Review

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Translating neurofilament light chain testing into clinical practice: a multidisciplinary implementation roadmap

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Abstract: Neurofilament light chain (NfL) has been identified as a sensitive and broadly validated biomarker of neuroaxonal injury across multiple neurological conditions, including multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and atypical parkinsonian syndromes. This position paper provides a multidisciplinary roadmap for translating circulating NfL testing into routine clinical practice, integrating analytical, interpretative, and organizational dimensions. It summarizes the biological basis and clinical evidence supporting NfL as a diagnostic, prognostic, and monitoring tool, emphasizing its high sensitivity to neuroaxonal injury across both acute inflammatory and chronic degenerative processes. Comparative analysis of immunoassay technologies identifies strengths and critical sources of variability, while operational guidelines highlight the need for pre-analytical standardization, inter-laboratory harmonization, and participation in quality control schemes. Confounders such as age, BMI, renal function, and comorbidities are shown to significantly influence interpretation, supporting the use of age-adjusted Z-scores, percentiles, and longitudinal normalization for accurate patient-level evaluation. From a clinical standpoint, the paper focuses on practical indications for NfL testing in MS, recommending its use for disease activity prediction and monitoring, treatment decisions and treatment response assessment. Integration of blood NfL with magnetic resonance imaging (MRI), glial fibrillary acidic protein (GFAP) and other biomarkers measurement is proposed as a core strategy for biomarker-driven precision neurology. The authors outline an implementation model encompassing laboratory validation, structured reporting and alignment with national neurological care pathways. They conclude that the transition of NfL into clinical use requires harmonized analytical procedures, interdisciplinary education, and sustainable governance frameworks. Priority actions include regulatory qualification, establishment of international reference materials, and development of pragmatic real-world trials to consolidate its clinical utility. When these measures are achieved, NfL will represent a cornerstone biomarker for precision neurology and neurodegeneration monitoring.

Keywords: biomarker standardization; real-world evidence; clinical validation; neurodegeneration; multiple sclerosis

Introduction

The clinical relevance of neurofilament light chain (NfL) as a biomarker of neuroaxonal damage is increasingly recognized in neurological research and clinical investigation [1].

While numerous studies have demonstrated the diagnostic, prognostic, and monitoring potential of circulating NfL across neurological disorders, including multiple sclerosis, neurodegenerative diseases, and acute brain injury [1–10], its integration into routine clinical practice remains limited and inconsistent.

NfL is a structural protein that reflects neuroaxonal damage, one of the central mechanisms underlying irreversible disability in both acute and chronic neurological disorders. Following neuro-axonal injury, cytoskeletal proteins such as neurofilaments are released into the extracellular space, subsequently reaching the cerebrospinal fluid

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(CSF) and, at lower concentrations, the peripheral blood [3]. For this reason, neurofilaments are considered among the most promising biomarkers of neurodegeneration and related pathological processes, and considerable efforts are currently directed toward their clinical translation.

The proteolytic degradation of neurofilaments generates fragments that expose epitopes normally concealed within intact neurons [11]. Advances in immunometric technologies now allow these epitopes to be reliably detected, enabling highly sensitive quantification of circulating NfL fragments. Commercially available assays rely on antibody-based immunometric approaches targeting soluble NfL or NfL fragments released after neuroaxonal injury, although antibody origin and epitope recognition differ across analytical platforms [12].

NfL has been extensively investigated as a diagnostic and prognostic biomarker in both clinical research and selected clinical settings across a wide spectrum of neurological disorders [5, 13–15]. Nevertheless, its transition into routine clinical practice remains constrained by critical issues such as assay standardization, pre-analytical variability, and the establishment of universally accepted reference ranges.

This position paper aims to address the current gap between scientific potential and clinical application by providing a multidisciplinary roadmap for the structured and sustainable implementation of circulating NfL testing. While the roadmap is intended to be broadly applicable across neurological diseases, multiple sclerosis is deliberately adopted as the reference implementation model, as it currently represents the condition with the most mature evidence base for longitudinal blood NfL use in routine clinical practice. This approach allows generalizable laboratory and clinical principles to be derived from a well-characterized disease model and translated to other neurological disorders, where clinical implementation is emerging but remains less standardized.

The overall goal is to develop practical recommendations to support laboratories and clinicians in adopting this biomarker in real-world settings, ensuring analytical robustness, clinical relevance, and organizational feasibility.

Biological and clinical rationale of the NfL test in neurological diseases

Neurofilaments are predominantly neuronal cytoskeletal proteins, highly enriched in axons. They belong to the family of intermediate filaments, with a diameter intermediate between that of actin microfilaments and myosin-containing thick filaments. Neurofilaments include light, middle, and heavy subunits. They are present in dendritic spines and in the neuronal perikaryon, but are predominantly organized within axons. In axons NfL is abundant, organized in arrays, with lateral branches that cross-bridge with each other but also with microtubules and actin fibres. Alpha-internexin and peripherin are distinct intermediate filament proteins, predominantly expressed in the central and peripheral nervous system, respectively [16]. Neurofilaments are incredibly stable *in vivo*, displaying a half-life of several months [17]. When neurons, axons, or both are damaged, NfL fragments are generated by proteolytic cleavage and can reach the cerebrospinal fluid and the blood through different, and not yet fully defined routes. Insights into the kinetics of NfL release into the bloodstream after neuronal injury have been obtained from time-defined events such as ischemic stroke, where elevations are detectable as early as 6 h post-injury [18]. Reported estimates of blood NfL half-life vary across studies and modeling approaches. Apparent half-life values ranging from several weeks to longer timeframes likely reflect not only plasma clearance kinetics, but also sustained release from injured neurons over time. These differences should therefore be interpreted as complementary modeling perspectives rather than contradictory measures of intrinsic NfL stability in blood [19]. This continuous axonal turnover likely explains the slow normalization of blood NfL concentrations following focal injury. In traumatic brain injury, NfL levels have been shown to return to baseline after approximately 6 months, 1 year, and several years after mild, moderate, and severe trauma, respectively [20].

