nature reviews neurology

https://doi.org/10.1038/s41582-024-00955-x

Review article

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Neurofilaments as biomarkers in neurological disorders — towards clinical application

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Abstract

Neurofilament proteins have been validated as specific body fluid biomarkers of neuro-axonal injury. The advent of highly sensitive analytical platforms that enable reliable quantification of neurofilaments in blood samples and simplify longitudinal follow-up has paved the way for the development of neurofilaments as a biomarker in clinical practice. Potential applications include assessment of disease activity, monitoring of treatment responses, and determining prognosis in many acute and chronic neurological disorders as well as their use as an outcome measure in trials of novel therapies. Progress has now moved the measurement of neurofilaments to the doorstep of routine clinical practice for the evaluation of individuals. In this Review, we first outline current knowledge on the structure and function of neurofilaments. We then discuss analytical and statistical approaches and challenges in determining neurofilament levels in different clinical contexts and assess the implications of neurofilament light chain (NfL) levels in normal ageing and the confounding factors that need to be considered when interpreting NfL measures. In addition, we summarize the current value and potential clinical applications of neurofilaments as a biomarker of neuro-axonal damage in a range of neurological disorders, including multiple sclerosis, Alzheimer disease, frontotemporal dementia, amyotrophic lateral sclerosis, stroke and cerebrovascular disease, traumatic brain injury, and Parkinson disease. We also consider the steps needed to complete the translation of neurofilaments from the laboratory to the management of neurological diseases in clinical practice.

Sections

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Key points

- Neurofilament proteins have emerged as one of the most important body fluid biomarkers of neuro-axonal injury in a wide range of neurological diseases.
- High-sensitivity analytical platforms enable reliable quantification of neurofilament light chain (NfL) levels in blood samples, paving the way for their use in clinical practice.
- Establishment of large reference databases of physiological blood levels of NfL adjusted for age and BMI was a major milestone towards the clinical use of NfL.
- Neurofilament levels can often not be used to diagnose disease entities but are useful as a diagnostic type biomarker in the preclinical phases of neurodegenerative diseases and as markers of disease progression, prognosis, and treatment response.
- Neurofilament levels are increasingly used as an outcome measure in clinical trials; FDA approval of tofersen was based on changes in blood NfL levels, marking a paradigm shift in the importance of biomarkers in regulatory approvals.
- Standardization and cross-compatibility of neurofilament measures taken with current emerging analytic platforms are key to completing the translation of neurofilaments into clinical practice.

Introduction

Neurofilament proteins have emerged as valid biomarkers of neuronal injury and loss, which is one of the major pathophysiological substrates of permanent disability in various acute and chronic neurological disorders[1]. Upon neuro-axonal damage and degeneration, neurofilament proteins are released into the cerebrospinal fluid (CSF) and, at lower concentrations, into the blood. Neurofilaments can be considered one of the most important fluid biomarkers of neurodegeneration, and efforts are focusing on translating their use into routine clinical practice[2].

A crucial milestone in the development of neurofilaments as biomarkers for clinical application was the introduction of highly sensitive platforms that enable reliable, high-throughput quantification of neurofilaments in various bodily fluids, particularly blood[1,2]. In some laboratories, measurement of neurofilament light chain (NfL) in the CSF[3] and, more recently, in plasma[4,5] has been used for several years as a biomarker to identify, exclude and grade neuro-axonal damage in clinical practice under the designation of a laboratory-developed test[6]. However, clinical validation in large cohorts has emerged only recently[2], and full regulatory approval for the use of NfL as a clinical biomarker is pending.

In a previous Review published in 2018, we summarized what was known at the time about neurofilament structure and function, analytical factors to be considered, and age-related changes in neurofilament protein levels, and provided a comprehensive overview of NfL as a biomarker in various neurological disorders[1]. Subsequently, the field has evolved rapidly through collaboration between basic scientists, clinical scientists and industry partners[2]. Now, changes in NfL levels are increasingly being used as secondary end points in clinical trials[7–10] and their importance as an outcome parameter has been recognized by regulatory authorities[11–13]. Indeed, the accelerated approval of tofersen (for treatment of SOD1-associated amyotrophic lateral sclerosis (ALS)) by the FDA based on NfL levels as a primary end point measure marks a breakthrough in the field of translational biomarker research[10]. However, this accelerated approval was in the context of an orphan or rare disease; all currently available assays for NfL are designated as for 'research use only' and further clinical and regulatory validation is needed to achieve the designation of 'in vitro diagnostic' that can be used in common diseases.

NfL levels in the serum or plasma provide quantitative, real-time information about the extent of ongoing neuro-axonal injury that can be combined with clinical and imaging metrics to assess disease activity. However, several hurdles need to be surmounted before NfL can be widely used in clinical practice. Several confounding factors, such as age, chronic kidney disease and BMI, affect the measurement of NfL concentrations[14,15], making it difficult to establish fixed cut-off levels based on absolute NfL concentrations[16]. Other issues that need to be addressed include NfL degradation and clearance, biological understanding of the analytical target[17], post-translational modifications and harmonization of different analytical platforms[2,18,19].

In this Review, we provide an update on the current knowledge of neurofilaments, analysis of NfL levels, the potential clinical value of neurofilaments for the main neurological disorders and the progress towards their translation into clinical practice. Further, we highlight the steps needed to complete the development and validation of NfL for its use as an in vitro diagnostic test.

Neurofilaments — structure and function

The cytoskeleton of neurons and axons contains five neurofilament protein isoforms[1]; NfL, neurofilament medium chain (NfM), neurofilament heavy chain (NfH), α-internexin and peripherin. Additional splice variants exist, but their distributions and significance are not yet clear because they have not been systematically examined[2]. Neurofilament proteins are obligate heteropolymers and contain intrinsically unstructured regions in which most mutations that cause or predispose to disease occur[2,20]. Presently, a total of 121 mutations in neurofilament isoforms have been associated with ALS, Charcot–Marie–Tooth disease or spinal muscular atrophy (SMA)[2] (Table 1).

The structures of neurofilament proteins are modified by post-translational modifications[21,22]. The most extensive post-translational modification is phosphorylation, which results in charge repulsion[2,23] (Table 1). Abnormal phosphorylation sites that result from mutations can promote formation of neurofilament protein hetero-aggregates, which are pathological features of Alzheimer disease and ALS[24]. Neurofilament protein aggregates associate through amyloidogenic elements[25–27], rendering them extraordinarily resistant to decay[28]. Other common post-translational modifications in neurofilament proteins are citrullination, glycosylation and glycation, all of which have roles in neurodegeneration and autoimmune pathology[2,29–31].

The relative quantities — the stoichiometry — of the different neurofilament protein isoforms is 7:3:2 in health and changes in disease, when NfL increases and NfM and NfH decrease. The shift in neurofilament protein stoichiometry also saves neurons energy in the face of progressive neurodegeneration[32], and this relationship explains why NfL is the most promising biomarker at the individual patient level — its greater abundance maximizes measurement sensitivity[2,22]. Therefore, the release of a stable, pathology-specific NfL cleavage product[17] makes

Table 1 | Characteristics of neurofilament isoforms

Characteristic	NfH	NfM	NfL	α-Internexin	Peripherin
Intermediate filament type	IV	IV	IV	IV	III
Anatomical distribution	CNS, PNS	CNS, PNS	CNS, PNS	CNS	PNS
Chromosome	22	8	8	10	12
Full length (amino acids)	1,020	916	543	499	470
Splice variant length (amino acids)	924	540	NA	NA	NA
MW based on DNA sequence (kDa)	112,477.6±7.2	102,470.8±6.6	61,400.8±4.0	55,390±3.6	53,650.3±3.5
MW based on processed DNA sequence (kDa)[355–359]	105.6	102.5	61.5	55.4	53.7
MW on SDS gel (kDa)[a]	190–210	150	68	66	57
Charge[b]	–11	–64	–49	–14	–15
Phosphorylation	+++[c]	++	+	+	+
O-glycosylation	++	++	+	–	–
Genetic risk for	ALS, SMA, CMT	ALS, PD	ALS, CMT	PD, LBD	ALS

ALS, amyotrophic lateral sclerosis; CMT, Charcot–Marie–Tooth disease; MW, molecular weight; LBD, Lewy body dementia; NA, not applicable; NfH, neurofilament heavy chain; NfL, neurofilament light chain; NfM, neurofilament medium chain; PD, Parkinson disease; SDS, sodium dodecyl-sulfate; SMA, spinal muscular atrophy. [a]Differs from calculated weights owing to post-translational modifications. [b]Calculated from amino acid sequence. [c]NfH is the most extensively phosphorylated protein of the human body.

this NfL peptide a good candidate for clinical use as a biomarker in individuals.

Beyond their main function of mechanical stabilization of the axonal cytoskeleton, neurofilament proteins have multiple functions, including modification of the axonal diameter[33–36], axonal flow[37,38], axonal transport[39–41], anchoring and distribution of mitochondria[42–44], and interactions with myelin proteins that govern the expression of neurofilament isoforms during development and myelination[2,45].

