Diagnostic value of plasma neurofilament light: A multicentre validation study

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Abstract

Increased cerebrospinal fluid neurofilament light (NfL) is a recognized biomarker for neurodegeneration that can also be assessed in blood. Here, we investigate plasma NfL as a marker of neurodegeneration in fifteen neurodegenerative diseases from two multicenter cohorts: King's College London (n = 847) and the Swedish BioFINDER study (n = 1464). Plasma NfL was significantly increased in all cortical neurodegenerative disorders, amyotrophic lateral sclerosis and atypical parkinsonian disorders. We further demonstrate that plasma NfL is clinically useful in identifying, i) atypical parkinsonian disorders in patients with parkinsonism, ii) dementia in individuals with Down Syndrome, iii) detect cases of frontotemporal dementia among psychiatric disorders such as moderate and severe depression, iv) identify frontotemporal dementia in patients with cognitive impairment. Data-driven cut-offs highlighted the fundamental importance of age-related plasma NfL cut-offs for disorders with a younger age of onset. Finally, our findings suggest that plasma NfL performs best when a concentration cut-off is applied to indicate no underlying neurodegeneration, with low false positives, in all age-related cut-offs.

Introduction

In the management of neurological disorders, reliable and easily accessible biomarkers are needed to recognise or rule out an underlying neurodegenerative process contributing to cognitive decline at the earliest stage. Cerebrospinal fluid (CSF) biomarkers for amyloid-β (Aβ42), total tau (T-tau), and phosphorylated tau (P-tau) work well to identify certain neurodegenerative disorders such as Alzheimer's disease (AD) and its underlying pathology [1] and are central to the biological definition of the disease [2], which is based on biomarker-based identification of pathology during life. However, at this time, no such fluid biomarkers are available for other common or rarer neurodegenerative disorders.

Axonal degeneration or injury is a predominant feature of many neurodegenerative disorders, resulting in irreversible impairment. In response to such damage, neurofilament light chain (NfL), a structural component of the neural cytoskeleton, is released into the extracellular space initiating a concentration increase in the CSF [3]. These elevations are observed in the majority of neurodegenerative disorders [4] along with inflammatory [5], traumatic [6] and vascular conditions [7]. However, even under normal circumstances, low levels of NfL are continuously released from axons in an age-dependent manner with typical NfL reference ranges in the CSF increasing by 2.5-fold between ages 20–50 years and doubling by the age of 70 [8,9]. A considerable drawback of CSF NfL, and all CSF biomarkers, is the perceived invasiveness or complexity attached to lumbar punctures which will undoubtedly limit use for routine clinical assessment.

Recent advances in ultrasensitive immunological assays [10–15] and immunoprecipitation mass spectrometry (IPMS) methods [16–18] have been developed to measure neurodegenerative biomarkers in blood. NfL can be quantified at femtomolar concentrations in plasma or serum, which has enabled the reliable detection of NfL not only in symptomatic patients but also in cognitively unimpaired (CU) individuals of all ages [19]. A key advantage of peripheral NfL over other postulated blood biomarkers, is that it shows a strong correlation to CSF NfL levels across several diagnostic groups, supporting the notion that blood NfL reflects central nervous system pathophysiology with negligible peripheral interference. Consequently, numerous CSF NfL findings have been replicated in blood, including increased concentrations of blood NfL in AD [13,20,21], frontotemporal dementia (FTD) [22] and several other disorders (for review, [23]). Interestingly, NfL is seemingly not elevated in Parkinson's disease (PD) in comparison to other neurodegenerative disorders and therefore a discrimination can be made from atypical parkinsonian disorders [24,25]. Furthermore, developing evidence demonstrates the potential use of using plasma NfL in discriminating FTD and primary psychiatric disorders [26,27] suggesting a potential differential diagnostic value of blood NfL in certain clinically relevant situations.

The context of use of a blood biomarker, such as NfL, is in primary care where it could be used as a rapid screening tool to identify or reject neurodegeneration as an underlying cause of cognitive decline [28]. To achieve this, at the individual level, reference values to indicate neurodegeneration need to be established which result in a low-rate of false positives. In this study, we examined 2311 individuals from two independent multicentre cohorts to firstly, demonstrate the distributions of plasma NfL in CU individuals and in patients on the AD continuum and a broad range of neurodegenerative disorders and depression. Secondly, to examine the diagnostic utility of plasma NfL in terms of effect size, area under curve (AUC), specificity and sensitivity when differentiating relevant neurodegenerative diseases from each other and CU individuals. Finally, age-related and data-driven plasma NfL concentration cut-offs were derived to indicate neurodegeneration and these were tested to predict the prevalence of abnormal NfL in neurodegenerative disorders and CU individuals.