Although more than 3,500 studies have been published over the past decade on NfL across a wide range of neurological diseases, its analytical and clinical validation is still in progress, and real-world implementation has only recently begun.

In amyotrophic lateral sclerosis (ALS), both CSF and plasma NfL show high sensitivity in differentiating ALS from disease mimics and provide valuable supportive information within diagnostic workflows [21]. From a prognostic perspective, circulating NfL represents the most extensively validated fluid biomarker in ALS, with higher concentrations consistently associated with faster disease progression at the group level, although its predictive accuracy at the individual patient level remains limited when used in isolation [21]. Notably, the clinical relevance of NfL has been underscored by its role as a pharmacodynamic biomarker in the development of the antisense oligonucleotide tofersen for SOD1-related familial ALS: despite the failure to meet the primary clinical endpoint, regulatory approval was supported by biomarker evidence demonstrating reductions in NfL levels [22].

In Alzheimer's disease (AD), early biological diagnosis increasingly relies on validated body fluid biomarkers within established frameworks, in which NfL is not included among core markers but is widely used as a complementary indicator of neurodegeneration. In this context, baseline plasma NfL levels have been shown to correlate with longitudinal measures of cognitive decline and global clinical status [22, 23]. However, in the phase II trial of the anti-amyloid antibody donanemab, changes in NfL concentrations were not associated with clinical outcomes, indicating that the utility of NfL as a pharmacodynamic biomarker in AD may be context-dependent and not sufficient when used as a standalone marker [24].

In Parkinson's disease (PD) and atypical parkinsonian disorders, circulating NfL levels show distinct patterns. In idiopathic PD, blood NfL concentrations are often within the normal range or only mildly increased compared with healthy individuals, whereas marked elevations are typically observed in atypical parkinsonian disorders. In this context, PD generally exhibits lower NfL concentrations than Parkinson's disease dementia (PDD), multiple system atrophy (MSA), dementia with Lewy bodies (DLB), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS) [25]. Among these disorders, MSA and PSP are associated with the highest NfL levels; however, diagnostic discrimination based on NfL alone remains limited [25].

In multiple sclerosis (MS), the incorporation of NfL into routine clinical practice for disease monitoring and therapeutic decision-making is approaching implementation [26]. NfL levels show stronger correlations with acute inflammatory relapses than with progressive neurodegenerative phases [9]. Nonetheless, substantial evidence supports its prognostic value for both short- and long-term disease activity [10]. Moreover, NfL measurement can assist in assessing treatment response [27] and in distinguishing true relapses from pseudo-relapses [10]. The impact of NfL testing in clinical practice has already been demonstrated [28], and its widespread adoption is anticipated as analytical accessibility improves.

Technological and laboratory considerations

From a laboratory perspective, the implementation of NfL testing requires careful consideration of the analytical context. Circulating NfL can be measured in both CSF and blood (serum or plasma), with recent advances enabling highly sensitive quantification in peripheral matrices. Several analytical platforms are currently available, including Single Molecule Array (Simoa), chemiluminescent immunoassays (CLEIA), ELISA, and electrochemiluminescence (ECLIA), each with specific strengths, limitations, and regulatory statuses.

The advent of ultrasensitive detection platforms has been pivotal in enabling large-scale measurement of NfL in blood.

A major technological advance in blood NfL quantification was the development of single molecule array (Simoa) technology [29], originally introduced by Quanterix and David R. Walt and colleagues, which enabled ultra-sensitive digital immunoassays. By employing miniaturized microwells and nanobeads to capture immunocomplexes, Simoa allows detection of positivity signals even when only a few neurofilament molecules are present, thereby enabling reliable quantification at ultra-low concentrations in peripheral blood.

Currently, several immunometric approaches are available for NfL measurement across body fluids. Table 1 summarizes the main analytical platforms currently available for the measurement of circulating NfL, highlighting substantial differences in analytical sensitivity, measuring range, precision, and regulatory status. Beyond analytical performance alone, differences in sensitivity, dynamic range, degree of automation, and regulatory status are not merely technical aspects but key determinants of clinical positioning. These characteristics define whether a platform is best suited for ultra-sensitive research applications or for standardized implementation in accredited clinical laboratories. Despite overall good analytical performance across platforms, variability in sensitivity and measuring range may affect longitudinal monitoring and inter-laboratory comparability, particularly at low NfL concentrations. These differences, together with the lack of certified reference materials, underscore the need for harmonization strategies and context-specific interpretation when implementing NfL testing in clinical practice.

The first ELISA assay for NfL was introduced by Rosengren and colleagues in 1996 [30], providing reproducible results but with limited sensitivity compared to more recent technologies. Subsequent advances included the development of highly sensitive assays such as Simoa, as well as immunoassays based on chemiluminescence (CLIA) and electrochemiluminescence (ECLIA) [31, 32]. These methods have expanded the clinical utility of NfL testing by enabling accurate detection in both CSF and blood, thus facilitating non-invasive monitoring of neurodegeneration.

In parallel, mass spectrometry-based approaches have been explored for NfL quantification, particularly in research settings requiring molecular characterization and assay orthogonality. While these methods offer high analytical specificity, their complexity and limited throughput currently restrict their use to specialized laboratories [33]. Unlike immunoassay-based methods, which depend on antibody specificity and are generally limited to single-analyte

Table 1: Analytical platforms for circulating neurofilament light chain (NfL) measurement.