Origins of neurofilaments in body fluids

In vitro studies have demonstrated a linear relationship between the number of degenerating neurons and neurofilament levels[1,46]. Brain microdialysis has shown that, in human traumatic brain injury (TBI), neurofilament protein cleavage products are released into the interstitial fluid adjacent to degenerating neurons[46]. This proteolytic breakdown of neurofilaments yields highly soluble and stable cleavage products[2,17,47]. The number of known cleavage products has increased rapidly since the first description of NfL[2,17,46,48–50], and their relevance to the clinical use of neurofilament biomarkers is becoming clear. For example, the Uman antibodies (monoclonal antibodies against purified mammalian spinal cord that became key reagents in a commercial assay[17]) interact with epitopes that are only accessible on NfL cleavage products released upon neurodegeneration-induced protease activity[17] (Fig. 1).

Neurofilament protein cleavage products are assumed to diffuse from the interstitial fluid into adjacent body fluid compartments and have been detected in CSF, blood, amniotic fluid, and anterior chamber and vitreous body in the eye[1,2,51]. In addition, the glymphatic system probably contributes to drainage of NfL from the brain into systemic circulation as demonstrated for other biomarkers such as glial fibrillary acidic protein (GFAP)[52]. In support of this idea, impairment of glymphatic flow in diseases, such as idiopathic intracranial hypertension, is associated with an increased CNS-to-serum ratio of NfL[53]. The half-life of neurofilament protein cleavage products has not been determined experimentally but applied mathematical approaches have been used to estimate that the half-life of measurable NfL peptides in the blood in TBI is ~500 h (refs. 54,55). However, serum concentration of NfL results from complex wash-in (leakage from the injured brain) and wash-out (clearance and elimination from the blood) processes with temporal dynamics that create a profile with an unusually long peak time and a decay rate that indicates ongoing pathology[56]. This decay of NfL after the peak is referred to as the effective half-life and can vary substantially under different conditions and over time, making it difficult to determine a general half-life for NfL cleavage products[56].

Most neurofilament proteins detected can be attributed to neurons but traces of NfL mRNA can be detected in glial cells[57] and minimal quantities of neurofilament proteins are detectable in erythrocytes, T cells, podocytes, oocytes, stem cells, testicular tissue, thymus tissue and cancerous tissue[2]. In addition, NfH, NfM and NfL are all expressed in the PNS as well as in the CNS[2]. Consequently, the most likely source of neurofilament proteins in body fluids can only be deduced in well-described clinical scenarios[2]. This uncertainty could be reduced in the future with the development of quantitative tests for intermediate filaments that are more specific for the CNS (α-internexin) and the PNS (peripherin)[58,59].

To determine the origin of neurofilament proteins and the associated pathology, a coherent and transparent approach to reporting quantification of these proteins and their cleavage products is essential. This approach will need to include reporting of the antibodies used. For this purpose, a simple nomenclature for labelling neurofilament proteins has been proposed in which the antibody clone used is added as a superscript[24]; for example, NfH^{SMI35} (ref. 60) and $NfL^{Umea\ 47:3}$ (ref. 61). This nomenclature can be further developed by adding a subscript to indicate the location of cleavage products on the consensus human sequence[2] (Table 1); for example, $NfL_{530–540}$ (ref. 50) or $NfH_{852–986}$ (ref. 46).

Technical challenges in clinical application

Analytical challenges

Advances have been made in the past 10 years in the techniques used to measure NfL in CSF and plasma or serum. The first major advance was single-molecule array (Simoa) ultrasensitive technology[62,63], which enabled a shift from measurements in CSF to measurements in serum and plasma, where concentrations are much lower and not detectable

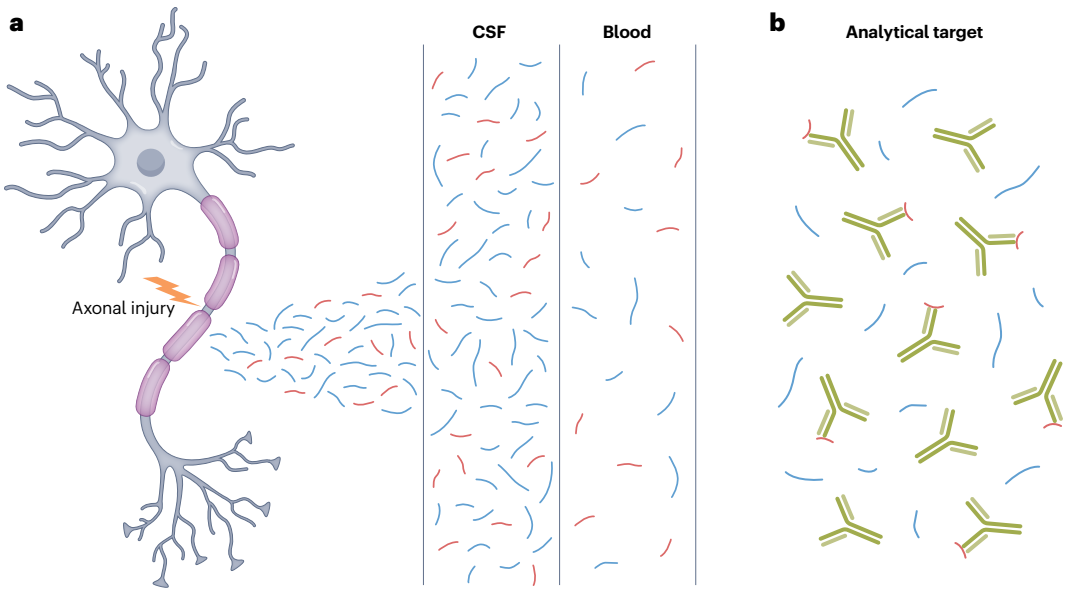

Fig. 1 | Neurofilament release after axonal damage and detection of cleavage products in the blood. a, When an axon is damaged (left), cytoskeletal proteins, including neurofilament proteins (blue), are released into the extracellular space and, subsequently, into the cerebrospinal fluid (CSF) and, at lower concentrations, into the blood. Degradation of neurofilament proteins produces cleavage products (red, blue) and can unmask epitopes[17] that are normally hidden in neurofilament proteins released by intact neurons during physiological metabolism. **b**, Highly sensitive immunoassays can reliably detect blood levels of certain neurofilament light chain fragments via these epitopes (red). Adapted from ref. 1, Springer Nature Limited.

with classical enzyme-linked immunosorbent assay (ELISA). Simoa technology is based on a digital ELISA, in which single nanobeads carrying immunocomplexes are counted as positive or negative based on secondary antibody recognition, which is then used to calculate the concentration of the analyte[64]. Combining this technology with NfL antibodies that were originally validated in CSF detection kits (UmanDiagnostics)[65] enabled the detection of minute quantities of NfL[5,66]. The same antibodies have subsequently been used in a microfluidic NfL assay (on an ELLA platform), which achieved similar performance as Simoa[67]. A third assay, known as Meso Scale Discovery, is similar to ELISA but relies on a more sensitive electrochemiluminescence technique for analyte detection[61]. Despite their sensitivity, these three technologies are designated for 'research use only' — multiple challenges hinder their translation into clinically approved diagnostic tests (Box 1).

Despite the challenges involved, growing interest in NfL quantification and the potential value of this biomarker in clinical practice has led to ongoing development of quantification methods that can be used for in vitro diagnostics. These methods are based on fully automated platforms[68] that are compatible with random-access workflows in clinical chemistry laboratories[19]. These assays typically enable measurement of NfL in both CSF and plasma or serum. In 2022, details of an immunoprecipitation mass spectrometry-based assay for measurement of NfL in CSF were published, showing a strong correlation between measurements made with this assay and those made with the most widely used ELISA for NfL in CSF[50]. This assay is now being considered as a possible reference method for NfL quantification[19].

Confounding factors

Normal ageing involves physiological phenomena that lead to neuroaxonal degeneration and release of neurofilament proteins into CSF and blood[69]. No convincing evidence suggests that these processes and the resulting neurofilament protein levels differ between the sexes[16,70]. In addition, comorbidities can affect the release and turnover of neurofilaments, and the prevalence of such comorbidities is highest among people in the upper age strata; therefore, these effects are superimposed on age-related changes in neurofilaments[71]. This combination means that defining normal reference values in these age groups is a major challenge. This problem is especially relevant to blood concentrations of neurofilaments, and major efforts have been made in the past 5–10 years to define relevant confounders.

The normal upper reference values for CSF concentrations of NfL increase more than twofold between the ages of 20 and 50 years and double again by age 70 years[72,73]. These increases are likely to reflect a faster rate of neuro-axonal degeneration — a notion supported by correlations between neurofilament levels and the rate of hippocampal atrophy in cognitively healthy older people without pathological increases in Alzheimer disease biomarkers[74] — but could also reflect changes in CSF fluid dynamics[75]. In the blood, concentrations of NfL increase by 2.2% per year between the ages of 18 and 70 years in healthy control individuals[76]. An international normative reference database for serum NfL concentrations in individuals with no evidence of CNS disease that is based on *Z*-score (percentile) transformed values adjusted for age and BMI has now been created — a major milestone towards clinical implementation of NfL as a biomarker[16] although the existing database is assay specific. In this database, blood concentrations of NfL increase exponentially with age until ~50 years of age, after which the rate of increase becomes even steeper.

Besides age, BMI is an important confounder for blood levels of NfL; lower levels are associated with a higher BMI owing to the larger volume of blood in which NfL is diluted[77]. Both age and BMI have been

included in the derivation of normative values[16], enabling BMI to be accounted for in clinical application. However, some laboratories use reference values that are adjusted for age but not BMI[16,78] on the basis that the effect of age on blood levels of NfL is considerably stronger than that of BMI.

