Different Clinical Contexts of Use of Blood Neurofilament Light Chain Protein in the Spectrum of Neurodegenerative Diseases

Giovanni Palermo, Sonia Mazzucchi, Alessandra Della Vecchia, Gabriele Siciliano, Ubaldo Bonuccelli, Carole Azuar, Roberto Ceravolo, Simone Lista, Harald Hampel, Filippo Baldacci

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100 Abstract

One of the most pressing challenges in the clinical research of neurodegenerative diseases (NDDs) is the validation and standardization of pathophysiological biomarkers for different contexts of use (CoUs), such as early detection, diagnosis, prognosis, and prediction of treatment response. Neurofilament light chain (NFL) concentration is a particularly promising candidate, an indicator of axonal degeneration, which can be analyzed in peripheral blood with advanced ultrasensitive methods. Serum/plasma NFL

		concentration is closely correlated with cerebrospinal fluid NFL and directly reflects neurodegeneration within the central nervous system. Here, we provide an update on the feasible CoU of blood NFL in NDDs and translate recent findings to potentially valuable clinical practice applications. As NFL is not a disease-specific biomarker, however, blood NFL is an easily accessible biomarker with promising different clinical applications for several NDDs: (1) early detection and diagnosis (i.e., amyotrophic lateral sclerosis, Creutzfeldt–Jakob disease, atypical parkinsonisms, sporadic late-onset ataxias), (2) prognosis (Huntington's disease and Parkinson's disease), and (3) prediction of time to symptom onset (presymptomatic mutation carriers in genetic Alzheimer's disease and spinocerebellar ataxia type 3).
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Different Clinical Contexts of Use of Blood Neurofilament Light Chain Protein in the Spectrum of Neurodegenerative Diseases

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Abstract

One of the most pressing challenges in the clinical research of neurodegenerative diseases (NDDs) is the validation and standardization of pathophysiological biomarkers for different contexts of use (CoUs), such as early detection, diagnosis, prognosis, and prediction of treatment response. Neurofilament light chain (NFL) concentration is a particularly promising candidate, an indicator of axonal degeneration, which can be analyzed in peripheral blood with advanced ultrasensitive methods. Serum/plasma NFL concentration is closely correlated with cerebrospinal fluid NFL and directly reflects neurodegeneration within the central nervous system. Here, we provide an update on the feasible CoU of blood NFL in NDDs and translate recent findings to potentially valuable clinical practice applications. As NFL is not a disease-specific biomarker, however, blood NFL is an easily accessible biomarker with promising different clinical applications for several NDDs: (1) early detection and diagnosis (i.e., amyotrophic lateral sclerosis, Creutzfeldt–Jakob disease, atypical parkinsonisms, sporadic late-onset ataxias), (2) prognosis (Huntington's disease and Parkinson's disease), and (3) prediction of time to symptom onset (presymptomatic mutation carriers in genetic Alzheimer's disease and spinocerebellar ataxia type 3).

Keywords Alzheimer's disease · Amyotrophic lateral sclerosis · Biomarkers · Creutzfeldt–Jakob disease · NFL · Parkinsonian syndromes

Introduction

Neurodegenerative diseases (NDDs) are currently considered as a continuum of disorders with common pathophysiological mechanisms, including misfolded protein deposition, neuronal synaptic disruption, axonal degeneration, neuroinflammation, and oxidative stress [1–3]. Therefore, the greatest current challenge in the field of NDDs is to provide biomarkers for the pathological mechanisms underlying each clinical picture [4], in order to improve the diagnostic and prognostic stratification of the patients and to allow early diagnosis and disease monitoring as well as to test treatment efficacy.

Within this multifaceted *scenario*, neurofilament light chain (NFL) is, at the present, the most promising candidate biomarker for an early identification of a general neurodegenerative process able to support disease diagnosis, prognosis, and progression, as well as monitoring an eventual disease-modifying treatment [5–7]. It is a component of neurofilaments (NFs) that, together with glial filaments, are the main types of intermediate filaments (IFs) of the nervous system [9–11]. Its physiological function is to confer mechanical stress resistance by preserving the characteristic cellular shape, intracellular traffic regulation between axons and dendrites, and, indirectly, nerve conduction speed modulation maintaining axon diameter [11]. Recent research suggests that they are also important for normal synaptic function [12]. Axonal dysfunction and degeneration are

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important steps in NDD pathogenesis, occurring long before neuronal cell death and often preceding detectable deposition of misfolded proteins [8]. During these processes, NFL is released into the extracellular space and, consequently, into body fluids, such as the cerebrospinal fluid (CSF) and blood.

Broadly speaking, mounting data reported increased CSF NFL levels in NDDs [5, 6, 13]. In the mid-1990s, through the first enzyme-linked immunosorbent assay (ELISA) developed for NFL, Rosengren and colleagues [14] demonstrated that CSF NFL concentration was increased in amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and vascular cognitive impairment (VCI). Although with different magnitudes, further studies revealed that CSF NFL increased also in other several NDDs, such as frontotemporal dementia (FTD) [15, 16], Parkinson's disease (PD) and atypical parkinsonisms (APs) [17, 18], Huntington's disease (HD) [19], mild cognitive impairment (MCI) [20, 21], and Creutzfeldt–Jakob disease (CJD) [22, 23], as well as in non-primary neurodegenerative disorders, such as multiple sclerosis (MS) [24], neuroinfectious conditions [25], traumatic brain injury [26], acute stroke [27], and cerebrovascular diseases [28]. Moreover, in NDDs, CSF NFL levels showed to correlate with poorer cognition, short survival times, brain atrophy, and disease severity and progression [29–31], supporting the notion that it could be useful not only as a diagnostic biomarker but also as a prognostic and progression biomarker [32]. As a result, it has been proposed as a dynamic biomarker for axonal degeneration [5, 6, 13] with the potential capacity to monitor treatment effectiveness [10, 33].

In the last few years, the interest in NFL research shifted toward blood. An ideal biomarker should be easily measurable, accurate, quantitative, reproducible, and employable to exactly categorize the population in line with a certain disease [34, 35]. To this end, blood-based biomarkers would have significant advantages in time efficiency and cost efficiency compared to CSF and neuroimaging [36, 37]. Moreover, they would offer potential applications at the population level as screening tools in primary care, as well as for longitudinal evaluations with repeated sampling during follow-up. It is not surprising that brain pathophysiological processes are reflected into the periphery. However, CSF proteins partially enters the blood flow, are subsequently diluted in a greater volume compared with CSF, and go through biochemical interactions with a large amount of plasma proteins. They are also cleared by blood cells and metabolized by other tissues. Finally, these processes overall hamper their measurement in plasma or serum using traditional techniques. Nevertheless, in the past few years, the development of analytical tools for ultrasensitive quantification—the immunomagnetic reduction (IMR) and the single molecule array (Simoa) techniques—by allowing an efficient measurement of NFL in blood [38], charted a tight correlation between CSF and blood NFL in different NDDs [39]. Therefore, blood NFL was suggested as a proxy of any neurodegenerative process, paving the way to its use in clinical practice as a reliable risk biomarker for neurodegeneration [40, 41]. Nonetheless, its potential application in real life remains unclear [42].

Biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic and pathogenic processes, or pharmacologic responses to a therapeutic intervention" [43]. From a clinical perspective, a biomarker can be also classified in further categories with some practical and conceptual overlaps: (1) antecedent biomarkers identifying a risk of disease development (*risk biomarkers*), (2) early biomarkers screening a subclinical condition (*screening biomarkers*), (3) biomarkers specifically recognizing a full-blown clinical picture (*diagnostic biomarkers*), (4) biomarkers categorizing disease severity (*staging biomarkers*), (5) biomarkers predicting future disease course (*prognostic biomarkers*), and (6) biomarkers predicting treatment response (*predicting or monitoring biomarkers*) [44]. Accordingly, it is crucial to define the context of use of a certain biomarker (primary care screening, diagnostic, risk of progression, disease monitoring, stratification for clinical trials, and pharmacodynamic or treatment response monitoring).

This review will attempt to summarize the current literature on blood (plasma or serum) NFL in NDDs, trying to translate research data in practical considerations, focusing on the context of use of blood NFL as a biomarker in the framework of the NDDs (Table 1).

Literature Research Methods

We conducted a systematic review of the literature until February 2020, using the key terms "NFL," "neurofilament light chain," and "neurofilament" to interrogate the PubMed database for articles published in English evaluating blood NFL concentrations (serum and plasma) in NDDs. Overall, we identified 38 studies. The use of internationally accepted clinical diagnostic criteria for each NDD, in particular AD [45–49], ALS [50–52], dementia with Lewy bodies (DLB) [53–55], FTD [56–58], PD [59–61], AP [62–65], and sporadic Creutzfeldt–Jakob disease (sCJD) [66, 67], has been checked out for any single study. The diagnostic performance of blood NFL concentrations to correctly allocate the participants to the different diagnostic groups was considered as follows: "excellent" (area under the ROC curve (AUROC) 0.90–1.00), "good" (AUROC 0.80–0.89), "fair" (AUROC 0.70–0.79), "poor" (AUROC 0.60–0.69), or "fail" (i.e., no discriminatory capacity) (AUROC 0.50–0.59) [68].

AD

AD is the most common form of dementia in the elderly, accounting for 50–70% of prevalent neurodegenerative

Table 1 Overview on the possible context of use of blood NFL as a biomarker in NDDs

	Diagnostic value			Prognostic value	Monitoring treatment
	Preclinical phase	Prodromal phase	Full-blown picture		
AD	±	+	+	+	±
PD	±	±	+	+	±
Atypical parkinsonisms (4R tauopathies)	±	±	+	+	±
DLB	±	±	±	±	±
FTD	−	±	+	±	±
ALS	±	±	+	±	±
CJD	±	+	+	−	±
HD	−	−	−	+	±
SMA	−	−	−	±	+
Sporadic late-onset ataxias	±	±	±	+	±
NDDs as a whole	±	+	+	+	±

Plus sign (+), potential use, supportive data are available; plus–minus sign (±), unknown; negative sign, negative evidences are available

AD Alzheimer's disease, *ALS* amyotrophic lateral sclerosis, *CJD* Creutzfeldt–Jakob disease, *DLB* dementia with Lewy body, *FTD* frontotemporal dementia, *HD* Huntington's disease, *NDD* neurodegenerative diseases, *PD* Parkinson's disease, *SMA* spinal muscular atrophy

dementia cases with an enormous health and economic impact [69]. The scientific and clinical research is nowadays shifting from dementia to the prodromal or even preclinical phases of AD to find effective therapeutic interventions that can delay or halt neurodegenerative progression [70, 71].