Manufacturer & analyzer	Assay type	LOD, pg/mL	Analytical measuring range, pg/mL	Precision, %CV	IVD/RUO status	Matrix	Preferred clinical use cases
Quanterix Simoa® HD-X	Digital ELISA	0.085	0.34–1,140	Intra: 4.8; inter: 6.4	RUO	Serum, EDTA plasma, CSF	High-sensitivity applications; early disease detection; research studies; longitudinal monitoring requiring detection of very low NfL concentrations
Fujirebio Lumipulse® G1200	CLEIA	3.0	2.0–5,000	Intra: 4.1; inter: 5.5	RUO	Serum, EDTA/-Li-hep plasma, CSF	Routine longitudinal monitoring; assessment of disease progression; settings requiring wide dynamic range and high analytical reproducibility
Roche Cobas® e801	ECLIA	0.140	0.17–1,000	Intra: 4.2; inter: 4.8	RUO	Serum, plasma, CSF	High-throughput laboratory environments; integrated automated workflows; exploratory clinical implementation within centralized laboratories
Siemens Atellica® IM	CLIA	1.3	2.5–3,000	Intra: 2.1; inter: 6.9	IVD	Serum, EDTA plasma	Routine clinical laboratories; standardized workflows; clinical implementation in accredited diagnostic settings
ProteinSimple Ella™	Microfluidic ELISA	0.5	2–6,000	Intra: 15.3; inter: 20.7	RUO	Serum, EDTA plasma, CSF	Research settings; small- to medium-sized cohorts; exploratory biomarker panels and method comparison studies

IVD/RUO status refers to availability for routine clinical use according to manufacturer declarations and current regulatory frameworks. Preferred clinical use cases reflect typical applications based on analytical characteristics and published validation studies. LOD, limit of detection; CLEIA, chemiluminescent enzyme immunoassay; CLIA, chemiluminescent immunoassay; ECLIA, electrochemiluminescent immunoassay; IVD, *in vitro* diagnostic; RUO, research use only; CV, coefficient of variation; CSF, cerebrospinal fluid.

detection, mass spectrometry provides superior analytical specificity and multiplexing capacity, allowing simultaneous quantification of multiple biomarkers within a single run. This capability is especially valuable for complex neurological conditions, where biomarker panels may yield more comprehensive diagnostic and prognostic information than NfL alone. Moreover, the reduced susceptibility of mass spectrometry to cross-reactivity and matrix effects positions it as a promising candidate for standardization efforts and the development of reference measurement procedures.

Methodological variability and lack of universal standardization currently limit the comparability of results across centres. Moreover, NfL is not yet uniformly certified as an *in vitro* diagnostic (IVD) test across platforms, which poses challenges for clinical adoption [34].

Significant differences exist among currently available NfL assays, which influence their suitability for specific clinical applications. Sensitivity is one of the most relevant aspects: the Quanterix Simoa® HD-X Analyzer™ demonstrates the highest analytical sensitivity, with a limit of detection (LOD) as low as 0.085 pg/mL. This makes it particularly valuable for detecting very low NfL concentrations, an advantage in screening or early disease detection. In contrast, the Fujirebio Lumipulse® CLEIA assay (LOD 3 pg/mL) and the Siemens Atellica® CLIA assay (LOD 1.3 pg/mL) are less sensitive, which may limit their ability to reliably quantify the lowest concentration ranges.

Another important factor is the measurement range. The Fujirebio assay covers the widest dynamic interval (2.0–5,000 pg/mL), a feature that supports its use in longitudinal disease monitoring and in clinical scenarios requiring reliable capture of large NfL fluctuations over time. By comparison, the Quanterix Simoa assay, while highly sensitive, offers a narrower range (0.345–1,140 pg/mL), which may reduce its ability to measure extreme values. A wide analytical measuring range is clinically relevant for longitudinal monitoring, as it allows reliable quantification of both low baseline NfL concentrations and marked increases observed during acute disease activity or rapid progression, without the need for repeat dilution or assay switching.

Assays also differ in terms of precision and reproducibility. The Lumipulse assay demonstrates the lowest intra-assay (<3.25 %) and inter-assay (<5.50 %) coefficients of variation, indicating excellent reproducibility. Atellica, by contrast, shows higher inter-assay variability (<12.4 %), which could introduce more uncertainty in serial measurements.

Overall, immunometric assays have demonstrated solid performance, but important challenges remain regarding methodological standardization and the establishment of

robust reference values. A meta-analysis by Forgrave et al. [35] revealed significant discrepancies in absolute NfL concentrations across studies, highlighting the pressing need for harmonization. At present, the absence of certified reference material complicates assay alignment, largely due to the structural complexity of NfL and the heterogeneity of its fragments and isoforms in biological fluids. Mass spectrometry-based characterization of these fragments will likely be essential for developing standardized reference materials and reference methods.

Collaborative initiatives are beginning to address these challenges. The Global NfL Consortium has aggregated large datasets from healthy populations to propose age-stratified reference values, confirming the strong correlation between NfL levels and age. In parallel, Arslan and Zetterberg [36] have outlined standardized pre-analytical procedures, covering sample type, collection, and storage, to reduce inter-laboratory variability. Disease-specific registries such as MSBase have contributed valuable data on NfL distributions in pathological contexts, while cross-platform comparison studies (Simoa, CLIA, ECLIA) have sought to align measurements, although complete harmonization remains elusive [37].

Population-based studies have also contributed to the establishment of reference models for clinical interpretation. Benkert et al. [38] developed an age- and BMI-adjusted dataset including more than 4,500 healthy individuals, introducing Z-scores to express how an individual's NfL value deviates from normative ranges. Similarly, Simrén and colleagues [39] proposed age-specific cut-offs from a large cohort of healthy subjects analysed using the Simoa platform. To further support clinical translation, online tools (https://mybiomarkers.shinyapps.io/Neurofilament) now allow clinicians to contextualize a patient's NfL value relative to both healthy controls and disease-specific populations.

These advances represent crucial steps toward integrating NfL measurement into routine clinical decision-making. Nonetheless, the absence of certified reference materials and persistent methodological variability continue to limit full standardization and comparability across platforms [37].