Another confounder of blood NfL concentration is renal insufficiency owing to reduced clearance of NfL from the blood[14,16,79,80] and, possibly, protein metabolism[81], although evidence for the latter is limited. Indeed, estimated glomerular filtration rate is negatively correlated with NfL concentrations in the blood when the rate is <60 ml/min/1.73 m^2, which is associated with a considerable increase in serum NfL levels[16]. In practice, therefore, this association is mainly relevant for those with certain renal diseases[82].

Other comorbidities that have been associated with increased blood levels of NfL include diabetes mellitus and cardiovascular conditions such as atrial fibrillation, heart failure and peripheral artery disease[81,83,84]. The effects of lifestyle factors on NfL levels are largely unexplored, although alcohol abuse has been associated with increased blood levels of NfL in a small study[85]. In mountain climbers, serum NfL levels increased after ascent to 4,559 m independently of the occurrence of acute mountain sickness[86], although another study showed no significant changes in NfL levels in normobaric hypoxia[87]. Intense bouts of aerobic exercise do not seem to affect CSF levels of NfL in the short term[88] but observations of blood NfL levels have differed between studies; lower levels and unchanged levels have been reported in association with aerobic exercise[89,90]. The influence of race and ethnicity on NfL levels has not been studied in adequately large healthy control populations.

Statistical analysis and reference values

Statistical analysis of NfL levels presents several challenges. NfL concentrations are continuous measures but their distribution is generally right-skewed and heavy-tailed (Fig. 2a). For this reason, NfL measures are often analysed after log transformation[78,91,92] to meet the assumption of a normal distribution of the residuals required in regression models, with median values or geometric means as summary statistics[92,93].

In our 2018 Review, we stated that "the main factors limiting the application of neurofilament measurements to disease monitoring individuals are the lack of normal values of neurofilament across all age groups […] and the need for thorough multi-centre analytical assay validation to achieve standardized and reliable measurements across different sites"[1]. Since then, considerable efforts have been made to standardize assessment of neurofilament proteins to create reference values and enable their use at the individual level[15,16,78,94,95].

As discussed above, NfL levels in the blood strongly increase with age and moderately decrease with higher BMI[16,71,77]. Consequently, NfL levels need to be interpreted in a physiological context; by contrast, most standard laboratory parameters are reported in relation to age-independent upper limits of normal. Adjustment for these confounding factors is an option at the group level (although complicated by non-linearity[16]), but alternative approaches are needed for application to individuals.

One possible approach is to define normal values in different age bins[78], but the costs are a loss of biological information dependent on the width of these bins (because the upper limit of normal is identical for individuals at the extremes of each bin) and potential loss of precision, depending on the number of samples per bin. These costs, which are particularly problematic when sample sizes are small, can be minimized by modelling associations across the entire age range. For example, reference curves can be generated that are analogous to child growth curves[96].

The association of NfL levels with age and BMI in individuals aged >20 years has been modelled based on a large, multinational dataset of control individuals in Europe and the USA using a generalized additive model for location, scale and shape[16]. Modelling of the skewed distribution of the data enabled precise estimation of *Z*-scores (which are interchangeable with percentiles; Fig. 2b). These NfL *Z*-scores express how strongly (in terms of the number of standard deviations) a given NfL measurement deviates from NfL values in control individuals adjusted for age and BMI. Hence, one value represents whether and to what extent NfL is pathologically increased. *Z*-scores are, by definition, normally distributed across age groups, which is advantageous for modelling. Following the modelling in people aged >20 years, international initiatives have broadened the range to include children, adolescents and young adults aged 0–20 years[97] (Fig. 3). The distribution of NfL levels in healthy children revealed an age-dependency that is distinct from that in adults, thereby highlighting the limitations of solely relying on statistical age adjustments. Furthermore, the use of the *Z*-score provided greater statistical power when distinguishing between healthy groups and groups with disease (Fig. 3).

An unmet need and potential threat in the development of valid and easy-to-use reference values for application at the individual level is inter-centre variability in NfL measures obtained with the same assay. In a round-robin study across 17 laboratories in Europe and the USA,

Box 1

From the laboratory to the clinic

Laboratory-developed tests can have important roles in health care, enabling the use of analytically and clinically validated biomarkers for clinical diagnostics. However, obtaining regulatory approval for an in vitro diagnostic method is a challenge for kit suppliers. Regulatory and quality requirements are strict, including demonstration of the control of reagent production and quality by the company; full analytical qualification of the test, including its performance (for example, its limit of detection, limit of quantification and linearity) and robustness; studies of interference by the presence of substances in the specimen; demonstration of the ability to supply batches that give comparable values over time; and clinical validation that the test provides the answer to a defined medical need[6].

For suppliers, these stages of production and analytical and clinical validation are expensive and are the legal responsibility of companies. The situation is further complicated by the requirement to use equipment for analysis that is itself certified for in vitro diagnostic use, has a low cost, and meets the ergonomic and user requirements of routine laboratories; functionality requirements include the ability to connect to IT systems, management of patient identities (a barcode reader), adaptation to clinical primary sample tubes and a random-access sample passage system. Consequently, only a small number of companies are developing in vitro diagnostic solutions and these are subject to intense global competition.

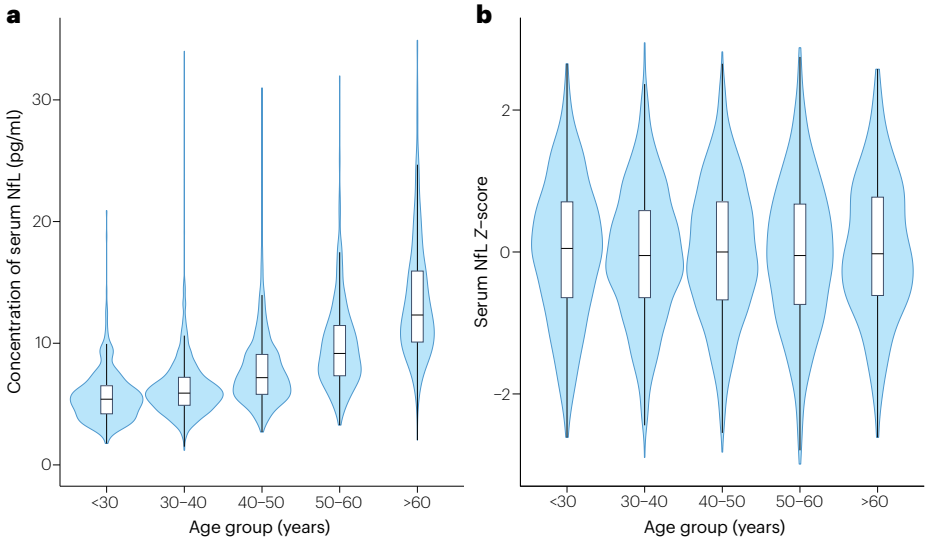

Fig. 2 | Physiological levels of NfL in different age groups. a, Distribution of raw serum concentrations of neurofilament light chain (NfL) by age group. As often observed for fluid biomarkers, the distribution of the concentrations is right-skewed with outliers. The effects of BMI are not included in these data. **b**, NfL *Z*-scores by age group. *Z*-scores provide a single measure of deviation from normal that accounts for age (and BMI). *Z*-score is the number of standard deviations from the mean in the reference population. Plots generated based on data from ref. 16.

coefficients of variation for nine serum samples ranged from 6.9% to 11.8% between laboratories[98]. However, larger variation is seen in inter-assay precision between centres and in clinical practice when compared with controlled experiments[65,98], necessitating rigorous procedures to develop robust in vitro diagnostic methods.

Summary and remaining challenges

Considerable methodological efforts have been made in the past 5–10 years to make NfL values applicable in clinical practice. Online tools are now available to assess whether an NfL measurement falls within the normal range accounting for age and BMI[16,97]. However, work remains to be done before broad clinical application is possible. For example, NfL measurement results would ideally be equivalent regardless of which analytical assay or platform is used, and efforts to produce certified reference material to calibrate assays and harmonize values across platforms are in progress[19]. Current age-dependent and BMI-dependent NfL reference ranges will need to be transformed to adjust for newly developed assays that produce different absolute NfL values.

Finally, data are accumulating from routine NfL assessments in real-life situations and are being used in pragmatic trials to examine how implementation of NfL as a biomarker in clinical practice affects the course of the disease[99]. In the next few years, these studies will provide us with insights into the practical utility of NfL in clinical settings.

Neurofilaments in neurological disease

Neurofilaments have been investigated as biomarkers in a wide range of neurological diseases. Below, we summarize developments in each disease with a focus on progress towards clinical application of neurofilament measurements in each context.

Neuroinflammatory disorders

Multiple sclerosis, neuromyelitis optica spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) are chronic diseases of the CNS that involve focal inflammation and brain-diffuse neurodegeneration and share typical clinical features such as relapses and focal white matter lesions. NMOSD and MOGAD have only recently been recognized as distinct nosological entities from multiple sclerosis following the discovery of pathogenic autoantibodies that target astrocytes in NMOSD and oligodendrocytes in MOGAD. Though these three diseases have different pathogenetic mechanisms and long-term clinical courses and require different therapies, an increase in levels of neurofilament proteins during acute disease states is a common denominator.