Biomarkers hold promise for improving early diagnosis in AD and establishing a tailored approach. The use of specific surrogate biomarkers (neuroimaging, blood [plasma/serum], and CSF) of AD pathology has been included in revised diagnostic criteria to distinguish AD from other forms of dementia since its early disease stages. However, postmortem studies demonstrate a high degree of neuropathologic heterogeneity in patients who received a clinical diagnosis of AD [72]. The pathogenesis of AD involves interacting pathophysiological cascades in which the deposition of amyloid plaques (Aβ) and the formation of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein would represent only the core events. The recently established "A/T/N" scheme proposes three binary biomarker categories which reflect AD pathophysiology, where "A" refers to Aβ pathology, "T" to tau pathology, and N to neurodegeneration [73, 74]. However, emerging evidence stresses the existence of additional molecular pathophysiological pathways, such as synaptic dysfunction and degeneration, innate immune response and neuroinflammation, vascular and cell membrane dysregulation, brain metabolic dysfunction, and axonal disruption [75]. The latter is prominent in AD, and it is more closely related to cognitive decline than Aβ pathology [76], thus leading to propose CSF NFL as a non-specific biomarker to detect early AD pathophysiological alterations [77]. In addition, an increased release of NFL molecules is a consequence of aging that contributes to an axonal degeneration due to subclinical cerebrovascular changes and neuronal atrophy [78]. In this regard, a recent prospective community-based study enrolling a cohort of cognitively intact subjects reported high variability of serum NFL levels above 60 years [79].

Several studies showed that CSF NFL levels are elevated in AD patients when compared with healthy controls (HCs) and higher NFL concentration is predictive of a rapid disease progression along core biomarkers of AD pathology [21]. Peripheral serum or plasma NFL strongly correlated with CSF NFL concentration, suggesting that it reflects the same pathological process [80]. In general, we found that the levels of NFL are higher in serum than in plasma, but the majority of studies used plasma to quantify NFL.

NFL as Diagnostic Biomarker

Current evidence revealed that plasma NFL allows to discriminate AD patients from HC subjects with a good/excellent diagnostic accuracy [77, 81] (Table 2). In addition, plasma NFL levels showed to be higher in the AD dementia group than in the MCI group and in Aβ-positive MCI patients than HC [77]. More recently, this finding has been replicated in a larger study in the Dominantly Inherited Alzheimer Network (DIAN) [82]. Other studies reported higher plasma/serum NFL levels in AD and MCI patients compared with controls [76, 83, 84], but with conflicting results about the differences between MCI and cognitively normal individuals. A recent meta-analysis by Wang and colleagues [85] confirmed these findings, supporting a possible contribute of plasma NFL in the AD diagnostic workup. Moreover, plasma NFL levels could also reflect NFT pathology (as determined by NFL immunostaining) and neurodegeneration at postmortem evaluation [76].

t2.1 **Table 2** The utility of NFL in the diagnostic and progression evaluation of neurodegenerative diseases

t2.2 First author (type of study)	Controls	Patients	Diagnostic/prognostic value of blood NFL	Notes
t2.3 AD				
t2.4 Preische, 2019 (longitudinal study)	HC (AD mutation non-carriers) $n = 162$	AD presymptomatic mutation carriers, $n = 243$	- Rate of change of serum NFL in differentiating non-mutation carriers vs presymptomatic mutation carriers, AUROC = 0.7 - Non-mutation carriers vs symptomatic non-mutation carriers, AUROC = 0.89 - Baseline serum NFL value in differentiating non-mutation carriers vs presymptomatic mutation carriers, AUROC = 0.49 (at the cutoff value of 37.4 pg/mL) - Non-mutation carriers vs symptomatic non-mutation carriers, AUROC = 0.85 (at the cutoff value of 27.9 pg/mL)	- Mutation carriers were subdivided into 3 groups: presymptomatic mutation carriers (individuals who scored 0 on the CDR scale across all visits), converters (CDR = 0 at baseline and CDR > 0 at subsequent visits), and symptomatic mutation carriers (CDR > 0 across all visits)
t2.5 Lewczuk, 2018 (cross-sectional study)	HC = 41	MCI-AD = 25 ADD = 33 AD = 58	- Plasma NFL in differentiating patients vs HC, AUROC = 0.85 (95% CI 0.772–0.934), unadjusted for other variables - AD vs HC, AUROC = 0.92 (95% CI 0.869–0.970), adjusted for age - At the cutoff maximizing Youden index, 25.7 pg/mL, the unconditional accuracy was 0.82	- MCI-AD were AD patients at the stage of MCI. - ADD were AD patients at the stage of early dementia. - AD included MCI-AD + AD patients
t2.6 Mattsson, 2017 (longitudinal study)	HC = 193	AD = 180 MCI = 197 pMCI = 109 sMCI = 65	Plasma NFL in differentiating AD vs CNC, AUROC = 0.87 (correcting for age, sex, educational level, and *APOE* ε4 genotype). When only correcting for age, sex, and educational level, the AUROC was reduced to 0.79	- Patients were recruited from the ADNI cohort. - MCI patients were divided into stable MCI (sMCI, with no progression to dementia during ≥ 2-year follow-up) and progressive MCI (pMCI, with conversion to dementia)
t2.7 FTD				
t2.8 van der Ende, 2019 (longitudinal study)	Non-carriers of a mutation in GRN, C9orf72, or MAPT = 127	Presymptomatic carriers of a mutation in GRN, C9orf72, or MAPT = 149 FTD (symptomatic carriers of a mutation in GRN, C9orf72, or MAPT) = 59	- Serum NFL in differentiating symptomatic mutation carriers vs presymptomatic mutation carriers, AUROC = 0.93 (95% CI 0.90–0.97), at the cutoff concentration of 17 pg/mL - Symptomatic mutation carriers vs non-mutation carriers, AUROC = 0.95 (95% CI 0.92–0.98), at the cutoff concentration of 17 pg/mL - Baseline serum NFL converters vs presymptomatic mutation carriers, AUROC = 0.93 (95% CI 0.89–0.98), at the cutoff level of 15.0 pg/mL	- Multicenter cohort study on families with genetic FTD in Europe and Canada - At least 2 serum samples were taken with a time interval of 6 months or more. - 9 presymptomatic carriers became symptomatic during follow-up (converters). - Presymptomatic carriers or non-carrier mutations were healthy relatives of first degree at risk of FTD
t2.9 Katisko, 2019 (longitudinal study)		FTD = 91 (bvFTD = 66; nfvPPA = 16; svPPA = 4; FTD-MND = 5) PPD = 34	- Serum NFL in differentiating FTD vs PPD, AUROC = 0.85 (95% CI 0.776–0.923), at the cutoff level of 19.9 pg/mL	- The PPD group included patients with a severe late-onset psychiatric disorder (psychotic, mood disorders, or both).

t2.10 **Table 2** (continued)

	First author (type of study)	Controls	Patients	Diagnostic/prognostic value of blood NFL	Notes
				- bvFTD vs PPD, AUROC = 0.82 (95% CI 0.732–0.908), at the cutoff level of 19.9 pg/mL	- In the FTLD group, 26 patients had a definite diagnosis due to the C9orf72 repeat expansion
t2.10	Al Shweiki, 2019 (cross-sectional study)	HC = 27	bvFTD = 20 Schizophrenia = 11 Depression = 28 Bipolar disorder = 11	- Serum NFL in differentiating bvFTD vs HC, AUROC = 0.94 (95% CI 0.87–0.99) - bvFTD vs depression, AUROC = 0.89 (95% CI 0.8–0.98), at a cutoff level above 35.7 pg/mL - bvFTD vs bipolar disorder, AUROC = 0.94 (95% CI 0.81–1.01), at a cutoff level > 26.5 pg/mL - bvFTD vs schizophrenia, AUROC = 0.9 (95% CI 0.77–1.03), at a cutoff level > 17.7 pg/mL	- 20 bvFTD consisting of 9 possible bvFTD, 5 probable bvFTD, and 6 genetic bvFTD (4 due to C9orf72 mutations, 2 MAPT mutations) - Serum NFL levels did not differ between psychiatric and control patients
t2.11	Steinacker, 2018 (longitudinal study)	HC = 15	bvFTD = 74 AD = 26 (excluding patients with normal Aβ42) AD = 11 (without the typical AD CSF biomarker pattern) MCI = 17 Subgroups were defined on the basis of both CSF Aβ42 and tau levels: - 30 bvFTD (excluding patients with decreased Aβ42) - 31 bvFTD (excluding patients with decreased Aβ42 and increased tau or p-tau)	- Serum NFL in differentiating bvFTD vs AD, AUROC = 0.67 - bvFTD vs MCI, AUROC = 0.9 (95% CI 0.84–0.97) - bvFTD vs HC, AUROC = 0.85 (95% CI 0.72–0.97) - bvFTD vs AD subgroups selected based on CSF Aβ42 levels, AUROC = 0.79, at a cutoff value of 33 pg/mL - bvFTD vs AD subgroups selected based on both CSF Aβ42 and tau/p-tau levels, AUROC = 0.77, at a cutoff value of 34.3 pg/mL	- Genetic screening of 67 patients with bvFTD revealed 7 carriers for C9orf72 repeat expansion. - Analysis of MAPT and GRN in 18 patients revealed 1 carrier of the MAPT mutation and 1 carrier of the GRN mutation
t2.12	Steinacker, 2017 (longitudinal study)	HC = 35	PPA = 99 (nfvPPA = 40, svPPA = 38, lvPPA = 21)	- Serum NFL in differentiating AUROC nfvPPA/svPPA vs HC, AUROC = 0.84 (95% CI 0.69–0.99), at a cutoff value of 25 pg/mL - nfvPPA/svPPA vs lvPPA, AUROC = 0.76 (95% CI 0.64–0.88), at a cutoff level of 31 pg/mL	- Patients with PPA met only clinical diagnostic criteria without knowledge of fluid biomarker concentration
t2.13	Wilke, 2016 (cross-sectional study)	HC = 46	FTD = 41 ALS = 25	- Serum NFL in differentiating FTD vs HC, AUROC = 0.81 (95% CI 0.72–0.91) at an optimal cutoff value > 36 pg/mL - ALS vs HC, AUROC = 0.99 (95% CI 0.98–1.00)	
t2.14	Meeter, 2016 (longitudinal study)	HC = 71	FTD (caused by a pathogenic mutation in *GRN*, *MAPT*, or C9orf72) = 101	- Serum NFL in differentiating FTD vs HC, AUROC = 0.97 (95% CI 0.93–1.00) - FTD vs presymptomatic carriers, AUROC = 0.93 (95% CI 0.87–0.98), at the cutoff level of 18.0 pg/mL	- Participants were recruited as part of GENFI or ascertained before participation in GENFI. - 4 subjects became symptomatic during follow-up (converters)