Box 1 – Pre-analytical workflow for blood NfL testing

- **Sample matrix:** Serum or plasma, according to assay specifications
- **Collection:** Standard venipuncture using validated tubes
- **Processing:** Timely centrifugation according to manufacturer instructions
- **Storage:** Aliquoting and storage at −80 °C for long-term preservation
- **Quality assurance:** Participation in **external quality assessment (EQA) schemes** is strongly recommended

Pre-analytical factors play a critical role in the reliability of NfL measurements. Key variables include the biological matrix (CSF, serum, or plasma), collection and storage conditions, and sample handling procedures. For blood-based assays, the type of collection tube can significantly influence results. In a study of 30 paired samples analysed on the Simoa platform, serum and lithium-heparin plasma yielded higher NfL concentrations compared with EDTA or citrate plasma, underscoring the need for matrix-specific reference values [40]. The choice of matrix is often influenced by study design and historical practice rather than intrinsic biological differences. For example, serum has become the preferred matrix in amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) research, largely due to its early adoption and validation in large cohorts. Conversely, plasma has been more frequently used in dementia studies, reflecting its integration in early biomarker discovery pipelines. Ultimately, both serum and plasma provide reliable measurements when appropriate protocols are applied, although comparisons across studies must take these methodological differences into account.

Several studies confirmed a strong correlation between serum and plasma NfL levels, with conversion formulas proposed to harmonize results across matrices [41]. Beyond sample type, stability studies have demonstrated that NfL remains robust under a range of conditions, including up to three or four freeze–thaw cycles and storage at 2–8 °C for as long as eight days [42]. Moreover, NfL exhibits a relatively long half-life, substantially longer than other neurodegeneration biomarkers such as glial fibrillary acidic protein (GFAP) [1, 43]. This property suggests that NfL may be less suited for capturing acute injury but particularly informative for monitoring chronic neurodegenerative processes.

Other pre-analytical factors, including haemolysis, lipemia, and icterus, can interfere with assay performance, highlighting the importance of standardized procedures. To address this, Arslan and Zetterberg [36] proposed detailed recommendations for each phase of the testing process. In the pre-analytical phase, serum or EDTA plasma is preferred, centrifugation should occur within 6 h of collection, and morning fasting samples are recommended to minimize diurnal variation. During the analytical phase, high-sensitivity platforms should be prioritized, and awareness of calibrator sources (bovine vs. recombinant human) is essential to ensure comparability, with correction factors applied when necessary. Mass spectrometry has been identified as a promising candidate for future reference methods. Finally, in the post-analytical phase, circulating NfL results should be contextualized using age- and BMI-adjusted Z-scores or percentiles derived from

appropriate reference populations. Post-analytical quality assurance may include internal controls, such as duplicate measurements and the use of validated reference materials, where available. Importantly, normal NfL concentrations do not exclude neurological disease, and elevated values should always be interpreted within a broader clinical and radiological context.

Interpretation of blood NfL values increasingly relies on large international reference datasets generated from healthy populations, which enable standardized age- and BMI-adjusted percentiles and Z-scores. The use of these reference frameworks supports harmonized interpretation across laboratories and analytical platforms and represents an important step toward consistent clinical reporting.

Box 2 – Practical recommendations for managing interplatform variability

- Longitudinal monitoring of individual patients should be performed using the **same analytical platform whenever possible.**
- Absolute blood NfL values obtained from different platforms are **not directly interchangeable.**
- If platform switching is unavoidable, results should be interpreted cautiously, avoiding direct numerical comparison.
- Use of **age-adjusted Z-scores or percentiles**, when available, may partially mitigate inter-platform differences.
- Laboratories should clearly report the analytical method used and highlight any changes over time.

Clinical interpretation of results

Several confounding factors substantially influence the interpretation of NfL levels, as circulating concentrations are associated not only with neurological damage but also with ageing and multiple non-neurological conditions [44]. Variability in cut-off selection further underscores the importance of standardized analytical methods. Approximately half of the analysed studies applied age-adjusted or previously published thresholds, whereas others used diverse approaches such as Z-scores, cohort-derived cut-offs, or models accounting for specific confounding factors [1]. The identification and thorough understanding of these factors, together with strategies to control for them, are fundamental to the successful implementation of NfL testing in clinical practice.

Age represents one of the most important confounding factors and is consistently associated with higher NfL levels [45], with roughly 2 % increase in NfL every year between 20 and 80 years of age [44]. The increase in NfL levels and their inter-individual variability over time in the healthy ageing population can significantly affect clinical interpretations especially in longitudinal monitoring [46].

Body mass index (BMI) and plasma volume are also recognized as potential confounders. However, available data remain partly contradictory, with some studies reporting no significant relationship [44] and others showing a significant association [47]. The inverse relationship between BMI and NfL concentrations may have a substantial impact on data interpretation, highlighting the importance of accounting for and controlling BMI, other body weight- or fat-related parameters, and blood volume [47–49].

A correlation with renal function has been consistently reported by several groups. Serum creatinine and the derived estimated glomerular filtration rate (eGFR) show the strongest associations with NfL after age [44]. Reports indicate a negative relationship between NfL and eGFR, with the strongest associations observed in older individuals [50] and sensitivity to sex-related differences [51]. Conversely, the urinary albumin-to-creatinine ratio (UACR) has been found to increase in parallel with NfL levels, as observed in chronic kidney disease [50].

Comorbidities such as cardiovascular disease [52], diabetes [53], and impaired renal function [54] can markedly influence blood NfL levels, representing an important confounding factor.

Increased NfL levels associated with atrial fibrillation [55], diabetic polyneuropathies [53, 56], chronic kidney disease [50], and metabolic syndrome [57] must be carefully considered during the analysis and interpretation of NfL as a biomarker for neurological conditions.