Multiple sclerosis. NfL was first described as a CSF-based biomarker in multiple sclerosis in 1998 (ref. 100). NfL levels were increased in people with multiple sclerosis[101], were highest immediately after an attack, took >200 days to return to average levels within the multiple sclerosis cohort[100] and correlated with the degree of clinical disability[100]. In the 25 years since, these findings have been confirmed and extended into different stages and states of multiple sclerosis[1]. Most recently, the development of high-sensitivity assays to quantify NfL in the blood has enabled longitudinal measurement to assess treatment responses.

Measurement of NfL levels in the 6 months after initiation of therapy has shown that the higher therapeutic efficacy of monoclonal antibodies (CD20, CD52 and α4β1-integrin antibodies) than those of oral therapies (S_1P receptor inhibitors, dimethyl fumarate and teriflunomide) and platform therapies (glatiramer acetate and IFNβ)[16,102] is reflected in a more pronounced decrease in NfL levels[16,103,104]. Furthermore, given that serum and plasma levels of NfL are associated with acute clinical features (relapse rate) and MRI features (lesion development and load) of disease[4,105], they have been used as an efficacy end point in some of the most recent trials of therapies for relapsing–remitting multiple sclerosis and secondary progressive multiple sclerosis[7,103,106–108]. The specificity of NfL as a drug response marker is further emphasized by the observation that treatment with drugs that have little or no clinical efficacy (for example, riluzol[109], acyclovir[100], vitamin D[110,111], IFNβ and glatiramer acetate[104] in relapsing–remitting multiple sclerosis and fingolimod in primary progressive multiple sclerosis[93]) is associated with minimal or no change in NfL levels.

With the introduction of high-efficacy multiple sclerosis therapies (natalizumab, ocrelizumab, ofatumumab, alemtuzumab and haematopoietic stem cell transplantation) that almost completely suppress acute inflammatory activity but have less impact on progression,

interest increased in whether NfL can predict the course of multiple sclerosis progression. Evidence is now accumulating that NfL levels are not reduced to normal values by these high-efficacy therapies, and the remaining elevation could reflect subclinical disease activity that leads to continuous neuronal damage and eventually disease progression[112]. In fact, studies have shown that NfL levels are quantitatively associated with the future risk of worsening disability and with MRI features of neurodegeneration such as optic nerve, brain, and spinal cord atrophy[4,16,109,113–119] and chronic white matter inflammation[120]. NfL *Z*-scores are elevated 1–2 years before but not concurrently with disability worsening independent of relapse activity, underlining the potential for NfL levels to predict the disease course more effectively than standard clinical and MRI assessments[121]. This association between elevation of NfL levels and an increased risk of disability worsening could form the basis of a new treatment goal; that is, achieving physiological NfL levels, reflecting optimal suppression of subclinical disease activity to minimize future disability.

Given that NfL levels specifically reflect neuronal damage, combining this measure with biomarkers that indicate other pathophysiological features of progression could provide additional diagnostic power. Indeed, a head-to-head comparison of NfL and GFAP (the intermediate filament equivalent of NfL in astrocytes) has demonstrated that the combination of these two biomarkers outperforms the ability of NfL alone to predict long-term disability[122]. In clinical practice, the primary uses of NfL in multiple sclerosis are likely to be quantification of clinical and subclinical disease activity and the monitoring of drug response[123]. For the prediction of long-term outcomes, the combination of NfL and GFAP might have higher predictive power to anticipate progression[122].

NMOSD and MOGAD. Elevated CSF levels of NfH in NMOSD were first reported in Japanese people with the disease in 2007 (ref. 124). Subsequently, increased serum levels of NfL have been described in NMOSD and MOGAD[125,126], and multiple studies support the clinical use of NfL in multiple sclerosis, NMOSD and MOGAD (Supplementary Table 1). As in multiple sclerosis, CSF and serum levels of NfL are highly correlated[127], are higher during clinical exacerbation than during remission[125,127–129], and correlate with clinical disease severity[125,127,130]. Comparison of neurofilament levels between the two diseases has shown significant differences at the group level but also strong overlap[124,129,130], such that these differences are not meaningful for differential diagnosis at the individual level. The same is true for the difference in levels between NMOSD and multiple sclerosis[127]. In essence, therefore, NfL and NfH are not diagnostic of NMOSD or MOGAD but are markers of disease activity and disease progression. In contrast to multiple sclerosis, little is known about whether NfL is a prognostic marker in NMOSD and MOGAD. The current evidence points towards GFAP being a more appropriate biomarker than NfL for longitudinal monitoring of NMOSD[128].

Neurodegenerative dementias

Evidence from many studies supports clinical use of neurofilaments in various neurodegenerative dementias, including Alzheimer disease, Parkinson disease dementia, dementia with Lewy bodies and frontotemporal dementia (FTD; Supplementary Table 2). We discuss the clinical utility for each of these conditions below.

Alzheimer disease. At the group level, people with Alzheimer disease can be differentiated from healthy control individuals based on NfL levels in CSF[73,131] and blood[132,133] with fair accuracy (area under the curve (AUC) ~0.7). However, NfL levels seem to be independent of cerebral amyloidosis but associated with neurodegeneration, especially of white matter axons[134–137]. The almost ubiquitous increase of NfL in neurodegenerative diseases[73,138] limits its ability to differentiate Alzheimer disease from other causes of dementia. Nevertheless, measures of NfL are relevant in specific clinical contexts. For example, NfL levels can differentiate primary progressive aphasia associated with Alzheimer disease from that associated with semantic variant FTD, which is associated with higher NfL levels[139]. Very high levels of NfL in Creutzfeldt–Jakob disease (CJD) also make it possible to differentiate this disease from Alzheimer disease[140] and rapidly progressive Alzheimer disease[141].

In autosomal dominant Alzheimer disease, changes in blood levels of NfL seem to precede the first clinical manifestations by more than a decade and also predict clinical progression within the Alzheimer disease continuum[142–144]. Therefore, NfL can be used to predict and follow the evolution of Alzheimer disease in people with a genetic risk for this disease such as people with Down syndrome[145] or with autosomal dominant inherited Alzheimer disease[142].

Parkinson disease dementia and dementia with Lewy bodies. Progression of Parkinson disease is often associated with cognitive decline and, in late stages, dementia[146]. However, during the initial stages of Parkinson disease, NfL levels are similar to those in healthy control individuals[138]. Consequently, CSF and blood levels of NfL have no early diagnostic value in Parkinson disease but could inform prediction of progression to dementia[146,147] and help to differentiate classical Parkinson disease from atypical parkinsonian disorders such as progressive supranuclear palsy (PSP), corticobasal degeneration and multiple system atrophy (MSA)[148]. This differential diagnosis is important because these pathologies differ greatly in their management, treatment and prognosis[149].

Blood NfL levels are also increased in dementia with Lewy bodies. NfL levels are similar to those in Alzheimer disease and lower than those in FTD, limiting their use for differential diagnosis but offering opportunities for the measurement of treatment responses[150,151].

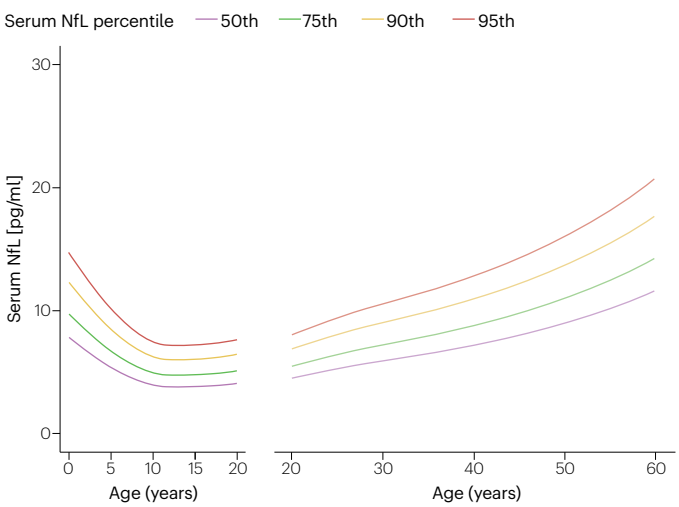

Fig. 3 | Age-related percentiles for serum NfL. Curves were generated by the use of a generalized additive model for location, scale and shape to model the non-linear association of serum neurofilament light chain (NfL) concentration with age in healthy individuals aged 0–20 years (left) and 20–65 years (right)[16,97]. Reproduced with permission from ref. 97, Elsevier.

Frontotemporal dementia. FTD — the third most common cause of neurocognitive impairment after Alzheimer disease and dementia with Lewy bodies — encompasses a heterogeneous group of neurodegenerative diseases characterized by behavioural, executive, and language deficits and caused by various underlying proteinopathies — aggregates of tau (~47.5%), inclusions of TAR DNA-binding protein 43 (TDP43; ~47.5%) or inclusions of the nuclear DNA/RNA-binding protein FUS (<5%)[152]. Approximately 30% of cases are familial, caused by mutations in *MAPT* (tau pathology), *C9Orf72* (TDP43 pathology) or *GRN* (TDP43 pathology)[153]. The most common form of FTD is behavioural variant FTD (bvFTD), which primarily affects personality, social behaviour and executive function. Semantic variant FTD affects language, corresponding to primary progressive aphasia.