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t2.15 **Table 2** (continued)

First author (type of study)	Controls	Patients	Diagnostic/prognostic value of blood NFL	Notes
		Presymptomatic carriers of a pathogenic mutation = 62	- Presymptomatic carriers vs HC, AUROC = 0.63 (95% CI 0.51–0.75), at the cutoff level of 8.3 pg/mL - Serum NFL was also associated with survival (HR in the higher tertile 3.10, 95% CI 1.09–8.76)	
ALS				
Thouvenot, 2019 (longitudinal study)	HC = 21	ALS = 198	- Serum NFL > 15.0 pg/mL in discriminating ALS vs HC, AUROC = 0.99 (95% CI 0.972–0.0999) - Patients with serum NFL levels ≥ 71.2 pg/mL had a higher risk of death (HR 4.7, 95% CI 3.0–7.4)	- Patients were prospectively followed up to 18.5 months
Verde, 2019 (longitudinal study)	Non-neurodegenerative controls = 50	ALS = 124 Disease controls = 44 FTD = 20 AD = 20 PD = 19 CJD = 6	- A cutoff NFL level of 49 pg/mL in differentiating ALS vs non-neurodegenerative controls, AUROC = 0.97 (95% CI 0.95 to 0.991) - A cutoff value of 62 pg/mL in differentiating ALS vs disease controls, AUROC = 0.87 (95% CI 0.81 to 0.935) - A cutoff value of 62 pg/mL in discriminating ALS vs all other categories considered together, AUROC = 0.88 (95% CI 0.849 to 0.926) - Patients with serum NFL above the median (125 pg/mL) had a shorter survival than patients with NFL ≤ 125 pg/mL, with an HR of 2.39 (95% CI 1.236 to 4.63)	- Disease controls included patients with conditions included in the differential diagnosis of ALS
Gille, 2018 (longitudinal study)		ALS = 149 (C9orf72 = 15, FTD = 15, PLS = 11, PMA = 6) ALS mimic = 19 Disease controls = 82 (GBS, CIDP, HSP)	- Serum NFL in differentiating ALS vs ALS mimic, AUROC = 0.85 (95% CI 0.79–0.90), at an optimal cutoff value of 93 pg/mL - ALS fast progressor vs ALS slow progressors, AUROC = 0.87 (95% CI 0.76–0.94), at an optimal cutoff value of 159 pg/mL - ALS vs GBS + CIDP, AUROC = 0.58 (95% CI 0.51–0.64), at an optimal cutoff value of 139 pg/mL - ALS vs HSP, AUROC = 0.84 (95% CI 0.78–0.90), at an optimal cutoff value of 55 pg/mL - ALS vs PLS, AUROC = 0.89 (95% CI 0.83–0.93), at an optimal cutoff value of 88 pg/mL - ALS vs PMA, AUROC = 0.71 (95% CI 0.63–0.78), at an optimal cutoff value of 86 pg/mL - ALS patients with serum NFL in the upper tertile had a HR = 5.34 (95% CI 1.39–20.56) vs patients with serum NFL in the low tertile.	- A subset of 16 ALS repeated serum sample showing a relative stability of NFL concentrations over time

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t2.19 **Table 2** (continued)

	First author (type of study)	Controls	Patients	Diagnostic/prognostic value of blood NFL	Notes
				- ALS patients with serum NFL in the mid-tertile had a HR = 4.47 (95% CI 1.08–18.63) vs patients with serum NFL in the low tertile	
t2.19	Feneberg, 2018 (cross-sectional study)		ALS onset ≤ 6 months = 54 ALS onset > 6 months = 135 Other MNDs (PLS, SMA, Kennedy disease) = 21 ALS mimics = 27 Neurologic disease controls = 60	- Serum NFL in differentiating early ALS vs neurologic disease controls, AUROC = 0.92 (95% CI 0.85–0.99, cutoff = 128 pg/mL - Early ALS vs MND mimics, AUROC = 0.99 (95% CI 0.97–1, cutoff = 97 pg/mL) - Late ALS vs neurologic disease controls, AUROC = 0.9 (95% CI 0.83–0.97, cutoff = 116 pg/mL) - Late ALS vs MND mimics, AUROC = 0.97 (95% CI 0.94–1, cutoff = 95 pg/mL)	- A minimum 3-month follow-up - Serum NFL concentrations were not significantly different between early and later symptomatic phases
t2.20	Steinacker, 2016 (longitudinal study)	HC = 28	ALS = 125	High NFL concentrations were associated with shorter survival (at least a 6-month follow-up) [numbers are not reported]	- Patients were subgrouped according to the progression rate at first examination into slow, intermediate, and fast progressors. - Blood NFL measures were taken every 6 months up to 3 years of follow-up and were stable over time
t2.21	Lu, 2015 (longitudinal study)	HC = 78 (London cohort = 42; Oxford cohort = 36)	ALS = 167 (London cohort = 103; Oxford cohort = 64)	- Serum NFL in differentiating ALS vs HC, AUROC = 0.86, at a cutoff level of 36 pg/mL (Oxford cohort) - Plasma NFL in differentiating ALS vs HC, AUROC = 0.87, at a cutoff level of 36.2 pg/mL (London cohort) - Patients with serum NFL (Oxford cohort) in the highest tertile had a HR = 6.05 (95% CI 1.68–21.87). - Patients with plasma NFL (London cohort) in the highest tertile had a HR = 3.78 (95% CI 1.68–8.50). - Patients with serum NFL (Oxford cohort) in the middle tertile had a HR = 2.68 (95% CI 0.87–8.27). - Patients with plasma NFL (London cohort) in the middle tertile had a HR = 1.91 (95% CI 0.86–4.23). - Patients with combined blood NFL in the highest tertile had a HR = 3.82 (95% CI 1.98–7.39). - Patients with combined blood NFL in the middle tertile had a HR = 2.08 (95% CI 1.09–3.97)	- Multicenter study (2 cohorts) - Authors also conducted analyses of the 2 cohorts combined, using the corresponding NFL data (serum or plasma) from each cohort, using cohort-specific tertile cutoff levels, and adjusting Cox regression and Kaplan–Meier survival analyses by center
t2.22	Gaiottino 2013 (cross-sectional study)	HC = 67	ALS = 46 AD = 20 GBS = 19	Serum NFL in differentiating ALS vs HC, with sensitivity of 91.3% and specificity of 91% at a cutoff level of 26.6 pg/mL	- Neurological control patients consisted of patients suffered from tension-type headache ($n = 21$), lower back pain ($n = 7$), psychiatric

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t2.23 **Table 2** (continued)

First author (type of study)	Controls	Patients	Diagnostic/prognostic value of blood NFL	Notes
		Neurological control patients = 68		disorders (n = 26), or miscellaneous non-specific symptoms for which no neurological explanation could be found (n = 14)
t2.23 Parkinsonian syndromes and degenerative ataxias				
t2.24 Oosterveld, 2020 (cross-sectional study)	HC = 52	PD = 139	Serum NFL in differentiating PD vs HC, AUROC = 0.64 (95% CI 0.55–0.73), at the cutoff value of 15.6 pg/mL	- The inclusion of serum NFL in a panel of CSF biomarkers (phosphorylated α-synuclein/total α-synuclein ratio and oligomeric α-synuclein/total α-synuclein ratio) yielded a sensitivity of 91% and a specificity of 81% (AUC 0.90, 95% CI 0.83–0.97)
t2.25 Ng, 2020 (longitudinal study)	HC = 50	PD = 149 (76 PD patients, 30 TD and 46 PIGD, had plasma NFL measured at the 2-year mark)	- Baseline plasma NFL in differentiating PD vs HC, AUROC = 0.83 (95% CI 0.77–0.89) - At year 2, plasma NFL in discriminating TD subtype vs PIGD subtype, AUROC = 0.65 (95% CI 0.53–0.77)	- Participants were included in the Early Parkinson's Disease Longitudinal Singapore Study which is an ongoing prospective cohort study analyzing the progression of early PD over a follow-up period of 5 years. - PD patients were classified into motor subtypes of "tremor dominant" (TD), "postural instability and gait disorders" (PIGD), or "indeterminate" based on MDS-UPDRS part II and III components
t2.26 Marques, 2019 (cross-sectional study)	HC = 53	PD = 55 AP (MSA = 22, PSP = 7) = 29	- Serum NFL in differentiating AP vs PD, AUROC = 0.91 (95% CI 0.83–0.98), at the cutoff value of 14.8 ng/L - AP vs HC, AUROC = 0.88 (95% CI 0.80–0.96), at the cutoff value of 13.6 ng/L	- Patients were recruited when the clinical diagnosis was still uncertain; definite clinical diagnosis was established after 3 years of follow-up and updated again after a maximum of 12 years of follow-up data
t2.27 Li, 2019 (cross-sectional study)	HC = 100 consisting of 2 cohorts: - Cohort A = 9 - Cohort = 91	Manifest SCA-3 = 107, consisting of 2 cohorts: - Cohort A = 17 - Cohort B = 90 Preclinical SCA-3 = 26 (all included in the cohort B)	- Serum NFL in differentiating manifest SCA-3 vs HC, AUROC = 0.98 (95% CI 0.96–1.00), at the cutoff value of 20 pg/mL - Preclinical SCA-3 vs HC, AUROC = 0.83 (95% CI 0.72–0.95), at the cutoff value of 10 pg/mL	- Participants in cohort B were classified into 3 subgroups according to the SARA scores: 26 preclinical SCA-3 individuals, 46 stage 1 SCA-3 patients, and 44 stage 2 SCA-3 patients
t2.28 Lin, 2019 (longitudinal study)	HC = 40	PD = 116 MSA = 22	- Plasma NFL in differentiating MSA vs PD, AUROC = 0.80, at the cutoff value of 24.06 pg/mL - PD vs HC, AUROC = 0.75, at the cutoff value of 12.34 pg/mL - Higher baseline NFL levels were associated with a higher risk of motor symptom progression (adjusted HR 1.03, 95% CI 1.01–1.07) and cognition progression (adjusted HR 1.03, 95% CI 1.01–1.05)	- PD patients with baseline NFL levels > 21.84 pg/mL were at higher risk of motor symptom progression. - PD patients with baseline NFL levels > 18.34 pg/mL were at higher risk of cognitive decline