Whilst critical issues in NfL interpretation considering confounding factors remain, incorporating these variables into NfL measurements could substantially enhance the biomarker's clinical utility. For instance, rather than using raw values, log-transformation of NfL data and the application of log-linear models controlling for age and other covariates may improve comparisons between healthy and diseased populations [58].

Approaches based on normalization to population-derived reference models, including Z-scores and percentiles, may improve the clinical contextualization of individual NfL values and better align biomarker interpretation with MRI and other clinical parameters. This approach could be crucial for improving diagnostic and prognostic assessment, as well as interpretation of treatment outcomes, as demonstrated in two independent European MS cohorts [38]. Longitudinal monitoring is clearly susceptible to confounding factors, particularly age, and the use of standardized

cut-offs based on NfL evaluation at multiple time points with data normalization may be beneficial [59].

Interpretation of NfL data should always consider confounding variables and be supported by relevant clinical information. In clinical practice, age-adjusted NfL Z-scores or percentiles may support decision-making by providing a quantitative estimate of neuroaxonal injury relative to reference populations. Rather than defining rigid cut-offs, interpretation should follow a graded risk model, whereby increasing Z-score values are associated with progressively higher risk of disease activity or progression, with clinically meaningful information already emerging at modest elevations above age-adjusted norms. Accordingly, graded increases in NfL values may inform context-dependent clinical actions, such as closer clinical follow-up, anticipation of MRI reassessment, treatment re-evaluation, or intensified monitoring. Importantly, NfL results should always be interpreted longitudinally and integrated with clinical findings, imaging data, and disease-specific factors, avoiding isolated or dichotomous use. Finally, the clinical context of use (i.e., context of use; [1]) in which NfL is employed as a biomarker must always be carefully considered, particularly regarding the specific pathological profile of the patient. This is essential to establish an appropriate standardization framework, including dedicated guidelines with targeted cut-offs and normalization procedures for different pathological conditions.

Defined clinical contexts of use for blood NfL testing

Blood NfL testing can be applied across several distinct clinical contexts, each associated with specific interpretative frameworks and varying levels of evidence. Among neurological diseases, multiple sclerosis (MS) represents the condition in which evidence is currently most mature, allowing a more structured interpretation across different clinical scenarios. Clear definition of the intended context of use is essential to ensure appropriate test ordering, reporting, and clinical interpretation. MS is presented here as a model disease, without implying disease specificity of NfL.

Diagnosis

Blood NfL is not disease-specific and should not be used as a standalone diagnostic biomarker. However, elevated levels may support the presence of active neuroaxonal injury. Within an MS-centered framework, NfL may contribute to differential diagnosis when interpreted alongside clinical evaluation, magnetic resonance imaging, and disease-specific biomarkers.

Prognosis and risk stratification

Baseline blood NfL levels, particularly when expressed as age-adjusted Z-scores or percentiles, may provide prognostic information regarding future disease activity or progression. This application is especially supported in multiple sclerosis, where higher baseline levels have been associated with increased inflammatory activity and worse long-term outcomes. Similar evidence is emerging in amyotrophic lateral sclerosis, although with different pathophysiological implications.

Disease monitoring

Longitudinal assessment of blood NfL, ideally performed using the same analytical platform, can reflect changes in neuroaxonal injury burden over time. In MS, dynamic changes in NfL parallel inflammatory activity and radiological disease burden, supporting its role as a complementary biomarker alongside clinical and imaging follow-up.

Treatment response

Reductions in blood NfL following treatment initiation or escalation may indicate suppression of neuroaxonal injury. This application is particularly relevant in inflammatory neurological diseases, with MS serving as the best-validated model. However, NfL should be interpreted within the broader clinical context and not as an isolated surrogate endpoint.

Implementation model in clinical practice

The integration of NfL testing into routine healthcare requires the development of structured implementation models that combine both laboratory and clinical perspectives. Italian multicentre experiences have highlighted the value of close interaction between specialized laboratories and clinical teams, ensuring that analytical performance, interpretative standards, and patient care pathways are harmonized.

Organizational models may differ according to the type of laboratory setting.

In this context, the choice of the analytical platform should be aligned not only with analytical performance, but also with the intended clinical use, laboratory workflow, and regulatory framework, as summarized in Table 1.

General clinical laboratories can adopt standardized workflows with automated platforms and predefined reference ranges, supporting broader accessibility and equity of testing across regional networks. Conversely, specialized laboratories can act as referral centres, providing advanced interpretative support, integration with disease-specific biomarkers, and the development of harmonized protocols for multicentre studies.

The successful implementation of NfL in routine healthcare requires a coordinated and multidisciplinary roadmap (Figure 1). The process begins with a careful definition of the clinical need, identifying those neurological conditions, such as multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, and traumatic brain injury, where NfL measurement may provide additional diagnostic, prognostic, or monitoring value. Once the clinical rationale has been established, laboratory preparation becomes essential, involving the selection of robust analytical platforms, the validation of performance characteristics, and the standardization of pre-analytical conditions. The next step focuses on embedding NfL within clinical pathways: developing evidence-based cut-offs, refining interpretative algorithms, and ensuring that clinicians are adequately trained to use the biomarker effectively. Integration into information systems, including LIS (Laboratory Information System) and EHR (Electronic Health Record) platforms, represents a key enabler, allowing automated reporting and seamless incorporation into patient care workflows. Importantly, broader governance and sustainability issues must be addressed, including cost-effectiveness evaluations, reimbursement mechanisms, and alignment with regional or national care pathways, to ensure equitable and scalable adoption. Finally, continuous monitoring through clinical audits, disease registries, and real-world evidence initiatives provides the feedback necessary to refine cut-offs, validate new applications, and update guidelines, thereby guaranteeing that NfL implementation remains dynamic and clinically relevant over time.