Many studies have investigated the diagnostic performance of NfL in FTD[154]. In clinical practice, differential diagnosis of FTD and primary psychiatric disorders (PPD) is challenging owing to the overlap of some behavioural symptoms with bvFTD. In this context, several studies have demonstrated that NfL concentrations in CSF and blood are higher among people with FTD than among people with PPD[155,156], with sensitivity and specificity values above 80%. Thus, as specified in current international recommendations[157], measurement of NfL in CSF or blood could be used in practice for the differential diagnosis of bvFTD and PPD[158], provided that validated thresholds could be defined. Making this differential diagnosis is key for patient management, which differs greatly between bvFTD and PPD.

In the context of FTD, blood levels of NfL differ according to the underlying mutation — they are highest in people with *GRN* mutations and lowest in people with *MAPT* mutations[159]. These levels rise in the presymptomatic stages of FTD, and the timing of preclinical increases differs with the underlying mutation. For example, increases begin ~30 years before symptom onset with *C9orf72* mutations, ~15 years with *GRN* mutations and at around the time of symptom onset with *MAPT* mutations[160]. The extent of increases in NfL in the presymptomatic stage has significant prognostic value with respect to conversion to clinical symptomatic disease (HR 6.7 for cross-sectional increases (baseline NfL *Z*-score ≥0.7); HR 13 for longitudinal NfL changes (annualized change ≥1.4)) in people with mutations[161]. Based on this evidence, increases in blood levels of NfL have been proposed as an inclusion criterion in trials that involve people with FTD who are at high risk of decline and as an additional biological outcome measure[160].

Amyotrophic lateral sclerosis

Upper motor neurons and lower motor neurons contain substantial amounts of neurofilament in their long and large myelinated axons, respectively. Consequently, levels of NfL and NfH in CSF and blood increase greatly in people with ALS compared with healthy control individuals[73,101,162–173]. Only CJD and HIV-associated neurocognitive disorder are associated with such high levels of neurofilaments[73,140,166,172,174,175]. Nevertheless, whether the degree of neurofilament elevation in ALS mostly reflects the extent of upper motor neuron loss and/or lower motor neuron loss is still a matter of debate[169,170,172,176,177]. Indeed, a correlation observed between NfL levels in CSF or blood and degeneration of the corticospinal tract (that is, upper motor neurons) assessed by diffusion tensor imaging[165,169] has not been replicated in large cohorts of people with ALS[166,172]. Reduced levels of neurofilament proteins in spinal cord tissue and increased concentrations in the CSF of people with ALS could reflect neurofilament protein loss from degenerating neurons[178]. However, on the other hand, a massive elevation in neurofilament levels in CSF and blood in the early symptomatic phase of ALS is followed by relatively stable protein levels later in the disease course[164,167,172,176,179–181] despite relatively low levels of other axonal and neuronal biomarkers, such as tau or β-synuclein[163,175], suggesting that the increase in neurofilament levels is not due to a simple loss of neurofilament proteins from neurons.

Despite the uncertainty over the source of neurofilaments in ALS, CSF and blood levels of neurofilaments can distinguish ALS from its mimics with a sensitivity and specificity of up to 80%, and this distinction is likely to be one of the most important clinical applications of neurofilament measures[164,166,168,170–172,176,177,182]. In this comparison, CSF levels of NfL and NfH and serum levels of NfL had high diagnostic accuracy and slightly outperformed serum NfH[171]. Furthermore, levels of NfL in CSF and serum maintained high diagnostic accuracy independent of whether the time from symptom onset to diagnosis was more or less than 6 months, a finding that is clinically relevant given that diagnosis of ALS is commonly delayed[182].

Given this diagnostic accuracy, use of these biomarkers could identify the first signs of neurodegeneration, enabling early initiation of a therapeutic intervention. In this context, several cross-sectional and longitudinal studies have been conducted to explore the role of CSF and blood neurofilament levels as candidate biomarkers of proximity to symptom onset. However, findings have been mixed; some studies have demonstrated a significant increase in blood levels of NfH and NfL 1–3.5 years before symptom onset or diagnosis in sporadic and genetic ALS[179,183–185] whereas others have not identified any difference in biomarker levels between presymptomatic and symptomatic phases[177,178,186]. These discordant results might reflect the interindividual and genetic heterogeneity of ALS or the different study designs[187].

With respect to prognosis in ALS, CSF and blood levels of NfL and NfH have been strongly associated with survival and disease progression rate[164–170,172,176,181,188–190], and this association has been confirmed on multiple analytical platforms[67]. Even if the ALS Functional Rating Scale–Revised remains the most widely used primary outcome measure in ongoing clinical trials, blood levels of NfL could provide a sensitive pharmacodynamic outcome measure with the potential to improve patient stratification and trial power, thereby reducing the required sample size, trial duration and individual burden for participants[180,181,191–193]. Indeed, neurofilament levels have been included as exploratory outcomes or secondary end points in ongoing trials; most notably, a longitudinal decline in neurofilament levels was seen during antisense oligonucleotide therapy for ALS associated with *SOD1* mutations[9,194,195]. Based on these findings, the FDA has granted accelerated approval of tofersen[11]. In another antisense oligonucleotide trial, an increase in blood levels of NfL, even in the absence of clinical symptoms, has been used as an inclusion criterion[196]. Nevertheless, evidence suggests that NfL concentrations are not useful for monitoring the therapeutic effect of the classic therapeutic agent riluzole[172,197].

Even though neurofilament levels are not included in the current diagnostic criteria for ALS[198], several centres are adopting use of the marker to improve clinical diagnosis, especially in the most complex cases[199]. The evidence collected to date reveals prognostic, monitoring and possible pharmacodynamic roles of the marker and supports its application as an end point in ongoing clinical trials (Supplementary Table 3).

Cerebrovascular disease

Serum, plasma and/or CSF levels of NfL and NfH are elevated in various subtypes of stroke[2,200–204]. Data on haemorrhagic stroke is scarce[203], but

studies of ischaemic stroke have consistently shown that blood levels of NfL are higher in people with ischaemic infarction than in those with a transient ischaemic attack[205,206]. Similarly, younger (median age 42 years) people (in whom the likelihood of concomitant chronic brain changes is low) with cervical artery dissections at the time of presentation with stroke had significantly higher serum levels of NfL than did people with transient ischaemic attack or isolated local symptoms[207]. The high sensitivity of blood NfL levels to acute ischaemic brain tissue damage has also been confirmed by studies that have demonstrated elevated levels in people with recent, small subcortical infarcts related to small vessel disease (SVD)[200].

Blood levels of NfL and NfH change over time after stroke onset[2,200–204,208]. In the days after onset, NfL concentrations increase continuously, peak after 2–3 weeks and stay elevated for 3–6 months[204]. These dynamics need to be considered when interpreting NfL levels observed in different studies as the timing of these ranges from hospital admission to >1 year after stroke. The concentration of NfL in the blood is also associated with stroke severity as it correlates with clinical scores and, to a lesser degree, the extent of tissue damage visible on brain imaging (CT and MRI)[201–203,205–207,209,210]. However, given the temporal dynamics of NfL in the acute phase, the association with infarct size is dependent on the time point of blood sampling. NfL levels measured in the hyperacute phase have limited correlation with infarct size, whereas levels at 1 week more robustly reflect brain tissue damage[201]. In intracerebral and subarachnoid haemorrhage, blood levels of NfL have been associated with haemorrhage volume[203].

Blood levels of NfL correlate with functional neurological outcome (modified Rankin scale score) and cognitive status after stroke[201–203,209,210]. Therefore, this marker could serve as a predictor of treatment response and functional outcome in people with stroke who undergo endovascular therapy for anterior circulation large vessel occlusion[211]. Moreover, accumulating evidence suggests that blood levels of NfL are predictive of vascular and all-cause mortality in people with cerebrovascular disease[203,212].

In cerebral SVD, which is an important cause of ischaemic and haemorrhagic stroke and of cognitive dysfunction and dementia, blood levels of NfL are related to the burden of disease assessed with brain MRI markers such as lacunes, white matter hyperintensities, microbleeds and ultrastructural tissue changes on diffusion tensor imaging[213–215]. Such associations have been demonstrated not only in age-related, sporadic SVD but also in the hereditary form known as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy[213,216,217], indicating that NfL levels truly reflect SVD pathology. Further evidence suggests that NfL in the blood is a valuable biomarker to monitor the disease as it has been related to the progression of SVD lesions, the future occurrence of stroke and new (often clinically silent) lesions on follow-up imaging[200,213,214,217]. Furthermore, blood levels of NfL have also been identified as a promising biomarker of covert brain infarction in the perioperative and postoperative period in various surgical procedures[218,219]. High levels of NfL in the blood measured at 1–10 days (median 4 days) after SVD-related stroke symptom onset have also been shown to indicate that more destructive lesions will develop in the long term (recent small subcortical infarcts that cavitate into lacunar infarcts)[220] and predicts cognitive decline and dementia during long-term follow-up[213,214,221]. Notably, blood levels of NfL can also predict the long-term risk of stroke in people without stroke in population-based studies[222] and in people with diabetes mellitus (significantly increasing the power of the Framingham Stroke Risk Score to predict incident stroke)[223].

In summary, blood levels of NfL are elevated in stroke and SVD, reflect disease severity, and could indicate progressive cerebrovascular disease. Furthermore, NfL could be a clinically relevant prognostic marker of functional neurological disability, incident cerebrovascular lesions, cognitive dysfunction and mortality. However, NfL has not yet been implemented as a clinical biomarker in people with cerebrovascular disease. Future studies of NfL in the context of cerebrovascular disease also need to account for factors that influence NfL levels such as age, renal function, presence of atrial fibrillation and BMI[14,224], which are likely to be particularly relevant and prevalent in this population.