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t2.29 **Table 2** (continued)

	First author (type of study)	Controls	Patients	Diagnostic/prognostic value of blood NFL	Notes
t2.30	Donker Kaat, 2018 (longitudinal study)	HC = 95	PSP = 131	- Serum NFL in differentiating PSP vs HC, AUROC = 0.87 (95% CI 0.83–0.92), at the cutoff value of 38.3 pg/mL - Higher NFL levels were associated with reduced survival (adjusted HR 1.5, 95% 1.1–1.9)	- Diagnosis of PSP was made according to NINDS-SPSP criteria (2003–2014); 23 patients had pathological confirmation
t2.31	Wilke, 2018 (cross-sectional study)	HC = 45	c-MSA = 25 SAOA = 25 SCA = 20	- Serum NFL in differentiating c-MSA vs SAOA, AUROC = 0.74 (95% CI 0.59–0.89) - SCA vs HC, AUROC = 0.91 (95% CI 0.81–1.00)	- SCA patients consisted of SCA-1 ($n = 6$), SCA-2 ($n = 3$), SCA-3 ($n = 8$), and SCA-6 ($n = 3$)
t2.32	Hansson, 2017 (longitudinal study)	- Lund cohort HC = 53 - London cohort HC = 26	- Lund cohort PD = 171 - Lund cohort AP = 54 (MSA = 30, PSP = 19, CBD = 5) - London cohort PD = 20 - London cohort AP = 71 (MSA = 30, PSP = 29, CBD = 12) - Early-stage disease cohort PD = 53 - Early-stage disease cohort AP = 56 (MSA = 28, PSP = 22, CBD = 6)	- Blood NFL in differentiating PD vs AP, AUROC = 0.91 (95% CI 0.87–0.95) (Lund cohort) - PD vs AP, AUROC = 0.85 (95% CI 0.72–0.98) (London cohort) - PD vs AP, AUROC = 0.81 (95% CI 0.73–0.90) (early-stage disease cohort)	- The study included 2 independent prospective cohorts of PD and AP patients and HC: the Lund ($n = 278$) and London ($n = 117$) cohorts. - The third cohort consisted of PD and AP patients with a disease duration < 3 years (early-stage disease cohort, $n = 109$)
t2.33	HD				
t2.34	Byrne, 2018 (cross-sectional study)	HC = 20	HTT mutation carriers (premanifest HD = 20; manifest HD = 40) = 60	- Plasma NFL in differentiating HTT mutation carriers vs HC, AUROC = 0.91 (95% CI 0.85–0.97) - Plasma NFL in differentiating premanifest HD vs manifest HD, AUROC = 0.93 (95% CI 0.86–0.99)	
t2.35	Byrne, 2017 (longitudinal study)	HC = 97	*HTT* mutation carriers (premanifest HD = 104, manifest HD = 97) = 201 They were divided into: - Early premanifest HD = 58 - Late premanifest HD =46 - Manifest HD stage 1 = 66 - Manifest HD stage 2 = 31	Higher baseline plasma NFL levels in premanifest HD were associated with clinical disease onset during the 3-year follow-up period, adjusted HR 3.03, 95% CI 1.07–8.60	- Participants were enrolled in the TRACK-HD* study. - At enrolment, participants with *HTT* CAG expansion mutations were classified as having premanifest or manifest HD based on the UHDRS-TMS. - 18 (17%) subjects with premanifest disease at baseline were newly diagnosed as having manifest HD during the 3 years of follow-up
t2.36	CJD				
t2.37	Staffaroni et al., 2019 (longitudinal study)		sCJD = 188	Higher baseline levels of plasma NFL levels were associated with shorter survival, HR 2.08 (95% CI 1.22–3.54)	Participants included patients with probable and definite (pathology-proven) sCJD with PRNP codon 129 polymorphism data available

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t2.38 **Table 2** (continued)

	First author (type of study)	Controls	Patients	Diagnostic/prognostic value of blood NFL	Notes
t2.39	Thompson, 2018 (cross-sectional study)	HC = 24	sCJD = 45	Serum NFL in differentiating sCJD vs HC, AUROC = 1.0	
t2.40	Kovacs, 2017 (cross-sectional study)	HC = 18	sCJD = 65 gCJD = 21 AD = 21 Other neurological pathologies = 25	- Plasma NFL in differentiating sCJD vs HC, AUROC = 0.99 (95% CI 0.98–1.0) - gCJD vs HC, AUROC = 1.0 (CI 1.0–1.0) - AD vs HC, AUROC = 0.99 (95% CI 0.97–1.0) - Other neurological disorders vs HC, AUROC = 0.96 (CI 0.90–1.0)	- The present study included 132 autopsy cases with rapidly progressive neurological syndromes. - Cases with a wide range of neuropathological alterations, including cerebrovascular disease, inflammation (meningoencephalitis), primary age-related tauopathy, non-AD tauopathies or Lewy body pathology
t2.41	Steinacker, 2016 (cross-sectional study)	HC = 40	sCJD = 33 gCJD = 9 GSS mutation carrier = 1 Demented controls (DCo) = 20	Serum NFL in differentiating sCJD + gCJD vs HC + DCo, AUROC = 0.95	- 39 CJD diagnoses were neuropathologically verified. - Patients with other diseases included AD ($n = 12$), MCI ($n = 4$), FTD ($n = 3$), and NPH ($n = 1$)

Aβ42 amyloid β-peptide 1–42; *AD* Alzheimer's disease; *ADNI* Alzheimer's Disease Neuroimaging Initiative; *ALS* amyotrophic lateral sclerosis; *AP* atypical parkinsonism; *APOE* apolipoprotein E; *AUROC* area under the receiver operating curve; *bvFTD* behavioral variant of frontotemporal dementia; *c-MSA* cerebellar variant MSA; *CBD* corticobasal degeneration; *CDR* cognitive dementia rating; *CI* confidential interval; *CIDP* chronic inflammatory demyelinating polyneuropathy; *CJD* Creutzfeldt–Jakob disease; *CSF* cerebrospinal fluid; *C9ORF72* chromosome 9 open reading frame 72; *DLB* dementia with Lewy body; *FTD* frontotemporal dementia; *FTD-MND* frontotemporal dementia with ALS; *gCJD* genetic Creutzfeldt–Jakob disease; *GBS* Guillain-Barre syndrome; *GENFI* genetic FTD initiative; *GSS* Gerstmann–Straussler–Scheinker disease; *GRN* progranulin; *HC* healthy controls; *HD* Huntington's disease; *HR* hazard ratio; *HSP* hereditary spastic paraplegia; *HTT* huntingtin; *lvPPA* logopenic variant PPA; *MAPT* microtubule-associated protein tau; *MCI* mild cognitive impairment; *MCI-AD* MCI converters to AD; *MDS-UPDRS* Movement Disorder Society-Unified Parkinson's Disease Rating Scale; *MND* motor neuron disease; *MSA* multiple system atrophy; *NFL* neurofilament light chain; *nfvPPA* non-fluent variant PPA; *NINDS-SPSP* National Institute of Neurological Disorders and Stroke and the Society for PSP; *NPH* normal-pressure hydrocephalus; *PD* Parkinson's disease; *PLS* primary lateral sclerosis; *PPA* primary progressive aphasia; *PMA* primary muscular atrophy; *PPD* primary psychiatric disorders; *PRNP* prion protein; *PSP* progressive supranuclear palsy; *p-tau* phospho-tau; *sCJD* sporadic Creutzfeldt–Jakob disease; *SAOA* sporadic adult-onset ataxia; *SARA* Scale for the Assessment and Rating of Ataxia; *SCA* spinocerebellar ataxia; *SMA* spinal muscular atrophy; *svPPA* semantic variant PPA; *UHDRS* Unified Huntington's Disease Rating Scale-Total Motor Score

*TRACK-HD is a multinational prospective observational study of HD that examines clinical and biological findings of disease progression in individuals with premanifest HD and early-stage HD

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NFL as Staging and Prognostic Biomarker

Studies in AD and MCI-AD patients found a correlation between plasma NFL concentration and cognitive impairment, MRI hippocampal volume loss and brain atrophy, and cerebral ^{18F}FDG-PET hypometabolism [76, 81–87]. Moreover, higher plasma NFL levels predicted faster cognitive deterioration and a higher rate of brain atrophy and hypometabolism in MCI patients over time [77]. Baseline plasma and CSF NFL levels were similarly associated with short-term declines in imaging measures of neurodegeneration and with global cognitive worsening, but not with change in amyloid ligand retention on PET [35], differently from CSF t-tau concentration that critically depends on cerebral Aβ burden [88]. Instead, increased plasma NFL was related to baseline and longitudinal glucose hypometabolism, which is an unspecific neurodegeneration marker, in AD-related regions of MCI Aβ+ individuals [87].

In a longitudinal analysis of NFL plasma levels in a large cohort of subjects enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI), Mattsson and colleagues [82] found increasing rates of NFL changes from preclinical AD stage to frank AD dementia through prodromal phase, suggesting NFL as a dynamic biomarker sensitive to AD disease progression. Of note, longitudinal NFL variations correlated with several baseline AD-related features (CSF biomarkers, imaging measures, and cognition) in the whole population, though with significant differences regarding clinical stage. Actually, the strictest associations were reported in MCI participants. Longitudinal NFL level was generally increased in patients who were classified as N+ (using temporal brain atrophy as N− indicator) and in those who were only T+. Therefore, NFL might reflect a neurodegenerative process that occurred independently from Aβ pathology. Noteworthy, the NFL rate of change, rather than NFL absolute concentration, was subject to a significant increase in mutation carriers compared with non-carriers. Moreover, the NFL rate of change strongly correlated with longitudinal precuneus cortical thinning in both symptomatic and presymptomatic mutation carriers [89].