Methods

Study Participants

In this study, 2311 individuals from two multicentre cohorts were included. The KCL cohort (cohort 1) represents a multicenter collection of participants (n = 847) collated at the Maurice Wohl Clinical Neuroscience institute, King's College London [5,29–41]. This consisted of CU individuals (n = 158), mild cognitive impairment (MCI, n = 86), early-onset Alzheimer's disease (EOAD < 65 years, n = 59), AD dementia (n = 102), FTD (n = 54), PD (n = 140), Parkinson's disease dementia and dementia with Lewy bodies (PDD/DLB, n = 59), cortical basal syndrome and progressive supranuclear palsy (CBS/PSP, n = 19), Down Syndrome (DS, n = 41; 12 with dementia), amyotrophic lateral sclerosis (ALS, n = 50), multiple sclerosis (MS, n = 42; no medication) and depression (HAM-D > 13, n = 37). The Lund cohort (cohort 2) consisted of 1464 participants enrolled as part of the prospective and longitudinal Swedish BioFINDER study (clinical trial no. NCT01208675) which recruited at the Neurology and Memory Clinics, Skåne University Hospital, Lund, Sweden, between 2008 and 2014 [42,43]. In addition, FTD cases were obtained from the Erasmus Medical Centre, Rotterdam, The Netherlands [44] and Lund Prospective Frontotemporal Dementia Study (LUPROFS) [45]. The Lund cohort included CU (n = 376), subjective cognitive decline (SCD, n = 209) and seven diagnostic groups in common with the KCL cohort (MCI, n = 280; EOAD, n = 23; AD dementia, n = 134; FTD, n = 150; PD, n = 171; PDD/DLB, n = 46; CBS/PSP, n = 24). In addition, the Lund cohort included patients with multiple system atrophy (MSA, n = 29) and vascular dementia (VaD, n = 22). In both cohorts, healthy controls underwent clinical, neurological and cognitive examinations and individuals with evidence of cognitive impairment or suspected parkinsonian signs were excluded from the study. Further description of contributing centers to the KCL and Lund cohorts are detailed in Supplementary Table 1.

To confirm findings related to the AD continuum, this study also obtained data from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (clinical trial no. NCT00106899) for 870 individuals (CU, n = 290; MCI, n = 442; AD dementia, n = 138). AD dementia participants had a Mini-Mental State Examination (MMSE) ranging between 20 and 26; Clinical Dementia Rating (CDR) 1 or above and met criteria for probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). Participants were classified as MCI if MMSE ranged between 24 and 30, CDR 0.5 (with the memory box score being 0.5 or greater) and did not meet criteria for dementia according to the NINCDS-ADRDA.

Determination of amyloid-β status

Individuals clinically classified as CU, SCD (Lund cohort only) and MCI were further categorized into Aβ-negative (Aβ-) or Aβ-positive (Aβ+). In the KCL cohort, Aβ cut-off values for assigning positivity were determined by CSF Aβ42, [^{11}C]PiB-PET or [^{18}F]AZD4694 as outlined in Supplementary Table 1. It was determined that 28/158 and 31/86 of CU and MCI were Aβ+, respectively. In the Lund cohort, Aβ-positivity was classified by CSF with Aβ42/Aβ40 < 0.091 by EUROIMMUN immunoassays (EUROIMMUN AG, Lübeck, Germany) [46]. This determined that 103/376, 75/209 and 165/280 of CU, SCD and MCI individuals were Aβ+, respectively. For ADNI, brain Aβ load—at the last available visit of each subject—was estimated using [^{18}F]florbetapir PET. The cut-off to determine Aβ-positivity was 1.11 SUVR, as suggested in the ADNI protocol. According to this criterion 100/290 and 247/442 CU and MCI were Aβ-positive, respectively.

Biochemical analysis

Blood sampling procedures for cohorts included in the KCL and Lund cohorts are summarized in Supplementary Table 1. Blood collection and processing procedures for ADNI have been detailed elsewhere [13]. Plasma NfL concentration was measured using two highly correlated versions of a Single molecule array (Simoa; Quanterix; Billerica, MA) method. For the KCL cohort, the commercially available NF-light assay was utilized (NF-light®; Quanterix; Billerica, MA) and all samples were analyzed at the Maurice Wohl Clinical Neuroscience Institute, King's College London, UK. Data acquisition spanned seventeen analytical runs and all the samples were above the lower limit of quantification reported for this assay (LLOQ, 0.174 pg/mL). For the low-concentration control sample (8.5 pg/mL), the intra-assay coefficient of variation was 7.5% and the inter-assay coefficient of variation was 12.8%, whilst for the high-concentration quality control sample (112 pg/mL), the corresponding coefficients of variation were 9.5% and 13.8%, respectively. For the Lund and ADNI cohorts, an in-house Simoa assay, utilizing the same antibodies and calibrator as the commercial kit, was used. The assay has been described in detail before [19], and was performed at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden. For the Lund cohort, data acquisition spanned twenty-three analytical runs and all the samples were above the lower limit of quantification (6.7 pg/mL). For the low-concentration control sample (12.2 pg/mL), the intra-assay coefficient of variation was 5.5% and the inter-assay coefficient of variation was 8.2%, whilst for the high-concentration quality control sample (107.3 pg/mL), the corresponding coefficients of variation were 9.3% and 9.4%, respectively. Data acquisition methods for NfL measurements in ADNI have been previously described [13,20].