Traumatic brain injury

One of the most important disease mechanisms in TBI is rotational injury to the brain tissue[225]. This injury type often results in axonal disruption and release of intra-axonal proteins, such as $NfH_{476–986}$, $NfH_{476–1026}$ and NfL, into the brain interstitial fluid, CSF and blood[46]. The dynamics of NfL changes after TBI are similar in CSF and serum, though the fold change seems to be slightly smaller in the serum than in CSF[226]. In people with acute moderate-to-severe TBI, serum NfL concentrations correlate with the ventricular CSF concentration and enable people with TBI to be distinguished from healthy people with an AUC of 0.98–1.0 (ref. 227). Serum levels of NfL measured within 48 h of injury have been shown to distinguish people with abnormal head CT findings from those with normal head CT findings with high accuracy[228–230].

Serum levels of NfL also seem to be altered for long periods after TBI. In people with a history of mild, moderate or severe TBI who were followed up with serial blood samples from 30 days to 5 years after injury, serum NfL levels enabled patients with mild, moderate or severe TBI to be distinguished from each other and from individuals without a history of TBI[231]. Similarly, serum NfL concentrations measured as long as 1 year after injury correlated with global brain volumes measured at the same time point and with diffusion tensor imaging measures of white matter integrity[231].

Further evidence for the value of neurofilaments in TBI comes from studies of sports-related injury. Measurement of NfL levels in professional ice hockey players with acute concussion revealed that levels in serum were higher among players with a delayed return to play[232]. Furthermore, serum levels of NfL performed better than plasma levels of total tau in distinguishing athletes with concussion from those without[232]. In the context of subacute and chronic repetitive head impacts, measurement of NfL concentrations in the blood of boxers 7–10 days after a bout showed that levels were increased compared with healthy individuals; these levels decreased after 3 months of rest but remained higher than in the control group[233]. Finally, in a study of professional athletes with a history of repetitive sports-related concussion who underwent lumbar puncture and blood assessment months after their most recent concussion, concentrations of NfL in the serum correlated with those in the CSF and could distinguish athletes with a history of concussion from those without with high accuracy[231].

Altogether, the evidence shows that CSF and plasma levels of NfL are promising biomarkers to quantify axonal injury in TBI. The conclusion of a systematic review published in 2022 was that, although the clinical usefulness of blood NfL for acute diagnosis of mild TBI is uncertain, the biomarker 'shows promise' for the prognosis of complications of mild TBI, neuroimaging findings and recovery when measured during the first days to weeks after injury[234]. Important to note is that NfL is a relatively slow biomarker in mild TBI — the maximum increase occurs as late as 2–3 weeks after injury, and the effective half-life of the biomarker after reaching its peak value is 2–3 months[226,235]. One limitation

of NfL as a biomarker of TBI is its expression in peripheral nerves[2,236] as trauma to extracerebral tissue could result in an increase in blood levels of NfL if peripheral nerves are injured. Whether this possibility really affects the diagnostic performance of NfL is unclear, and more research on CNS-specific neurofilament proteins, such as α-internexin, in the context of TBI would help to resolve this uncertainty[2]. Blood levels of NfL are currently not used in clinical practice in TBI but the availability of standard laboratory instruments will enable rapid and reliable results to be obtained facilitating its implementation.

Hypoxic–ischaemic brain injury

The first evidence that levels of NfL and NfH increase after cardiac arrest as a correlate of subcortical neuronal injury in hypoxic–ischaemic brain injury came from studies of small cohorts[237–240] and focused on analysis of CSF samples or use of standard ELISA methods to analyse blood samples. The introduction of highly sensitive Simoa technologies has enabled analysis of the blood compartment, and several studies using this approach have demonstrated that NfL levels are strongly associated with survival and long-term neurological outcomes after cardiac arrest[241–250].

In general, blood levels of NfL increase within the first 24 h after cardiac arrest and the increased levels persist for days to months, although more studies with sampling time points beyond a few weeks would be informative[251]. In single-centre and multi-centre studies, adults and children with unfavourable neurological outcomes had higher levels of NfL at several time points or a greater change from baseline than those with favourable outcomes[242–245,247,248,250–257]. Indeed, meta-analyses indicate that high blood levels of NfL at 48 h after cardiac arrest predict poor neurological outcomes with an AUC of 0.92–0.96 (refs. 255,257,258). Sub-analyses of populations who were treated with targeted temperature management and who had an out-of-hospital cardiac arrest produced similar results[255], though the predictive value of the biomarker was lower after intra-hospital cardiac arrest[251]. Furthermore, the absolute values of NfL and its early kinetics seem to accurately differentiate severe hypoxic–ischaemic brain injury from other causes of poor outcomes after cardiac arrest[252].

Current European and USA guidelines for neurological prognostic evaluation after cardiac arrest recommend a multimodal diagnostic approach, including head CT, electroencephalography (EEG) and measurement of neuron-specific enolase levels in blood[259,260]. However, several studies have shown that the prognostic value of blood NfL in this context is higher than that of other blood biomarkers (for example, neuron-specific enolase, S100 and tau), clinical tests, neuroimaging and neurophysiological investigations[242,246–248,255,261,262]. Moreover, the extent of brain injury as assessed by blood levels of NfL correlated with EEG and head CT findings — NfL levels were high in people with highly malignant EEG patterns and reduced ratios of grey matter to white matter[262–264]. For the prediction of poor clinical outcomes after cardiac arrest, applying a high cut-off for blood levels of NfL results in very high specificity and a low percentage of false positives but at the cost of lower sensitivity and false negatives[242]. Inclusion of NfL in multivariable models or algorithms that also include clinical scores and diagnostic parameters could increase the sensitivity and specificity of the models[248,250].

NfL in the blood shows promise as a prognostic marker after cardiac arrest. We suggest that its addition to future guidelines and clinical algorithms could widen its application, enabling more accurate and homogeneous identification of patients with good and poor outcomes after cardiac arrest[241].

Parkinson disease

Diagnosis of Parkinson disease remains challenging owing to the large clinical overlap with so-called atypical parkinsonian syndromes, such as MSA, PSP and corticobasal syndrome[265]. With respect to diagnostic biomarkers, seed amplification assays, including the Real-Time Quaking-Induced Conversion assay, have very high sensitivity and almost complete specificity for the diagnosis of Parkinson disease and other synucleinopathies in vivo[266,267], but measurement of neurofilaments in biofluids have provided interesting insights.

Since the late 1990s, cross-sectional and longitudinal studies have demonstrated that CSF NfL and NfH levels are higher among patients with atypical parkinsonian syndromes than among those with Parkinson disease[267–272]. These findings have been replicated in blood samples[267,272–274] and validated by comprehensive meta-analyses[73,275]. These analyses indicated that CSF and blood levels of NfL could distinguish between Parkinson disease and atypical parkinsonian syndromes with an area under the curve of 0.94 and 0.87, respectively[275]. Possible explanations for these findings are that neurodegeneration is faster and more extensive in atypical parkinsonian syndromes than in Parkinson disease and that subcortical large myelinated axons are more prominently involved in atypical parkinsonian syndromes than in Parkinson disease[272,275].

Combined assessment of CSF or blood NfL with Real-Time Quaking-Induced Conversion yielded very-high-accuracy discrimination between Parkinson disease and atypical parkinsonian syndromes or MSA. For the diagnosis of Parkinson disease, NfL levels needed to be lower than a chosen cut-off and α-synuclein seeds needed to be present[267,276]. Moreover, blood and/or CSF levels of NfL correlated with disease severity as assessed by motor or cognitive scores[267,272,277–285], the future rate of neurological and functional progression in Parkinson disease[274,278,280–282] and atypical parkinsonian syndromes[274], striatal dopamine transporter uptake in Parkinson disease[277,286], and specific regional atrophy in MSA and PSP[278,280]. Of note, NfL levels were associated with cognitive function in Parkinson disease at follow-up whether tested alone or in combination with other biomarkers[147,279,281,282,287]. Furthermore, high NfL levels were associated with shorter survival in Parkinson disease, PSP and MSA[267,277,280–283]. NfL levels were also elevated in the blood or CSF of people with prodromal α-synucleinopathies (that is, pure autonomic failure and rapid eye movement behavioural disorder) to a similar or even higher degree than in people with clinical Parkinson disease[267,272] and predicted phenoconversion to Parkinson disease, MSA or dementia with Lewy bodies[288–290].

Though NfL is not yet a routine clinical marker in parkinsonian syndromes, it could be a sensitive initial test for the differential diagnosis of these syndromes before more specific tests, such as seed amplification assays, are performed. Moreover, NfL can easily be implemented in prognostic evaluation, tracking of clinical progression and monitoring of therapeutic efficacy in Parkinson disease and atypical parkinsonian syndromes (Supplementary Table 4).