NFL as Risk/Screening Biomarker

Blood NFL levels seem to predict the progression to AD dementia in patients with subjective memory complaints [77]. An association between regional hypometabolism in the right hippocampus and higher plasma NFL levels was reported in cognitively normal participants from the ADNI database [87]. Hu and colleagues [90] explored the predictive role to develop AD of plasma NFL at the preclinical stage. Interestingly, plasma NFL concentrations were already abnormally high in cognitively normal individuals with significant Aβ-related pathological changes. Baseline plasma NFL levels did not differ in normal elderly volunteers who remain cognitively intact during the follow-up, independently from an initial amyloid PET positivity status. Instead, a trend toward elevated plasma NFL concentration was observed in Aβ+ individuals with subjective memory complaints compared to subjects without memory complaints who were Aβ+, and plasma NFL resulted to be inversely associated with cognitive performance [91]. By contrast, other groups investigated the correlation between serum NFL levels with cerebral metabolism in MCI patients. Regional hypometabolism in bilateral parahippocampal gyri, right fusiform, and middle temporal gyri was independently predicted by plasma NFL [92].

Weston and colleagues [93] reported increased serum NFL concentrations also in symptomatic and presymptomatic familial AD (FAD) mutation carriers, showing a significant correlation with the estimated years to/from symptom onset across all mutation carriers as well as with cognitive decline and MRI atrophy. This finding suggests that increases in serum NFL precede the onset of AD symptoms. A large study in the DIAN cohort confirmed NFL as a sensitive marker of early neurodegeneration, finding significant increased serum NFL levels in AD mutation carriers (Aβ precursor protein (APP) or presenilin 1 (PSEN1) or presenilin 2 (PSEN2)) 16 years before disease onset [89]. The rate of change of serum NFL peaked in mutation carriers during the conversion phase to clinically evident cognitive impairment and reached a plateau in symptomatic carriers; absolute values of NFL showed a trend toward a slow increase over time (Table 2). Interestingly, the increase in plasma NFL concentration during the follow-up (15–30 months) in 79 elderly participants without dementia, including 15 subjects with MCI, was associated with a significant decline in both attention and global cognition and with an increase in cerebral amyloid PET uptake [32]. More recently, baseline serum NFL was shown to be a strong and independent predictor of brain volume loss and subtle cognitive changes in a longitudinal study cohort of neurologically intact individuals [79].

NFL as Predictive Biomarker

Although not yet in humans, transgenic mice models treated with a β-secretase (BACE) inhibitor showed beneficial effects on AD-relevant downstream markers, including reduced plasma NFL concentrations [30].

FTD

The term FTD indicates a heterogeneous spectrum of NDDs inexorably conveying to a dementia syndrome characterized by predominant behavioral—behavioral FTD (bvFTD) [94]—or language—primary progressive aphasia (PPA) [58]—

impairment. It is the third most common neurodegenerative dementia after AD and DLB and is typically diagnosed in middle age [95]. bvFTD is the most prevalent phenotype (55–60% of cases) whereas PPA (40–45% of cases) can be further classified as non-fluent/agrammatic variant (nfvFTD) and semantic variant (svFTD) [96]. Notably, an additional clinical PPA variant, named logopenic variant, can show AD pathological features in more than half of subjects [97]. Finally, a clinical overlap between FTD and ALS is described and about 10–15% of cases with ALS report a dementia syndrome in the FTD spectrum (ALS-FTD) [98]. The underlying pathologies in FTD, essentially abnormal accumulations of either tau or TAR DNA-binding protein 43 (TDP-43) proteins, may be recognized only on postmortem examination. These misfolded protein aggregates lead to atrophy (structural MRI) and hypometabolism (^{18F}FDG-PET) of frontal and/or anterior temporal lobes depending on phenotype [41].

Unfortunately, with the exception of causative mutations in genetic forms (about 10% of cases: hexanucleotide repeat expansions near the chromosome 9 open reading frame gene (C9orf72), progranulin (GRN), and microtubule-associated protein tau (MAPT) [99]), CSF poly(GP) detection in C9ORF72 expansion carriers, and decreased CSF/blood progranulin levels in GRN mutation carriers, specific pathophysiological biomarkers for FTD are completely lacking. However, the diagnosis of FTD remains very challenging, especially for behavioral variant as its diagnosis is mainly based on clinical assessment and because its symptoms show a significant overlap with primary psychiatric disorders [100].

In this context, blood NFL is a promising candidate biomarker for FTD, especially for disease differential diagnosis, monitoring, and prognosis [101, 102]. Recent evidence proved that blood and CSF levels of NFL are tightly related and significantly higher in FTD subjects than in HC [103], without gender differences [104]. Furthermore, although NFL levels generally increase with aging, this association seems not present for FTD patients [16, 105–107]. Blood NFL levels tightly correlate with CSF values in almost all studies measuring this biomarker in both fluids, thus suggesting that the peripheral concentrations of NFL substantially reflect the pathophysiological modification within CNS leading to NFL increase in CSF.

NFL as Diagnostic Biomarker

Serum NFL levels distinguished FTD patients from controls with good/optimal diagnostic accuracy (Table 2) [105, 108–110]. Interestingly, this biomarker was higher in bvFTD individuals in comparison with psychiatric patients affected by depression, schizophrenia, and bipolar disorders [106], in which bvFTD misdiagnosis is common [100]. The diagnostic accuracy to differentiate bvFTD from psychiatric disorders was above 80% (Table 2), independently from the specific psychiatric condition (mood or psychotic disorders) [106, 107].

Only one study compared both FTD and AD subjects, reporting that serum NFL levels were higher in bvFTD patients [109], and separated the two groups with a sensitivity and specificity of 93% and 61%, respectively (Table 2), after a preliminary exclusion of bvFTD patients with an AD biomarker profile and clinical AD subjects without a core biomarker confirmation. Moreover, serum NFL concentrations seemed to be higher in nfvPPA and svPPA than lvPPA subjects [105], though with only moderate accuracy. A further study showed no significant differences between PPA subtypes [110].

Finally, although FTD individuals had higher serum NFL levels than subjects with other cognitive disorders such as AD, ALS patients reported even more elevated concentration [108]. Notably, ALS subjects present a TDP-43 pathology in about 95% of cases, suggesting a potential association between increased NFL concentration and TDP-43 pathology. Further confirmation of this hypothesis is supported by observing that FTD forms expected to be TDP-43 positive (C9ORF72 and GRN mutation carriers, or svPPA phenotype) reported higher concentration of this biomarker than FTD subtypes expected to be tau positive (MAPT mutation carriers and nfvPPA) [16, 99, 104].

NFL as Staging and Prognostic Biomarker

In different studies, serum NFL increases overtime in FTD subjects, independently from the phenotypes, with the exception of lvPPA [105, 109]. A longitudinal Mini-Mental State Examination (MMSE) decline was correlated with baseline serum NFL levels [107]. Disease duration was not associated with NFL concentration although one study reported a poor survival in FTD subjects in the higher tertile of serum NFL levels (Table 2).

Additionally, peripheral blood NFL concentrations appeared to reflect specific regional brain atrophy related to clinical phenotypes (PPAs or bvFTD). In bvFTD patients, serum NFL was associated with a low cognitive score and a reduction in whole-brain volume and was correlated with brain atrophy, including frontal and subcortical regions [99, 104, 109, 110].

Serum NFL was correlated with baseline cognitive impairment, cognitive decline overtime, and atrophy progression of the left frontal lobe and the right middle frontal gyrus in PPA individuals and nfvPPA/svPPA subjects, respectively [105, 107]. On the other hand, although only in one study, serum NFL concentration was not related to specific hypometabolic regions on ^{18F}FDG-PET in PPA subjects [110].

NFL as Risk/Screening Biomarker

Noteworthy, serum NFL was higher in genetic FTD with a full-blown clinical picture but not in presymptomatic carriers

compared to HC, independently from the gene mutation. Moreover, it was consistently higher in converters than in non-converter carriers [104] and increased overtime in converters but not in symptomatic FTD as well as non-converters. Therefore, serum NFL could differentiate genetic FTD patients from presymptomatic carriers, with an excellent diagnostic accuracy (Table 2) [16]. By contrast, the discriminatory accuracy of the biomarker relative to presymptomatic genetic carriers and controls resulted quite poor (Table 2).

ALS

ALS is a progressive neurological disease in which upper motor neuron (UMN) and lower motor neuron (LMN) degenerate, leading to paralysis and death, typically within 3–5 years from symptom onset. To date, there is no definitive diagnostic test for ALS, and confirmation of diagnosis is based on clinical findings, electromyography results, and exclusion of mimics [52]. Despite efforts to increase the sensitivity of diagnostic criteria, often the diagnosis is made only after the onset of symptoms for both sporadic and familial ALS [111, 112]. An early diagnosis would be paramount, since it was observed that the benefit of riluzole is related to its early administration.

A large body of research exists on neurochemical ALS biomarkers [113, 114], among which phosphorylated neurofilament heavy chain (pNFH) and NFL have been postulated as the most interesting candidates [115, 116]. This is not surprising, given the axonal impairment that characterizes the disease already at the early stage, with a large release of NF in CSF [117]. Additionally, previous studies indicate cytoskeletal proteins as one of the key factors contributing to neurodegeneration in ALS [118–123]. Other evidences provide additional support that NFL aggregation is an early event in motor neuron disease [124], and that NFL is involved in the aggregation and neurotoxicity of other proteins in motor neurons [125]. At the same time, elevated NFL levels in ALS may be explained by the higher content of axonal proteins in motor neurons compared to other neuronal populations [126]. Nonetheless, several data highlighted that NFL is able to discriminate ALS patients from healthy and disease controls [14, 117, 127, 128], especially in cases with predominant UMN signs, and correlates with clinical disability [129], disease stage, progression, and/or prognosis [130, 131], probably reflecting the burden of motor neuron degeneration. Although CSF NFL remains the more robust fluid biomarker for ALS because of its directly reflecting alterations in the CNS, a high correlation between CSF and blood NFL concentrations has been reported [132]. Moreover, at odds with other neurodegenerative diseases such AD, NFL concentrations do not correlate with age in ALS individuals [133–135].