From a laboratory perspective, the implementation of NfL requires a structured process to ensure analytical robustness and reproducibility across settings. The choice of the analytical platform, whether ultra-sensitive immunoassays such as Simoa, electrochemiluminescence-based methods, or validated ELISAs, should be guided by considerations of sensitivity, throughput, automation, and regulatory approval. Rigorous validation of assay performance is mandatory, encompassing precision, linearity, limits of detection and quantification, as well as inter- and intra-assay variability. Equally important is the harmonization of pre-analytical conditions, including the type of biological matrix (serum, plasma, or cerebrospinal fluid), sample handling, storage stability, and freeze–thaw tolerance, all of which may significantly influence measured concentrations. Laboratories should also establish internal quality control procedures and participate in external quality assessment schemes to guarantee comparability of results across centres. The standardization of reporting formats, including units of measurement and reference intervals, is essential to support clinical interpretation and to minimize variability in practice. Laboratory reports should not only provide absolute values but also contextualized information, including reference ranges, Z-scores or percentiles, and longitudinal trends where available. By ensuring that these laboratory requirements are met, healthcare providers can build a reliable foundation for the integration of NfL into clinical decision-making.

Furthermore, integration with complementary biomarkers, such as GFAP for astroglial injury and the kappa free light chain index (k-index) for intrathecal immunoglobulin synthesis, can enhance diagnostic accuracy and pathophysiological interpretation, particularly in complex neurological conditions.

Box 3 – Conceptual framework for multimarker interpretation

Blood NfL primarily reflects the **intensity of neuroaxonal injury**, whereas GFAP reflects **astroglial activation**. Combined interpretation may help distinguish inflammatory activity from chronic neurodegeneration and refine risk stratification in selected clinical contexts. At present, such approaches should be considered exploratory and complementary to established clinical assessments.

The consensus emerging from joint expert contributions underlines that a coordinated approach, linking laboratory practice, clinical decision-making, and health system organization, is essential to enable the effective and sustainable implementation of NfL testing in neurological clinical pathways.

Operational recommendations in multiple sclerosis

Serum NfL re-baselining

It typically takes several weeks for NfL to reach peak levels in peripheral blood following neuroaxonal injury and release

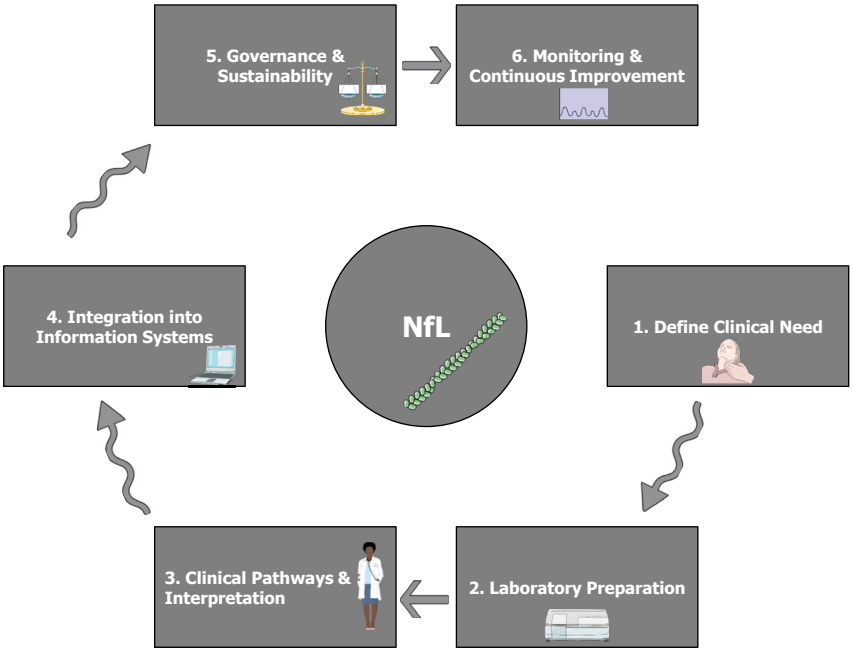

Figure 1: Multidisciplinary roadmap for the clinical implementation of NfL. Key multidisciplinary steps required for translating NfL into routine clinical practice, from defining clinical needs to laboratory validation, integration into clinical pathways, health system governance, and continuous monitoring.

from CNS tissue. Kinetic data from acute neurological injuries indicate a delayed peak of CSF and circulating NfL around one-month post-injury, with a gradual return to baseline values usually within six months [60]. In MS patients developing gadolinium-enhancing (Gd+) lesions, circulating NfL peaks mostly appear after lesion appearance, after a median of 8 weeks (interquartile range 0–12) [61] and subsequently declines gradually over the following months [42].

It is important to keep this delay in mind when interpreting circulating NfL measurements in clinical practice. Re-baselining is essential after each relapse or new Gd+ lesion, as post-inflammatory increases followed by gradual declines over several months can otherwise confound longitudinal interpretation. Following a clinical relapse or the appearance of a new Gd+ lesion, it is recommended to delay the establishment of a new baseline until approximately three months post-event, when blood NfL levels have peaked and begun to decline, and to confirm stability or a downward trend with a follow-up sample at six months [62].

Risk stratification and therapeutic decision-making scenarios

Circulating NfL levels clearly correlate with inflammatory disease activity and therapy response and may predict disability worsening at a group level [32, 63, 64]. To interpret NfL levels in the individual patient for clinical follow up and prognostication is necessary to compare the obtained concentration to reference values.

Several published datasets of healthy controls exist, that identified reference values of NfL concentration in serum or plasma, with different statistical methods (cut-off values, percentiles, Z-score), using diverse analytical platforms [38, 39, 65, 66, 67].