Huntington disease

In Huntington disease, highly sensitive assays for NfL in the blood have enabled evaluation of NfL as a robust marker of neurodegeneration and of the burden of subclinical neurodegeneration in people who are asymptomatic but have CAG repeat expansions that will ultimately lead to the formation of mutant Huntingtin protein. In a study of 201 people with *HTT* CAG repeat expansions, plasma levels of NfL were 2.6-fold higher in participants with Huntington disease, even in the early premanifest phase (predicted onset >10 years away) than in people without mutations

in *HTT*[291]. Elevated NfL levels were associated with clinical worsening assessed with severity scales such as the symbol digit modalities test and the Unified Huntington's Disease Rating Scale[291]. Moreover, NfL concentrations correlated with the extent of localized and global MRI atrophy[291]. As expected, NfL levels increased during follow-up at a faster rate in people with *HTT* mutations than in healthy controls[291]. A higher number of CAG repeats was associated with higher NfL concentrations and a faster rate of NfL increase[291]. Participants with low NfL concentrations were more likely to remain at the premanifest stage without noticeable abnormalities on the Unified Huntington's Disease Rating Scale[291].

Several other studies have replicated the finding that concentrations of NfL are increased in people with *HTT* mutations in the premanifest phase[292,293], even in individuals who are >24 years from clinical onset[294], and have replicated the correlations between NfL levels and years to onset[295,296], clinical severity[297,298], and MRI atrophy[297]. Notably, when compared with CSF levels of NfL and the mutant huntingtin protein, plasma levels of NfL had the strongest association with clinical severity and could differentiate between individuals in the premanifest phase and those in the manifest phase with an AUC of 0.93 (ref. 297) and 0.95 (ref. 298), respectively. Similarly, NfL levels had a high accuracy for distinguishing between premanifest Huntington disease and juvenile-onset Huntington disease and between people with premanifest Huntington disease and healthy individuals (AUC 0.90 and 0.96, respectively)[292]. Including NfL in clinical studies as a treatment outcome parameter would substantially increase the statistical power and reduce the sample sizes needed to a more considerable extent than use of mutant huntingtin protein in CSF alone[297,298].

Peripheral neuropathies

The first peripheral neuropathy in which CSF and blood levels of neurofilaments were successfully quantified was Guillain–Barré syndrome (GBS). In GBS, high NfL levels are seen in both CSF and serum[299–303]. High levels are associated with disease severity and axonal variants[304–306], and have been identified as independent prognosticators of poorer outcomes in adults and children[301,305,307]. Combining measures of neurofilaments in CSF and blood could enable conclusions to be drawn about whether neurofilaments originate from the CNS or the PNS[304].

These observations in GBS are analogous to observations in critical illness neuropathy[308], giant axonal neuropathy[309], chronic inflammatory demyelinating polyneuropathy[302,310–313], multifocal motor neuropathy[314], chemotherapy-induced peripheral neuropathy[312,315,316], vasculitic neuropathy[317], paraproteinaemia-related demyelinating polyneuropathy[314], Charcot–Marie–Tooth disease[318] and inherited neuropathies[319–321]. Higher blood levels of neurofilaments indicate more severe pathology, axonal damage and poorer outcomes in these conditions, and these levels are reduced in people who respond to treatment[313]. People with symptomatic hereditary transthyretin amyloidosis have particularly high blood levels of NfL[321,322]. In this disease, blood levels of NfL could have a role as a presymptomatic marker in families with disease-causing mutations[321], as a prognostic marker of clinical outcome[321,323] and as a marker of response to disease-modifying therapies[322,324].

Overall, evidence suggests that neurofilament concentrations are elevated and provide prognostic information in most acute and chronic neuropathies regardless of cause. Furthermore, from studies in a limited set of conditions, neurofilament levels also seem to reflect treatment responses and could be used for monitoring during therapy. However, the evidence base for qualitative differences in levels and temporal dynamics of the various neurofilament isoforms is less robust than that in more prevalent CNS conditions such as multiple sclerosis.

Spinal muscular atrophy

Elevated CSF and blood levels of neurofilaments are observed in children with SMA[325–328]. Greater neurofilament levels are associated with younger-onset and more severe forms of the disease; the highest levels are seen in individuals with two or more copies of *SMN2* (refs. 328–330). Impressive reductions in both CSF and serum NfL are seen in association with approved and emerging gene therapies in different forms of SMA[327,328,330–332]. Though these reductions in NfL are a valuable clinical trial end point and future regulatory threshold, the clinical value of these treatment-related reductions remains unclear. Pharmacokinetic studies from clinical trials of the antisense oligonucleotide therapy nusinersen in infantile-onset SMA indicate dose-related reductions in NfH levels as well as clinical improvements[333]. However, in a study of people with type 3 or 4 SMA, which have later onset and milder symptoms, CSF levels of NfH and NfL were only marginally different between those who were treated and those who were not[334].

Other neurological diseases

A variety of studies have investigated neurofilament concentrations in a range of other neurological diseases or conditions with neurological involvement. In rare genetic ataxias[335–338], some evidence suggests that neurofilament levels could be used as a marker of treatment response[339]. Blood NfL levels are modestly increased after a single self-limited tonic-clonal seizure[340] and can serve as a biomarker of acute neuronal injury in status epilepticus[341,342]. Autoimmune encephalitides are, to varying degrees, associated with elevated levels of NfL; levels are higher in anti-leucine-rich glioma-inactivated protein 1 (LGI1)-mediated disease than in anti-NMDA receptor encephalitis[343,344]. Though the prognostic value of NfL in anti-NMDA receptor encephalitis is unclear[343,345], elevated blood levels enabled this condition to be differentiated from first-episode psychosis[345]. Blood NfL concentrations can be used as a marker of brain injury in Wilson disease in addition to the clinical and neuroimaging disease severity scales[346]. Prion diseases, such as sporadic CJD, are characterized by highly elevated levels of NfL[140,174,175], with more moderately increased concentrations in more slowly progressing forms, for example, fatal familial insomnia[174,347].

Infections characterized by neurological involvement are a large and heterogeneous group of conditions in which neurofilaments have been studied. Among the most studied are HIV-associated neurological complications, including dementia, in which neurofilament levels have potential as a marker of treatment response[5,348]. Since 2020, an increasing number of studies have explored the potential of NfL levels in the blood as a prognostic marker in COVID-19 (refs. 349–352). Collectively, various forms of neurofilaments, particularly NfL, have shown potential as differential diagnostic, prognostic and/or treatment response markers across a spectrum of conditions.

Due to the inherent non-specificity of NfL for a particular disease aetiology, elevated levels of NfL in an individual can be the result of neuro-axonal injury from multiple concurrent clinical and subclinical pathologies. This possibility has potential value in identifying the existence of multiple pathologies that could have otherwise been missed but also complicates the interpretation of one-off NfL measurements and underscores the need for the clinical context to be considered. Integration of neurofilament measures with other emerging biomarkers with greater diagnostic specificity (for example, isoforms of amyloid-β in the case of Alzheimer pathology)[353] could help to refine the application of neurofilament as a biomarker, perhaps even enabling the relative contributions of concurrent pathologies to neuro-axonal injury.

Conclusions and future outlook

Neurofilament proteins, and particularly NfL, have become one of the most intensely studied blood-based biomarkers in neurological and neuropsychiatric diseases[1,10,354]. Their capacity to reflect, in real time, neuro-axonal injury as the substrate of persistent disability has attracted many researchers from basic, translational and clinical sciences to explore the potential of neurofilaments for use in epidemiological studies, in the diagnostic work-up of individuals and as an end point in clinical trials. One particular advantage of NfL is that its levels in plasma or serum reflect neuronal damage as effectively as its levels in CSF, enabling minimally invasive longitudinal monitoring of the biomarker and giving it great potential for clinical application.

Use of NfL in clinical practice has become closer to reality owing to the establishment of large reference databases for physiological serum levels in adults and children, which enable more precise interpretation at the individual level rather than just at the group level. Nevertheless, differences between serum and plasma preparations preclude the use of reference values for both matrices equally. Similarly, the various high-sensitivity assay platforms that are expected to be launched for clinical use generate different absolute values of NfL, which precludes comparison of data generated with different platforms until reference 'exchange rates' are established. Current evidence suggests that serum samples are preferable to plasma samples for measurement of NfL in large-scale clinical laboratories as they provide the same biological information but the production of serum samples is simpler and more easily standardized.

Like any biomarker, NfL levels cannot be interpreted outside of the clinical context and must be used to address a specific question. Furthermore, these levels are specific only for neuronal damage and cannot be used to diagnose a nosological disease entity owing to the extent to which levels overlap across diseases[73]. In addition, the interpretation of NfL levels differs according to disease stage or state; for example, it can be a diagnostic type biomarker in the preclinical phase of neurodegenerative processes whereas, at later stages, it is most useful as a prognostic marker and for monitoring of disease progression and treatment response. In this context, the FDA approval of tofersen based on longitudinal reductions in blood levels of NfL during therapy in *SOD1*-associated ALS — that is, as a treatment response marker — signals a paradigm shift in the value of biomarkers in the regulatory approval of investigational new drugs. Many of the diseases discussed above are currently without effective therapies but are associated with increased levels of NfL, which could be a viable efficacy measure for the development of novel therapeutic approaches to all of these diseases. Of the neurofilament proteins, NfL holds the most promise for smaller and shorter clinical trials as NfL assays enable detection of drug effects earlier and with higher sensitivity and accuracy. Given that levels of NfL increase years before clinical symptoms in primary neurodegenerative diseases, measurement of NfL could provide a window of opportunity for early therapeutic intervention when damage to the nervous system is limited.