Harmonization of KCL and Lund cohorts

Quality control (QC) samples provided by the Lund cohort (n = 30) were quantified at random in the KCL analysis. High concordance (r = 0.925, $P < 0.0001$, Supplementary Fig. 1A) was achieved between the QC samples despite the absolute values in the KCL cohort being significantly higher ($P = 0.025$, Supplementary Fig. 1A). Based on this QC data a correction factor of 1.18 was applied to all Lund and ADNI samples to adjust the data to the KCL cohort for all subsequent analyses.

Statistical analysis

Associations between continuous variables were tested with Spearman's rank-order correlation with a partial correlation adjusting for age. Group differences were assessed by Mann-Whitney test or one-way Kruskal–Wallis test by ranks, with *post-hoc* Dunn's test where appropriate. To measure the specificity and sensitivity of plasma NfL we calculated the AUC of the receiver operating characteristics (ROC) using the 'AUC' package for R. Cut-off concentrations for plasma NfL were defined in the KCL cohort and three variations were investigated a) 90%, 95% and 99% confidence interval of CU Aβ-, b) mean plus 2 standard deviations of the CU Aβ- and c) Gaussian mixture modelling (GMM). Hedges' g statistical unit was used to report the effect size. SPSS (IBM, Armonk, NY) and the R programming language (version 3.4.3) were used for statistical analysis and Graph Pad PRISM for data visualization.

Results

The demographic and clinical data for the KCL and Lund cohorts are displayed in Tables 1 and 2. A full description of the demographic variables and the relation of plasma NfL with age, sex, *APOE* ε4 carrier status and CSF NfL are fully are presented in Supplementary Results 1 and 2.

Table 1
Demographics of the KCL cohort

		Cognitively unimpaired		Cognitive impairment						Parkinsonian		Other		
Characteristics	**Overall**	CU Aβ-	CU Aβ+	MCI Aβ-	MCI Aβ+	EOAD	AD	FTD	PDD/DLB	PD	CBS/PSP	DS (n = 29)	DSAD (n = 12)	ALS (n = 50)
	(n = 847)	(n = 130)	(n = 28)	(n = 55)	(n = 31)	(n = 59)	(n = 102)	(n = 54)	(n = 59)	(n = 140)	(n = 19)			
Age, years, mean (SD)	63.6 (14.6)	64.0 (14.1)	66.3 (7.7)	70.7 (6.36)	69.6 (7.0)	57.8 (5.2)	76.1 (6.2)	65.4 (9.5)	77.5 (6.4)	65.5 (10.0)	70.4 (8.4)	50.4 (11.4)	59.1 (9.8)	62.2 (12.5)
Female, n (%)	413 (48.5)	63 (54.8)	13 (46.4)	29 (52.7)	16 (51.6)	32 (49.2)	55 (53.9)	23 (42.6)	32 (54.2)	52 (37.1)	10 (52.6)	10 (34.5)	4 (33.3)	13 (26.0)
APOE e4 carriers, n (%) [missing]	131 (35.1) [479]	18 (28.1) [51]	8 (30.8) [2]	20 (38.5) [3]	15 (50.0) [1]	8 (34.8) [42]	43 (47.3) [11]	9 (25.0) [18]	NA	NA	3 (25.0) [7]	4 (14.8) [2]	3 (25.0)	NA
MMSE, mean (SD)	24.3 (5.8)	29.4 (1.0)	29.0 (1.0)	26.0 (3.2)	26.0 (2.2)	22.2 (6.5)	19.9 (6.7)	25.9 (4.5)	20.2 (4.2)	27.3 (2.6)	23.1 (5.3)	NA	NA	NA
CSF Aβ, mean (SD)	675 (352)	999 (317)	405 (103)	921 (385)	413 (120)	NA	434 (155)	NA	NA	NA	NA	NA	NA	NA
Aβ PET	1.55 (0.5)	1.29 (0.1)	1.81 (0.3)	NA	NA	NA	2.08 (0.4)	1.22 (0.3)	NA	NA	1.61 (0.2)	NA	NA	NA