NFL as Diagnostic Biomarker

Mounting evidence reports significantly higher blood NFL levels in ALS patients when compared to controls (Table 2) [40, 135–138]. The diagnostic performance of serum NFL in discriminating ALS and non-neurodegenerative subjects showed excellent sensitivity and specificity (Table 2) [139, 140]. These findings led authors to propose the introduction of serum NFL measurement into clinical practice as supportive diagnostic tool. In addition, serum NFL showed significantly elevated concentration in ALS even at the onset of the first symptoms, confirming its potential role as a biomarker for early detection of symptomatic sporadic ALS. In this regard, serum NFL concentrations demonstrated optimal sensitivity and specificity also in distinguishing early symptomatic ALS from other neurologic diseases or motor neuron disease mimics, independently whether diagnosis was definite, probable, or possible, following the El Escorial criteria (Table 2) [134]. Interestingly, Gille and colleagues [135] reported an increase of serum NFL as a function of the number of regions (i.e., cranial, cervical) affected by UMN degeneration. Accordingly, in a MRI-based study, elevated CSF and serum NFL concentrations were significantly associated with lower diffusion tensor imaging (DTI) fractional anisotropy and increased radial diffusivity in the corticospinal tract of ALS patients, as well as with clinically UMN score burden [133]. On the other hand, previous studies have shown that NFL levels were not increased in Kennedy disease and spinal muscular atrophy (strictly LMN diseases) [128, 134]. As a consequence, a subclinical involvement of the UMN is likely in ALS patients with isolated LMN symptoms and elevated serum NFL concentration [117]. However, the neuroanatomical correlate of NFL increase is not yet clear since Verde and colleagues [139] showed a lack of association with DTI-MRI measurements of the integrity of cerebral white matter tracts in the brain of ALS patients. Finally, serum NFL levels were reported relatively lower in patients with primary lateral sclerosis (PLS) and hereditary spastic paraplegia (HSP), two UMN-isolated syndromes, compared with ALS subjects, suggesting in such patients different pathophysiological processes and rates of neurodegenerative diseases [134, 135].

NFL as Staging and Prognostic Biomarker

Several studies reported that blood NFL levels correlate with disease severity parameters, such as the decline in the ALS Functional Rating Scale-Revised (ALSFRS-R) score and the ALS Milano-Torino Staging (MITOS) system score [135, 137, 140, 141].

Furthermore, serum NFL levels at recruitment or at the time of diagnosis predicted survival independently from other clinical variables and were negatively associated with disease duration (Table 2) [136, 138, 142]. Thouvenot and colleagues

[140] evaluated the largest series ever of serum samples taken from ALS patients, finding that NFL concentration was the most important parameter related to ALS survival in multivariate models (Table 2). Likewise, serum NFL concentrations in the middle and high tertile were associated with an increased HR compared with those of patients in the lowest tertile (Table 2) [135]. Interestingly, unlike pNFH, NFL levels seem to change minimally throughout the course of the disease, maintaining distinct temporal profiles from controls, and a steady trajectory [136].

However, not all studies confirmed blood NLF as a robust prognostic biomarker in ALS patients, even if all proved lower NFL concentration in slow disease progressors [141]. Notably, the ALSFRS-R and the ALS MITOS system better correlated with CSF than serum at the baseline [137]. No significant correlation has been found between blood NFL levels and cognitive dysfunction in ALS [135].

NFL as Risk/Screening Biomarker

In contrast with AD, CSF and blood NFL levels are reported normal in presymptomatic ALS mutation carriers (C9orf72, SOD1, FUS/TLS, or TARDBP), but they increase suddenly with symptom onset in symptomatic mutation carriers as demonstrated by Weydt and colleagues [143]. Furthermore, using the parental age of disease onset as a proxy for assumed age of clinical onset, any trend toward an increase of NFL concentration was observed in asymptomatic mutation carriers. Despite recent longitudinal data on a large cohort of presymptomatic, SOD1 mutation carriers provided evidence that an increase in CSF and blood NFL levels occurs at least 1 year before of any clinical manifestations of the disease [144].

NFL as Predictive Biomarker

It would be interesting to determine whether riluzole reduces blood NFL levels over time given its neuroprotective effects and, although minimally, its capacity to slow disease progression. Currently, no studies investigated blood NFL as an indicator of treatment response in ALS, and no difference in blood NFL levels between patients treated and not treated with riluzole has been reported so far [136, 139]. On the other hand, recent studies on spinal muscular atrophy (SMA), a group of severe autosomal recessively inherited neurodegenerative disorders characterized by degeneration of the spinal alpha motor neurons, have highlighted an emerging role of NFL in tracking disease progression and response to treatment. Of note, recent data provided evidence that CSF NFL levels normalize and correlate with motor improvement in children with SMA treated with nusinersen, with a greatest benefit found in children who received treatment earliest during the course of disease [145]. Nusinersen, an antisense oligonucleotide delivered intrathecally by a spinal tap, is the first drug clinically approved for the treatment of all SMA types, with a rather dramatic impact on phenotype [146]. The levels of two additional biomarkers of neurodegeneration (CSF tau and glial fibrillary acidic protein (GFAP), an intermediate filament present in astrocytes) decreased together with CSF NFL after nusinersen administration, indicating that the neuronal and astroglia damage can be restored by nusinersen treatment [145]. Moreover, the decrease of NFL concentration was much larger than that of tau and GFAP, suggesting NFL as an early treatment response biomarker in SMA patients, helpful to select those patients will benefit to continue such an invasive treatment. Further studies with a long follow-up are needed, but these preliminary results in SMA indicated NFL as a promising marker for upcoming disease-modifying therapies in diseases beside SMA. Conversely, it is worth mentioning that the diagnostic and monitoring value of NFL in CSF and blood has not been confirmed in adolescent and adult SMA-type (SMA types 2 and 3) patients treated with nusinersen [134, 147]. It was hypothesized that NFL release is lower in late-onset SMA than in the infantile-onset subtype. Actually, the first phenotype is characterized by a long-lasting and chronic disease course while the foster by an acute and highly aggressive onset. Additionally, subjects with infantile-onset SMA report a significantly better response to nusinersen therapy when compared to individuals with adult-onset one. In this regard, the finding of normal blood levels of NFL in SMA could be used in a diagnostic panel of biochemical markers to help differentiate patients presenting with motor neuron deficits, separating SMA from ALS. Indeed, a substantial proportion of patients with SMA initially receive a diagnosis of ALS [148].

Degenerative Parkinsonisms

PD is the most common degenerative parkinsonism, with evidence of progressive loss of dopaminergic neurons in the pars compacta of the substantia nigra. Diagnostic criteria have been recently revised to improve diagnostic accuracy imaging biomarkers as supportive features [61]. However, early diagnosis and progression prediction remain challenging for physicians. Of note, the differential diagnosis between PD and AP can be difficult, mainly at the early clinical stage. Similarly, evolution of diagnostic criteria for AP improved accuracy, but misdiagnosis rates are still high [149–152]. The APs that most commonly mimic PD are progressive supranuclear palsy (PSP) and multiple system atrophy (MSA), whereas among APs, the lowest diagnostic accuracy regards the corticobasal degeneration (CBD).

Currently, biochemical biomarkers for PD and AP are an unmet need, but many CSF/serum molecules are under evaluation. CSF NFL concentration overlaps in patients with PD, PD with dementia (PDD), and DLB and are comparable with

those in HC [31]. In contrast, it has been demonstrated that CSF NFL levels are markedly increased in AP patients. Accordingly, it might discriminate between PD and AP with a high degree of diagnostic accuracy [17, 18, 153, 154]. This is in line with the remarkable axonal degeneration of large myelinated axons occurring in AP as well as with the rapid neuronal loss in such conditions [155]. Furthermore, CSF NFL concentration correlates with measures of disease severity and other clinical variables, demonstrating its capability to reflect neurodegenerative mechanisms. However, to overcome the well-known limits related to CSF examination, blood-derived NFL would be a more favorable biomarker. In this regard, the strong correlation between blood and CSF NFL levels in parkinsonian syndromes holds potential for an application in clinical practice [156].

PD

NFL as Diagnostic Biomarker

Similar to CSF results, NFL concentration in serum/plasma is considered useful for a differential diagnosis between PD and AP [157]. This has been tested for the first time in three independent prospective cohorts of PD, PSP, MSA, and CBD patients, compared with HC. Blood NFL levels in AP were significantly elevated compared with those in PD, showing a diagnostic accuracy ranging from good (in the early cohort with disease duration < 3 years) to excellent (in the Lund cohort) (Table 2) [156]. Conversely, blood NFL levels were not able to accurately separate PD from HC. A subsequent study in subjects with an uncertain diagnosis at the time of inclusion confirmed similar results (Table 2) [158]. Nevertheless, recent studies support the promise of plasma NFL as a diagnostic biomarker also in PD, demonstrating relatively higher NFL levels in cases vs controls [159] and a good diagnostic accuracy in differentiating PD patients from HC (Table 2) [160]. Moreover, higher serum NFL levels were found even at early stages of the disease and in participants at risk of disease progression (prodromal PD and symptomatic and asymptomatic mutation carriers of known PD genetic mutations), indicating the presence of active disease and potential for conversion to either PD or parkinsonian syndromes [161].

NFL as Staging and Prognostic Biomarker

Heterogeneous results regarding possible correlations between blood NFL and PD clinical features in three independent PD cohorts have been provided in the prospective and longitudinal study of Hansson and colleagues [156]. In general, higher blood NFL levels were observed in more advanced PD and, in the Lund PD cohort, a higher blood NFL concentration was associated with disease duration and more severe motor symptoms (measured as Hoehn and Yahr (H&Y) stage, Unified Parkinson's Disease Rating Scale (UPDRS) III motor score, Timed Up and Go Test, and Tandem Gait Test). Conversely, no clinical correlations were described in the London cohort and the early-stage disease cohort [156]. However, further studies confirmed the positive relation between plasma NFL levels and motor symptom severity (measured as H&Y stage and UPDRS part III score) and proved a significant correlation between plasma NFL concentration and cognitive dysfunction at MMSE [161, 162]. In another study, PDD patients reported higher plasma NFL level compared with PD subjects without dementia [84], supporting an association between plasma NFL and cognitive function in PD patients [84]. Furthermore, higher baseline plasma NFL concentrations in PD patients were found to be longitudinally associated with a higher risk of progression for both motor and cognitive symptoms, suggesting that serum NFL may be a biomarker of clinical progression in PD (Table 2) [160–162].