Taking into account this methodological variability, most studies agree on the strict correlation between serum and plasma levels, particularly highlighting the significance of the age factor. This concordance of results supports the identification of clinically useful ranges around 10 pg/mL in subjects under 40 years of age and 15 pg/mL in those under 60 years of age, with sharp increases observed in advanced age, the clinical significance of which should be treated with caution. When interpreted using web applications based on percentiles or Z-scores, in patients with MS values above 95 % or a Z-score greater than 1.5 are associated with an increased risk of future disease activity.

Anyway, a correct interpretation of circulating NfL measurement requires to consider their kinetics, the phase of the disease (asymptomatic, relapsing or progressive), the ongoing (disease-modifying therapy) DMT and the eventual above mentioned confounding factors.

It has been demonstrated that blood NfL levels significantly increase years before clinical onset of the disease [68–70]. In pre-clinical MS (defined MS according to the 2024 revised diagnostic criteria) high blood NfL levels are associated with evidence of disease activity and early clinical conversion [71, 72], suggesting a promptly initiation of a DMT.

After the clinical conversion, during the diagnostic and prognostic workup the timing from the onset of neurological

symptoms or from the detection of MRI activity must be considered in the evaluation of blood NfL measurement, taking into account the above mentioned NfL kinetics in the body fluid compartments [42, 60–62].

After re-baseline, persistently elevated circulating NfL levels are indeed indicative of highly active disease and thus a potentially more aggressive course, even more so when combined with high blood GFAP levels [73]. These data, along with other well-known negative prognostic factors [74] should guide the treatment choice towards a high efficacy therapy (HET).

Evaluation of treatment response

Blood NfL measurement may help clinicians to monitor the treatment efficacy. As for MRI, baseline blood NfL levels should be established through reassessment after an appropriate interval, taking into account both the time elapsed since the last clinical or radiological activity and the time required for treatment onset of action. This strategy should be applied to every therapeutic switch.

Ideally, circulating NfL levels should be reassessed 6–12 months after starting treatment to evaluate the response and guide any necessary therapy adjustments. If a baseline value has not been previously established, the initial observed reduction may reflect both the physiological kinetics of NfL, as well as a decrease in neuroinflammation. Indeed, after starting a DMT, a reduction in blood NfL levels is generally observed, with the magnitude of decline reflecting the efficacy of the selected treatment, whether high- or low-to-moderate-efficacy, until values are brought back within the normal range identified in healthy controls [75, 76]. In longitudinal NfL assessment, a sustained elevation of NfL despite treatment may indicate unresolved neuroinflammation and suboptimal therapeutic efficacy, potentially warranting treatment adjustment [26].

In fact, the presence of elevated blood NfL values in apparently stable patients correlates with an increased risk of losing no evidence of disease activity (NEDA) in the subsequent year [38, 70, 77].

If a low-to-moderate efficacy DMT has been chosen based on prognostic factors or the presence of comorbidities, closer patient monitoring may be required. In such cases, regular measurement of blood NfL every 3–6 months can provide valuable insights into the effectiveness of the current treatment in controlling disease activity, complement MRI data, and allow for more timely therapeutic escalation when needed [26].

In fact, also a relatively short delay in switching to HETs is relevant to the long-term prognosis, as demonstrated by clinical trials and real-world studies [78–81].

If the chosen treatment is a HET, persistently elevated blood NfL levels correlate with Progression Independent of Relapse Activity (PIRA) [82, 83], likely reflecting ongoing smouldering neuroinflammation. At present, there are no effective therapies targeting this process, although novel strategies are hopefully on the horizon. In this future scenario blood NfL monitoring of the patient could become highly informative, anticipating the extent of clinical response to treatment. The overall impact of NfL implementation in clinical practice will be evaluated in a pragmatic randomized trial, aimed at confirming the added value of neurofilaments in supporting personalized treatment decisions [84, 85].

In summary, although not specific for MS, circulating NfL measurement enables disease monitoring that closely reflects underlying biological mechanisms. Blood NfL reflects the overall burden of neuroaxonal injury and may capture pathological processes occurring in regions that are less sensitively detected by routine imaging, including the spinal cord. However, NfL should be interpreted as a marker of global neuroaxonal damage rather than region-specific pathology. In healthcare settings where resources are limited and MRI scans cannot be performed at short intervals, blood NfL cannot replace but rather complement imaging findings within a multimodal approach that includes comprehensive clinical evaluation.

Integration of blood NfL with MRI and clinical assessments in complex clinical scenarios

The integration of blood NfL measurement with routine MRI and clinical follow-up allows for a more temporally granular assessment of inflammatory activity. MRI remains the gold standard for structural visualization of CNS lesions. However, while periodic MRI is typically carried out every 6–12 months, circulating NfL can be assessed more frequently, which can be particularly valuable in different scenarios such as: a) JCV-positive patients treated with natalizumab (NTZ), at risk for Progressive Multifocal Leukoencephalopathy (PML), b) following induction treatment (such as alemtuzumab or cladribine), c) following treatment discontinuation for any reason, including pregnancy and lactation, d) following treatment de-escalation or extended interval dosing, and e) in individuals with radiologically isolated syndrome (RIS). A rise in blood NfL levels in these scenarios can prompt a biomarker-driven rationale for earlier MRI reassessment, or to adjust/initiate treatment.

Patients with natalizumab-associated PML have at least a 10-fold rise in blood NfL levels at disease onset [86, 87].

A value of 8.4 pg/mL resulted in the best cut-off to classify pre-PML and control NTZ-treated patients after 2 years of NTZ treatment. Blood NfL levels also distinguished PML from MS relapses: the value that best differentiated PML from MS relapses was approximately 52.5 pg/mL [86, 87], while a threshold of 61.3 pg/mL yielded a specificity of 100 % (with a sensitivity of 88.0 %) for PML [87].

Following induction treatment with alemtuzumab, low blood NfL levels (<8 pg/mL) were associated with absence of clinical or MRI disease activity (specificity: 0.95). Circulating NfL peaks (>20 fold) were associated with clinical or MRI activity and started to increase 5 months before at the earliest [88].