Standardization and cross-comparability of measurements taken with current and emerging analytical platforms, which require reliable reference ranges, will be key steps in moving towards broader clinical use of NfL. Addressing these remaining challenges will position neurofilaments, and especially NfL, as an important tool in precision and personalized medicine for many neurological diseases over the coming years.

Published online: 12 April 2024

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Acknowledgements

C.E.T.'s research is supported by the European Commission (Marie Curie International Training Network, grant agreement No. 860197 (MIRIADE), Innovative Medicines Initiatives 3TR (Horizon 2020, grant No. 831434), European Platform for Neurodegenerative Diseases (IMI 2 Joint Undertaking (JU), grant No. 101034344) and European Union Joint Programme – Neurodegenerative Disease Research (JPND; bPRIDE), National MS Society (Progressive MS alliance), Alzheimer Association, Health Holland, the Dutch Research Council (ZonMw), Alzheimer Drug Discovery Foundation, The Selfridges Group Foundation, and Alzheimer Netherlands. C.T.E. is a recipient of ABOARD, which is a public–private partnership receiving funding from ZonMw (#73305095007) and Health Holland, Topsector Life Sciences & Health (PPP-allowance; #LSHM20106). C.E.T. is recipient of TAP-dementia, a ZonMw-funded project (#10510032120003) in the context of the Dutch National Dementia Strategy. S.L. is supported by the Fondation pour la Recherche Médicale (FRM), the Claude Pompidou Foundation, H2020 Marie Skłodowska-Curie Actions MIRIADE project, and the European Metrology Programme for Innovation and Research Neuromet2 project. M.O. was supported by the JPND networks Genfi-

Prox (01ED2008A) and bPRIDE (01ED2001), the German Federal Ministry of Education and Research (FTLDc 01GI1007A, Moodmarker 01EW200), the EU (MIRIADE 860197, FAIR-PARK II 633190), the German Research Foundation/DFG (SFB1279), the Foundation of the State Baden-Württemberg (D.3830), Boehringer Ingelheim Ulm University BioCenter (D.5009) and the Thierry Latran Foundation. F.P. is funded by the Swedish MRC (grant no. 2020-02700), the Knut and Alice Wallenberg Foundation, and the Swedish Brain Foundation. S.B. is supported by the Deutsche Forschungsgemeinschaft (DFG, SFB CRC-TR-128 and CRC-TR-355) and the Hermann and Lilly Schilling Foundation. T.G. acknowledges research support from the Austrian Science Fund and the Austrian Neurological Society. S.A.-R. received support from the Medical Faculty of Martin-Luther-University Halle-Wittenberg (Clinician Scientist-Programm No. CS22/06). S.T. is supported by the MS Canada for Postdoctoral Fellowship. H.T. acknowledges research support by DMSG (German Multiple Sclerosis Society), MWK-BW (Ministry of Science, Research and Arts of the State Baden-Württemberg), University of Ulm, and Chemische Fabrik Karl Bucher. A.P. acknowledges support from the National Institute of Health and Care Research Biomedical Research Centre at Moorfields Eye Hospital and the UK Department of Health. K.B. is supported by the Swedish Research Council (#2017-00915 and #2022-00732), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721 and #AF-968270), Hjärnfonden Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF agreement (#ALFGBG-715986 and #ALFGBG-965240), the JPND (JPND2019-466-236), the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495), and the Alzheimer's Association 2022-2025 Grant (SG-23-1038904 QC). H.Z. is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2022-01018 and #2019-02397), the European Union's Horizon Europe research and innovation programme under grant agreement No. 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Bluefield Project, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 860197 (MIRIADE), the JPND (JPND2021-00694), the National Institute for Health and Care Research UCL Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at UCL (UKDRI-1003). D.L. is supported by the International Progressive MS Alliance (grant PA-2002-36227). J.K. is supported by the Swiss MS Society, the Swiss National Research Foundation (320030_189140/1 and 212534/1), the University of Basel and the International Progressive MS Alliance. Parts of the manuscript have been generated by members of the BioMS-eu consortium.

Author contributions

All authors contributed to all aspects of the manuscript.

Competing interests

M.K. has received funding for travel and speaker honoraria from Bayer, Biogen Idec, Merck, Novartis, and Teva Pharmaceutical Industries and serves on scientific advisory boards for Biogen Idec, Merck Serono, Novartis, and Roche outside the submitted work. C.E.T. has collaboration contracts with ADx Neurosciences, Eli Lilly, and Quanterix and has performed contract research or received grants from AC Immune, Axon Neurosciences, BioConnect, Bioorchestra, Brainstorm Therapeutics, Celgene, EIP Pharma, Eisai, Fujirebio, Grifols, Instant Nano Biosensors, Merck, Novo Nordisk, PeopleBio, Roche, Siemens, Toyama, and Vivoryon. She is editor of *Alzheimer Research and Therapy*, and serves on the editorial boards of *Medidact Neurologie*/Springer and *Neurology: Neuroimmunology & Neuroinflammation*. S.L. has served as a consultant or on advisory boards for Biogen, Fujirebio-Europe, Lilly, Roche Diagnostics and Shimadzu. M.O. has given scientific advice to AXON, AviadoBio, Biogen, Fujirebio and Roche. F.P. has received research grants from Janssen, Merck KGaA and UCB, has received fees for serving on Data Monitoring Committees (DMC) in clinical trials with Chugai, Lundbeck and Roche, and has prepared an expert witness statement for Novartis. T.Z. has contributed to scientific advisory boards and/or has consulted for Biogen, Celgene, Novartis, Merck and Roche, has received compensation for serving on speakers bureaus for Biogen, Celgene, Merck, Novartis, Roche and Sanofi, and has received research support from Biogen, Merck, Novartis, and Sanofi. S.B. has received honoraria from Biogen Idec, Bristol Meyer Squibbs, Hexal, Merck Healthcare, Novartis, Roche, Sanofi-Genzyme and Teva. M.P.S. has received consulting fees from Alexion, Biogen, Immunic, Merck, Novartis, Roche and Sanofi. T.G. has received travel grants and speakers' honoraria from Amgen, Bayer, Boehringer Ingelheim, Novartis and Pfizer outside the submitted work. A.A. has received research grants from Denali Therapeutics and Roche, outside the submitted work. A.G. has received research grants from Denali Therapeutics and F. Hoffmann-La Roche. A.G. has received personal fees from *JAMA Neurology*, Neurona and Pipeline Pharmaceuticals outside the submitted work. A.G. also has a patent pending for a small-molecule drug for remyelination. The institutes of L.K. (University Hospital Basel and the Research Center for Neuroimmunology and Neuroscience Basel) have received the following exclusively for research support: steering committee, advisory board and consultancy fees from Bayer, Biogen, BMS, Janssen (J&J), Merck, Novartis, Roche, Sanofi, Santhera and TG Therapeutics; speaker fees from Bayer, Biogen, Merck, Novartis, Roche and Sanofi; support of educational activities from Merck, Novartis, Roche and Sanofi; license fees for Neurostatus products; and grants from Bayer, Biogen, the European Union, Innosuisse, Merck, Novartis, Roche, the Swiss MS Society and the Swiss National Research Foundation. M.C. has received compensation for consulting services and speaking honoraria from Bayer Schering Pharma, Biogen Idec, Bristol Myers Squibb, Genzyme, Merk Serono, Novartis, Teva Pharmaceuticals and Sanofi-Aventis. H.T. has participated in meetings sponsored by or received honoraria for acting as an advisor or speaker for Alexion, Bayer, Biogen, Bristol Myers Squibb, Celgene, Diamed, Fresenius, Fujirebio, GlaxoSmithKline, Horizon, Janssen-Cilag, Merck, Novartis, Roche, Sanofi-Genzyme, Siemens and Teva. M.S.F. has received research or educational grants from Sanofi-Genzyme Canada, has received honoraria or consultation fees from Alexion/Astra Zeneca, BiogenIdec, EMD Inc./EMD Serono/Merck Serono, Find Therapeutics, Hoffman-La Roche, Novartis, Quanterix, Sanofi-Genzyme, and Teva Canada Innovation, is a member of company advisory boards, boards of directors or similar for Alexion/Astra Zeneca, Atara Biotherapeutics, Bayer Healthcare, Celestra Health, EMD Inc./Merck Serono, Find Therapeutics, Hoffman-La Roche, Actelion/Janssen (J&J), Novartis, Sanofi-Genzyme, and Setpoint Medical, and has participated in a company-sponsored speaker's bureau for EMD Serono and Sanofi-Genzyme. A.P. has received grant support for remyelination trials in multiple sclerosis to the Amsterdam University Medical Centre. K.B. has served as a consultant and on advisory boards for Acumen, ALZPath, BioArctic, Biogen, Eisai, Lilly, Moleac, Novartis, Ono Pharma, Prothena, Roche Diagnostics and Siemens Healthineers; has served on data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials and participated in educational programmes for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work in this paper. H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work in this paper. D.L. is Chief Medical Officer of GeNeuro. J.K. has received speaker fees, research support, travel support, and/or served on advisory boards from Alnylam, Bayer, Biogen, Bristol Myers Squibb, Celgene, Immunic, Merck, Neurogenesis, Novartis, Octave Bioscience, Quanterix, Roche, Sanofi and Stata DX. S.A.-R., S.T. and P.B. declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41582-024-00955-x.

Peer review information *Nature Reviews Neurology* thanks T. Cronberg, G. Shaw and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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