PSP

NFL as Diagnostic Biomarker

Two other studies confirmed the diagnostic value of blood NFL in PSP patients showing good capability to discriminate between PSP and HC (Table 2) [163, 164]. In contrast, blood NFL is not suitable to separate PSP from other forms of AP [156] and similar levels are reported in patients with MSA and PSP [158].

NFL as Staging and Prognostic Biomarker

Greater baseline NFL levels in serum/plasma seem to correlate with disease severity and clinical progression in PSP patients, though with conflicting results. Such heterogeneity may reflect differences in study design, since PSP patients have been evaluated as a separate group in some studies but not in others where PSP, MSA, and CBD patients have been combined as a whole group. Specifically, blood NFL levels positively correlated with motor symptom severity, evaluated as H&Y stage and with UPDRS III motor score, but not with disease duration or other clinical assessments, in the AP group (including also MSA and CBD patients) [156]. Similarly, serum NFL concentration at baseline correlated with motor performances, measured with the International Cooperative Ataxia Rating Scale score and Tandem Gait Test in another cohort of AP patients including PSP [158].

In studies focusing exclusively on PSP patients, higher serum NFL levels were related to more severe motor, functional, and cognitive disability as well as shorter survival but not with age at symptom onset or disease duration (Table 2) [163]. Notably, NFL levels in the higher tertile were

associated with worse survival (Table 2) [164]. Higher baseline plasma NFL levels also predicted greater whole-brain and superior cerebellar peduncle volume loss at 1-year follow-up [163].

MSA and Degenerative Ataxias

NFL as Diagnostic Biomarker

As for PSP, studies indicate elevated blood NFL concentration in MSA patients, suggesting its use in discrimination of MSA from PD and HC with a good diagnostic accuracy (Table 2). However, as aforementioned, NFL cannot discriminate among APs [158].

Moreover, blood NFL was proposed to improve the differential diagnosis of degenerative ataxias. In a pilot study evaluating serum NFL levels in patients with a clinical diagnosis of probable cerebellar-MSA (c-MSA) subtype, sporadic adult-onset ataxia (SAOA), and frequent repeat-expansion spinocerebellar ataxias (SCAs 1, 2, 3, and 6) and in HCs, serum NFL concentration was found to be higher in SCA patients and in the c-MSA group compared with controls. This is probably the result of the diffuse involvement of spinocerebellar and corticospinal tracts in these multisystemic neurodegenerative ataxias [165]. However, the performance of serum NFL differentiating c-MSA from SAOA was only moderate (Table 2), in contrast with a higher accuracy previously reported for CSF NFL (AUC = 0.93) [166]. NFL levels were significantly lower in SAOA and comparable with those of HC. A further study investigated serum NFL concentration in large cohorts of SCA-3 subjects and demonstrated higher levels in both preclinical and manifest SCA-3 individuals compared with HC [167]. Serum NFL levels discriminated manifest SCA-3 from HC with excellent accuracy, and the diagnostic performance remained good in distinguishing preclinical SCA-3 subjects from HC (Table 2) [167]. Recently, plasma NFL concentrations resulted higher also in patients affected by Friedreich's ataxia (FA), which is the most common autosomal recessive ataxia caused by CAG repeat expansion in the ATXN3/MJD1 gene, compared with aged-matched controls [168].

NFL as Staging and Prognostic Biomarker

Serum NFL in c-MSA patients does not seem to correlate with clinical disease severity (as assessed by the Scale for the Assessment and Rating of Ataxia (SARA)) or disease progression [165]. Similarly, a recent study in 99 patients with genetically confirmed FA did not find a correlation with disease severity (as defined by SARA score), age at onset, or disease duration [169]. Moreover, serum NFL concentration remains stable in a subgroup of 14 FA patients who received a 2-year follow-up evaluation [169].

Conversely, serum NFL concentration increased with disease severity in a large cohort of SCA-3 patients, including manifest and preclinical individuals, and correlated with both clinical scales (according to SARA and International Cooperative Ataxia Rating Scale (ICARS) scores) and reduction of cerebellar and brainstem volume [167]. Preclinical SCA-3 group was divided in early and late preclinical subgroups using the median predicted number of years to onset of manifest disease. Serum NFL concentrations resulted higher in manifest than preclinical SCA-3 subjects and in late preclinical SCA-3 subjects compared with early preclinical SCA-3 individuals. However, no differences were observed between early preclinical subjects and HC [167]. Despite CAG repeat count is a well-known prognostic factor for SCA-3 and FA, a correlation between serum NFL and CAG repeat lengths has been inconsistently reported [168, 169].

NFL as Risk/Screening Biomarker

In their study on 133 SCA-3 patients, Li and colleagues [167] demonstrated higher serum NFL concentrations in 26 preclinical *ATXN3* mutation carriers (patients with SARA score < 3) compared with controls. Moreover, a correlation between motor symptoms, neuroimaging markers, and serum NFL was found in all *ATXN3* mutation carriers, suggesting that NFL may serve to track neurodegeneration and disease progression already in pre and prodromal SCA-3 phases.

HD

Among the most common neurodegenerative diseases, HD is unique, since the major part (≈ 99%) of individuals presenting a HD phenotype have a mutation in the same gene [170]. Indeed, HD is an autosomal dominant inherited neurodegenerative disease with the typical manifestations of involuntary movements, psychiatric symptoms, and cognitive decline. The etiological basis is the deleterious expansion of polyglutamine encoding CAG repeats in the huntingtin (HTT) gene, leading to the expression of neurotoxic mutant huntingtin (mHTT) and extensive degeneration of neurons primarily occurring in the striatum and cortex [171]. The disease usually starts in midlife, with age of onset inversely correlating to CAG repeat number [171]. Although the cause is known, disease-modifying treatments are not yet available. In HD, a reliable genetic test confirms a clinical diagnosis in symptomatic people or predicts disease onset in asymptomatic mutation carriers [172]. As a consequence, a novel biomarker should be directed to track disease progression and predict a treatment response to targeted therapies. Although not conclusive, the results of the available studies display that blood NFL

could have a role in this context. Previous reports on CSF NFL indicate elevated concentrations in HD subjects [19, 173, 174]. This is not surprising because mtHTT interacts with other proteins altering their function and finally leading to abnormal protein aggregation and impaired axonal transport [175]. Furthermore, the level of misfolded mtHTT protein correlates with NFL concentration in CSF, thus suggesting a contemporary releasing of both proteins from damaged neurons [176].

NFL as Staging and Prognostic Biomarker

In the first retrospective study investigating NFL concentrations in the blood of premanifest HD (preHD) and early-stage HD patients enrolled in the TRACK-HD cohort, Byrne and colleagues [177] showed higher baseline NFL levels in 201 HTT mutation carriers, including 58 with early premanifest and 46 subjects with late premanifest disease, than in controls. Moreover, NFL concentration reflected baseline motor and cognitive deficits in HD patients and differed significantly with increasing disease stage. Positive associations were found between plasma NFL concentration, age, and CAG triplet repeat counts, with higher CAG lengths being associated with earlier and steeper increases in plasma NFL [177]. Therefore, NFL is the first biofluid marker showing a direct relationship with a causative gene expansion [178].

Of note, baseline plasma NFL predicted rates of brain atrophy, cognitive decline, and worsening of functional ability and motor performance in mtHTT carriers [177]. Interestingly, it was closely associated with the rate of whole-brain atrophy than with the rate of striatal one, suggesting that plasma NFL reflects more the rate of global neuronal degeneration than that of a specific brain area [177]. Additionally, in the same TRACK-HD cohort, Johnson and colleagues [179] showed voxel-wise region-specific associations between plasma NFL levels and both cross-sectional and longitudinal MRI cortical thinning and white matter volume reduction, highlighting the value of NFL as a dynamic and robust marker of brain atrophy. Notably, higher concentrations of NFL in plasma were associated with lower volume in regions known to be affected in HD and predicted subsequent occipital gray matter atrophy and widespread white matter reduction over the 3-year follow-up, independently of age and CAG length repeats [179]. NFL increased significantly from baseline both in individuals with premanifest HD and in those with manifest HD [179]. Remarkably, in a subsequent study, Byrne and colleagues [180], combining CSF/plasma NFL and CSF mutant huntingtin protein (mHTTp) as biofluid biomarkers, demonstrated that NFL levels were more accurate than mHTTp to discriminate between premanifest and manifest HDs and correlated with severity of symptoms better than mHTTp in manifest HD.

CJD

CJD is the most common human prion disease. Approximately 85% of cases are sCJD, but in a minority of cases, CJD can be genetically determined (gCJD) [181]. The disease is a rapidly progressive and fatal neurodegenerative condition, whose different phenotypes depend, at least in part, by polymorphisms on the gene encoding prion protein (PrP) [182]. Diagnosis is frequently tardive and relies on clinical World Health Organization (WHO) criteria supported by detection of the 14-3-3 protein and, more recently, t-tau in the CSF [66, 183, 184]. Also, CSF NFL recently demonstrated to be a reliable biomarker in the CJD diagnostic workup. Although few studies explored its role as a biomarker in CJD patients so far, CSF NFL levels are significantly increased in CJD (including those with more slowly progressive and atypical disease course) compared with AD, FTD, other NDDs (dementia), and controls, indicating a massive synaptic degeneration and neuroaxonal damage in CJD [22, 23, 185, 186]. Additionally, it is noteworthy that NFL concentrations in CSF appear highly variable among different sCJD subtypes, with higher NFL levels in those with more rapidly progressing disease [187]. Importantly, plasma NFL correlates with CSF NFL concentration and recent studies suggest that blood NFL can accurately reflect the massive neurodegeneration in CJD patients.

