression coefficient=−0.488, 95% CI −0.783 to 0.192, p=0.001) over 5 years.

Also, longitudinal NfL changes have shown a similar prognostic value compared with 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 to 6.3, p<0.001) increase in new Gd+ lesions over the same period. 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. [57] 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 to 0.91, p=0.001] over the same period).

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). 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 intermediately CSF NfL values (<60 ng/L, p=0.01; 60–386 ng/L, p=0.03, respectively).

With regard to the prognostic value of blood NfL, the timing of longitudinal measurements is an important issue. Indeed, within 2 months after CIS, serum NfL does not seem to be dependent from the interval between CIS onset and blood sampling. On the contrary, 6 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. Therefore, while the first assessment of NfL can be performed at any time within 2 months after the first clinical event without expecting any significant variations in its levels, a second measurement 6 months later could have a more specific prognostic value.

NfL in the prognostic evaluation of AD and FTD

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). 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 at the follow-up (regression coefficient for plasma NfL=0.1, p<0.001). 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 4 years of follow-up.

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 mutation carriers (correlation coefficient=0.75, p<0.001 for serum NfL). This finding points to the possibility of evaluating the effects of drugs in subjects with preclinical AD in clinical trials.

In patients with FTD, higher baseline CSF NfL levels are independently correlated with a worse prognosis and shorter survival. For instance, while the 5-year survival of patients with FTD with a baseline CSF NfL <1989 pg/mL is 73%, it decreases to 36% when the baseline CSF NfL is >3675 pg/mL (estimated HR 1.7, 95% CI 1.3 to 2.1, p<0.001). Such a prognostic effect is superior to that of CSF p-tau/t-tau (estimated HR 0.7, 95% CI 0.56 to 0.86, p=0.001). 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). CSF NfL has shown a positive correlation with the magnitude of annual MMSE score loss in patients with FTD (correlation coefficient=0.3, p=0.003). In other studies, CSF and serum NfL did not show a significant association with the progression of cognitive impairment. However, changes over time could have been less detectable in patients with low executive function scores already at the baseline.

Finally, since serum NfL correlates with functional impairment and brain atrophy in FTD at different disease stages, a potential application would be the identification of patients who might currently have possible bvFTD and are likely to develop
probable bvFTD, that is, 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 ALS

According to cross-sectional studies, baseline CSF NfL is lower in patients with ALS with slower progression who are referred to neurologists after 1 year from symptom onset compared with those with faster progression seeking medical attention earlier. 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 patients with ALS. [S28]

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). Also, CSF NfL is predictive of the time to the generalisation of motor symptoms (HR=7.9, 95% CI 2.9 to 21.4, p<0.0001, over about 1 year of follow-up). According to prediction model performs better with the addition of other biomarkers, such as CSF Aβ42 (HR for CSF NfL/Aβ42 ratio >1=up to 6.7, 95% CI 1.5 to 30.5, p=0.01). In PSR, higher baseline CSF and blood NfL values seem to correlate with faster worsening of motor and cognitive symptoms. For example, patients with baseline plasma NfL levels ≥36.7 μg/mL were found to have more severe worsening of the PS rating scale score (mean increase in score=36.5%, 95% CI 28.8% to 44.3%) over 1 year than patients with baseline plasma NfL levels <36.7 μg/mL (mean increase in score=28.9%, 95% CI 22% to 35.9%). The combination of NfL with other biomarkers (ie, the CSF NfL/p-tau ratio) further improved the ability to predict the annual change in the PSP rating scale scores (p=0.003). In addition, the longitudinal 1 year change in CSF NfL is inversely correlated with the changes in superior cerebellar peduncle volumes over 1 year (correlation coefficient=−0.45, p=0.04). [S29]

Finally, among movement disorders, blood NfL might have a prognostic value in patients with HD, 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 TBI

TBI is a risk factor for both short-term (eg, postconcussion syndrome and post-traumatic epilepsy) and long-term neurological sequelae (eg, 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.

In patients with mild TBI, serum NfL values measured at 1 and 36 hours after the trauma can be used to differentiate between patients with rapidly resolving symptoms and patients with prolonged postconcussive syndrome (AUC=0.82, 95% CI 0.6 to 1 at 1 hour; AUC=0.83, 95% CI 0.6 to 1 at 36 hours). [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 postconcussive syndrome. [S31] In addition, 1 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). Serum NfL (measured 6 days after sport-related TBI) shows even higher accuracy in identifying ice hockey players who resign due to prolonged postconcussive syndrome (AUC=0.89, p<0.005). 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).

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 DMTs targeting immune-mediated CNS injury are available. CSF NfL is decreased in patients with RRMS and SPMS after 6 and 12 months of treatment with natalizumab, 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.

In addition, in a randomised clinical trial, patients with RRMS treated with fingolimod showed a significant decrease in CSF NfL values after 12-month treatment compared with placebo. [S33] Similar results have been reported for the blood NfL 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). 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 3 months, [S7] it can be hypothesised that blood NfL measurement every 3 months might be useful to profile the ongoing axonal injury in patients with MS. 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 mutation 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 (eg, 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 workup 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 patients with MS, 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 patients with ALS and APDs.

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. Standardisation 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 (eg, 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.

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 to 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.

Multicentre 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

► 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 standardisation 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

► 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 HC samples were still not measurable due to their low concentrations of the biomarker. [S6]

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 onwards. This technique has sharply increased the sensitivity for NFL measurement in blood and has allowed a reliable quantification in blood samples from young HCs. [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. [S7] [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% to 6.9%. Therefore, it can detect group-level changes that are quite small and intrapatient changes exceeding its analytical variation.
Neurodegeneration

Panel 3. Unanswered questions and future directions

- Certified reference methods and materials for global assay standardisation have to be developed to allow external calibration of the assays. This would increase the comparability of studies.
- Multicentre and round robin studies (ie, interlaboratory testing of the same samples with the same analytical methods) have to be performed in order to validate the available assays and to standardise 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-subject 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 has 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 have 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


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