Following discontinuation of a first-line DMT in the DOT-MS trial (NCT04260711) delta blood NfL had the highest ability to detect significant disease activity, with the best calculated cut-off of a 46.4 % increase (sensitivity 0.57, specificity 0.96) [89]. After NTZ cessation, blood NfL levels peaked up to-16-fold in 37/50 RRMS patients and were linked to return of disease activity in 19/37, with rises seen up to three months before onset of disease activity in some patients [90].

Periodic circulating NfL level assessment can also provide valuable information for clinicians in the context of de-escalation or extended interval dosing regimens, which can contribute to optimizing long-term drug safety. For example, de-escalating rituximab dose (from 1,000 mg to 500 mg/6 months) results in stability of clinical, radiological, and blood NfL levels [91] and patients both on standard (every 4 weeks) and extended interval (every 6 weeks) NTZ dosing had stable blood NfL and no evidence of disease activity [92].

Patients with RIS who do not meet revised diagnostic criteria for MS [93] could be monitored not only with periodic MRI, but also with more frequent blood NfL testing, particularly in patients with high NfL Z-scores. To this regard, in a recent study on 273 RIS patients, NfL Z-scores higher than 1.67 were associated with a shorter time to MS symptoms [72].

In complex clinical settings, blood NfL testing provides a safe and practical alternative in patients for whom MRI is difficult or contraindicated, for instance, those with non-MRI-compatible cardiac devices or with severe claustrophobia requiring general anaesthesia.

Furthermore, blood NfL concentrations may prove to be a valuable support in differentiating true relapses from pseudo-relapses, which are not associated with new Gd+ lesions, and which are often temperature- or infection-related.

Future perspectives and conclusions

The integration of neurofilament light chain (NfL) testing into clinical practice is approaching a decisive turning point [8]. Over the last decade, NfL has evolved from a purely research biomarker to a credible clinical indicator of neuroaxonal damage, with growing recognition by regulatory agencies and inclusion in interventional studies. Future directions must now focus on consolidating its translational potential, expanding its role in clinical trials, personalizing neurological care, and ensuring robust international standardization.

Potential value in clinical trials

Circulating NfL is increasingly used as an objective, quantitative, and dynamic biomarker in neurological clinical trials. Its sensitivity to both inflammatory and degenerative processes enables early assessment of neuroprotective effects and treatment response, even before overt clinical or radiological changes. In multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer's disease, NfL has demonstrated strong correlation with disease activity and progression, supporting its qualification as a surrogate endpoint for axonal damage. The regulatory acceptance of NfL as a biomarker, already initiated in the tofersen trials for SOD1-ALS, represents a landmark for future drug development.

Expanding its application to early-phase trials may accelerate go/no-go decisions and reduce trial duration and sample size, while integration into real-world evidence frameworks could improve post-marketing surveillance and comparative effectiveness studies. Future interventional studies should also explore composite biomarker models combining NfL with glial, synaptic, or neuroinflammatory markers to refine therapeutic stratification and monitor multidimensional responses to treatment.

Role in personalized medicine

Beyond its role as a clinical endpoint, NfL holds major promise for personalized medicine. As an accessible blood-based biomarker reflecting cumulative neuroaxonal injury, it enables individualized monitoring of disease activity, treatment response, and subclinical progression. The integration of NfL with MRI data and clinical scoring can generate biomarker-informed decision pathways, allowing timely therapy optimization and more rational treatment sequencing.

Age- and BMI-adjusted reference models, combined with longitudinal sampling and Z-score interpretation, may support the definition of patient-tailored baselines. In diseases such as multiple sclerosis, this approach already supports early therapeutic intervention and re-baselining

strategies. In neurodegenerative and cerebrovascular disorders, serial NfL assessments may contribute to precision prognostication and risk stratification.

Moreover, digital health platforms and machine learning models can exploit NfL dynamics to develop predictive algorithms, integrating clinical, imaging, and molecular data for adaptive patient management. Such approaches are expected to advance the shift from reactive to proactive neurology, enabling early detection of disease reactivation and prevention of irreversible disability.

Outlook for international standardization

Despite its proven biological and clinical value, harmonization remains the main barrier to full clinical implementation. Variability among analytical platforms, lack of certified reference materials, and differences in reporting units currently limit comparability across studies and centres.

International initiatives, such as the Global NfL Consortium and the IFCC working groups on neurodegeneration biomarkers, are developing reference measurement procedures, calibrator materials, and multi-platform validation studies. The establishment of traceable reference systems, supported by mass spectrometry-based methods and large normative datasets, will be essential to achieve analytical alignment and regulatory qualification as an *in vitro* diagnostic tool.

Moving forward, consensus guidelines should define standardized preanalytical protocols, clinical indications, and interpretative algorithms, ensuring consistent use across laboratories and healthcare systems. Collaboration among academic centres, diagnostic industries, and regulatory bodies will be pivotal to establish an internationally recognized NfL standardization roadmap.

Concluding remarks

The translation of NfL from research to routine clinical practice symbolizes a paradigm shift in neurology, toward a biomarker-driven, data-informed, and personalized model of care. Achieving this vision requires coordinated action: harmonized laboratory standards, cross-disciplinary education, and integration of NfL testing into clinical pathways and health information systems.

By bridging analytical rigor with clinical utility, and research innovation with regulatory sustainability, NfL has the potential to become a core biomarker of neuroaxonal integrity, not only in specialized centres but also within everyday neurological decision-making worldwide.

Future integration of NfL into personalized medicine frameworks will depend on harmonised assays, international reference standards, and pragmatic trials assessing real-world clinical utility.

Overall, the clinical translation of blood NfL will depend on graded, age-adjusted interpretation models, longitudinal assessment on consistent analytical platforms, and integration with clinical and imaging data within clearly defined contexts of use.

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