NFL as Diagnostic Biomarker

Diagnostic accuracy of serum NFL for discrimination between CJD and controls was excellent (Table 2) [185]. These findings have been independently replicated in other two studies. Serum NFL distinguished patients from controls with 100% sensitivity and 100% specificity in 45 sCJD patients enrolled in the National Prion Monitoring Cohort [188]. Noteworthy, Kovacs and colleagues [189] reported high sensitivity and specificity of plasma NFL concentration in discriminating CJD subjects from non-CJD controls in a cohort of 132 pathologically classified patients (sCJD, gCJD, and AD cases) showing a rapidly progressive neurological picture. However, in this study, the diagnostic value in the differentiation between prion and other disease cases resulted lower than previously reported investigations (Table 2) [189]. Moreover, serum NFL values have been elevated since the early phases of the disease, suggesting a possible role as a screening biomarker [188]. Conversely, serum NFL concentration overlapped between ALS and CJD patients in a recent prospective study, even though the size of CJD group was very small [139].

NFL as Staging and Prognostic Biomarker

Longitudinal changes in serum tau and NFL levels were investigated in the aforementioned study of Thompson and

colleagues [188]. However, despite a trend toward increasing concentrations of both tau and NFL over the last 12 months before death, at variance with tau, no association was found between serum NFL concentration and speed of decline on the Medical Research Council (MRC) Prion Disease Rating Scale [188]. Also, a recent study strengthens the tight association of plasma tau levels with the rate of disease progression and survival time in sCJD [190].

NFL as Risk/Screening Biomarker

gCJD forms are linked to mutations in the prion protein gene (PRNP) inherited with an autosomal dominant pattern and variable penetrance [191]. The relationship between genotype and phenotype remains a matter of debate, and index cases do not always have a family history [192]. Under this scenario, the discovery of biomarkers serving as surrogates of outcome in clinical trials may be crucial because *PRNP* mutation carriers might benefit from a presymptomatic intervention. PrP-lowering therapeutics are now in preclinical development, and to this end, CSF total PrP has demonstrated to be a strong candidate fluid biomarker showing stable low levels in an ongoing natural history study including presymptomatic mutation carriers and normal controls [193].

Conclusions

The impact of physiological variables on blood NFL concentration, such as sex and age, has not been systematically investigated across published studies. Other variables possibly affecting its modification in peripheral blood, including systemic comorbidities and concomitant drug therapies, were not taken into account. Liver and renal clearance as well as blood cell counts and plasma protein composition could affect biomarker concentrations [194]; however, these factors were not investigated. Indeed, blood NFL concentration alterations associated with renal and hepatic dysfunctions remain unknown, thus representing a potential relevant methodological bias, especially in subjects with NDDs, being generally old and frequently exhibiting vascular comorbidities. In general, blood NFL concentrations correlate with aging due to a subtle axonal degeneration and vascular changes in elderly. However, such an association was clearly absent in CJD, ALS, and AP. This suggests that the probable contribution of aging on NFL concentrations and neurodegeneration becomes trivial in highly aggressive forms of NDDs [195].

Blood NFL concentrations are reported to be massively elevated in ALS patients, even in early disease stages, indicating its value as an efficacious, yet unspecific, biomarker in the differential diagnosis of ALS from ALS mimics. Moreover, blood NFL concentrations can reflect disease severity and/or progression in ALS, suggesting that peripheral NFL could contribute to support ALS prognosis. Other confounding variables, such as the discordant disease progression in the different clinical subtypes of ALS, should be elucidated in further studies. Actually, blood NFL could help better stratify the multifaceted clinical presentation of ALS phenotypes. However, further studies are needed to confirm its prognostic value. In contrast with genetic forms of AD, blood NFL concentrations are not increased in presymptomatic ALS mutation carriers appearing to tightly link to the symptomatic phase of the disease.

Another NDD where blood NFL increases result impressively is CJD. Because of early diagnosis remains challenging, NFL might be a reliable screening blood-based biomarker with a potentially high negative predictive value for CJD subjects, ruling out more common and less aggressive neurodegenerative dementia, such as AD. Definitely, blood NFL could be of usefulness as a first-step examination to promptly detect CJD during its prodromal phase and to start future disease-modifying treatments. On the other hand, preliminary findings do not support a potential use of NFL as a predictor of longitudinal disease progression in CJD, and its specificity should be further substantiated in comparison with other highly aggressive forms of NDD.

All reported studies showed a good or even excellent diagnostic performance of blood NFL in distinguishing patients with neurodegenerative disorders from HC. The potential contribution of this biomarker candidate to discriminate between different dementia disorders remains ambiguous given the lack of pathognomonic specificity. However, mounting data suggest that blood NFL could be a useful diagnostic tool in the diagnostic workup of FTD, to distinguish FTD (especially FTD with a TDP-43 pathology) from AD patients and to identify PPA with a likely underlying AD pathology, including lvPPA. Moreover, it can represent a biomarker tracking the disease progression and potentially identifying the transition phase from the presymptomatic to the symptomatic stage of the genetic forms of the disease. Most importantly, serum NFL is assumed to be a promising screening tool to rule out an underlying neurodegenerative disease in individuals with psychiatric disorders.

In AD, blood NFL may predict progression to dementia in individuals with MCI at high risk and identify preclinical AD before the conversion phase. Moreover, NFL captures early neurodegenerative changes in presymptomatic familiar AD mutation carriers in which blood NFL concentrations correlate with the predicted time to symptom onset. Further studies are crucial to calculate the negative predictive value of blood NFL as a screening tool in large and selected cohorts of individuals at risk of neurodegeneration and AD, such as individuals with subjective memory complaints and/or decline, late-onset psychiatric disorders, cerebrovascular disease, and diabetes, as well as in aging and elderly individuals in general.

Because of the high negative predictive value of elevated blood NFL concentrations in excluding PD, this candidate biomarker can represent a valid screening tool for clinicians in the early differential diagnosis between PD and AP in cases with confounding clinical presentations. In contrast, blood NFL measurements do not suffice to differentiate PD patients from controls and cannot be used to separate PSP, MSA, and CBD from each other. Nevertheless, blood NFL may be a prognostic tool in clinical practice in both PD and AP patients. Currently, to the best of our knowledge, there are no studies investigating blood NFL in patients with DLB, and few studies are available for PDD patients.

Regarding other relevant clinical neurological presentations, blood NFL may support the classification of sporadic late-onset ataxias, notably helping in differentiating c-MSA-C from SAOA. In choreic patients, blood NFL appears to be a robust prognostic biomarker of HD disease onset and progression and holds potential as a predictive biomarker of response to disease-modifying agents in clinical trials.

Finally, blood NFL seems to be a promising candidate predictor of the timing of clinical phenoconversion in presymptomatic mutation carriers with AD, HD, and SCA-3. Conversely, blood NFL concentrations are mostly normal in premanifest ALS and FTD mutation carriers but promptly increase with the onset of clinical symptoms.

In general, given the rapid advances in elucidating the pathophysiological mechanisms of diseases, at the molecular diagnostic level, biomarkers are excellent flexible tools to improve and inform all phases of drug discovery and development by enabling validation of mechanisms of actions [196, 197]. For this reason, NFL is assumed to act as an innovative molecular mechanistic biomarker supporting in vivo detection and the measurement of definite pathophysiological mechanisms across the spectrum of different NDDs. Together with other innovative molecular indicators, NFL will help establish panels of biomarkers—i.e., molecular signatures—encompassing the entire spectrum of molecular events of the NDD spectrum disorders. Applying these molecular signatures in longitudinal investigations will be critical to provide information to depict the pathophysiological processes characterizing different NDDs [198]. These innovative biomarkers will enable the selection of the most appropriate therapies for individual patients by defining which molecular pathophysiological events account for the patient's clinical symptoms at different stages of the disease [199, 200]. This will establish the grounds to develop effective targeted treatment strategies—i.e., "molecularly" targeted therapies—for the accurate treatment of specific molecular pathophysiological pathways. Future developments in investigating NDD heterogeneity will allow clinicians to deliver targeted interventions that are "customized," i.e., tailored, to the definite profiles of the individual NDD patient, according to the precision medicine paradigm. Such a precision medicine–based strategy is now increasingly facing the clinical and biological/genetic complexity and heterogeneity of the various forms of NDD [198]. Precision medicine emphasizes the need of clinical medicine to focus on the pathophysiology of the individual patient, with his/her own distinctive, diverse, and complex matrix of multisystem features [200]. Concerted global efforts will pave the way for a future of neurology, in which drugs will timely and effectively support the prevention and treatment of diseases with very precise biomarker-guided targeted approaches for the right patient at the right time [201].

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Compliance with Ethical Standards

Competing Interests S.L. received lecture honoraria from Roche and Servier.

H.H. is an employee of Eisai Inc. and serves as Senior Associate Editor for the Journal Alzheimer's & Dementia and does not receive any fees or honoraria since May 2019; before May 2019, he had received lecture fees from Servier, Biogen, and Roche; research grants from Pfizer, Avid, and MSD Avenir (paid to the institution); travel funding from Functional Neuromodulation, Axovant, Eli Lilly and company, Takeda and Zinfandel, GE Healthcare and Oryzon Genomics; and consultancy fees from Qynapse, Jung Diagnostics, Cytox Ltd., Axovant, Anavex, Takeda and Zinfandel, GE Healthcare, Oryzon Genomics, and Functional Neuromodulation, and he also participated in scientific advisory boards of Functional Neuromodulation, Axovant, Eisai, Eli Lilly and company, Cytox Ltd., GE Healthcare, Takeda and Zinfandel, Oryzon Genomics, and Roche Diagnostics.

He is co-inventor in the following patents as a scientific expert and has received no royalties:

- In Vitro Multiparameter Determination Method for the Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388
- In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784
- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300
- In Vitro Multiparameter Determination Method for the Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463
- In Vitro Method for the Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286
- In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822
- In Vitro Method for the Diagnosis of Neurodegenerative Diseases Patent Number: 7547553
- CSF Diagnostic in Vitro Method for Diagnosis of Dementias and Neuroinflammatory Diseases Publication Number: 20080206797
- In Vitro Method for the Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966
- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20080131921

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Q1. Please check if the affiliations are captured and presented correctly.
Q2. Please check if the section headings are assigned to appropriate levels.
Q3. Please check the provided expanded form for the abbreviation "MMSE" if correct.
Q4. Please check the word "diseases" inserted after the word "neurodegenerative," which can be found in the sentence starting "Finally, serum NFL levels were reported relatively lower," for completeness if correct.
Q5. References [19, 174], [22, 186], [200, 203] based on original manuscript we received were identical. Hence, the latter was deleted and reference list and citations were adjusted. Please check if appropriate.
Q6. Please provide complete bibliographic details of this reference.