Table 2
Demographics of the Lund cohort

		Cognitively unimpaired				Cognitive impairment							Parkinsonian	
Characteristics	**Overall**	CU Aβ-	CU Aβ+	SCD Aβ-	SCD Aβ+	MCI Aβ-	MCI Aβ+	EOAD	AD	FTD (n = 150)	PDD/DLB	VaD	PD	CBS/PS
	(n = 1,464)	(n = 273)	(n = 103)	(n = 134)	(n = 75)	(n = 115)	(n = 165)	(n = 23)	(n = 134)		(n = 46)	(n = 22)	(n = 171)	(n = 24)
Age, years, mean (SD)	70 (7.1)	72 (6.5)	73 (5.5)	69 (5.7)	72 (5.3)	69 (5.6)	72 (4.5)	59 (3.5)	75 (5.2)	64 (8.8)	73 (6.9)	64 (5.7)	65 (10.7)	72 (4.4
Female, n (%)	734 (50.1)	160 (58.6)	64 (62.1)	77 (57.5)	32 (42.7)	36 (31.3)	79 (47.9)	14 (60.9)	79 (59.0)	77 (51.3)	15 (32.6)	13 (44.8)	63 (36.8)	15 (62.
APOE e4 carriers, n (%) [missing]	507 (40.4) [210]	46 (17.1) [4]	65 (63.1) [0]	33 (24.8) [1]	48 (64.0) [0]	27 (23.5)) [0]	116 (70.3)) [0]	6 (54.5)) [12]	81 (66.4)) [12]	NA	18 (41.9) [3]	9 (45.0) [2]	44 (29.9) [24]	7 (35.0 [4]
MMSE, mean (SD) [missing]	26.8 (3.8) [96]	29.0 (1.1) [2]	28.8 (1.1) [0]	28.7 (1.3) [4]	28.2 (1.6) [5]	27.3 (1.9) [2]	26.8 (1.8) [4]	20.6 (4.5) [1]	21.3 (4.0) [3]	23.3 (5.8) [51]	22.3 (3.9) [7]	22.3 (4.5) [11]	28.0 (2.0) [0]	25.5 (4.5) [4
CSF Aβ42/Aβ40	0.10 (0.04)	0.13 (0.02)	0.06 (0.02)	0.13 (0.02)	0.06 (0.02)	0.13 (0.03)	0.06 (0.02)	0.06 (0.04)	0.07 (0.03)	N/A	0.11 (0.04)	0.13 (0.02)	0.12 (0.03)	0.11 (0.03)
CSF NfL	1321 (1158)	894 (515)	1107 (770)	935 (470)	1332 (887)	1599 (1495)	1549 (1162)	1066 (870)	1845 (1572)	4590 (1230)	1589 (1030)	1758 (794)	888 (640)	2577 (1345)

Plasma NfL concentrations in cognitively unimpaired and neurodegenerative disorders

Plasma NfL levels (unadjusted for age) for cognitively unimpaired and diagnostic groups in the KCL and Lund cohorts are displayed in Fig. 1A and 1B, respectively. In the KCL cohort, the concentrations of plasma NfL were significantly increased in all cognitively impaired, parkinsonian and other conditions compared to the CU Aβ- group ($P < 0.0001$, Fig. 1A), with the exception of PD, MS and EOAD groups. However, when adjusting for age, individuals classified as EOAD had significantly higher NfL levels as compared to those of CU Aβ- group ($P = 0.001$). Likewise, highly significant increases of plasma NfL were observed in all cognitively impaired and parkinsonian groups as compared to PD ($P < 0.0001$). However, FTD and ALS were the only groups showing significantly higher plasma NfL levels in comparison to AD dementia ($P < 0.05$ and $P < 0.0001$, respectively). Plasma NfL levels in CU Aβ + were also significantly higher as compared to CU Aβ- individuals ($P < 0.05$). Similar findings were found in the Lund cohort where the concentrations of plasma NfL were significantly increased in all disorders when compared to the CU Aβ-, CU Aβ+, SCD Aβ-, SCD Aβ + groups ($P < 0.0001$) and MCI groups ($P = 0.001$), with non-significant differences in PD and EOAD. Age adjustment did demonstrate a significant increase in the EOAD group in comparison to Aβ- ($P = 0.001$) but not to Aβ + control groups. However, unlike the KCL cohort, when comparing within the unimpaired groups, no significant increase was observed in CU Aβ+, SCD Aβ- and SCD Aβ + when compared with CU Aβ- individuals. AD dementia was significantly increased compared with all CU, MCI and PD groups ($P < 0.0001$), as well as EOAD ($P < 0.005$), but not significantly different from EOAD after age correction.

When combining the two cohorts, the largest effect sizes against CU Aβ- group were observed for DS with AD (DSAD, Hedges $g = 1.87$), MSA (Hedges $g = 1.25$), ALS (Hedges $g = 1.19$), CBS/PSP (Hedges $g = 0.96$) and FTD (Hedges $g = 0.84$). Medium effect sizes (Hedges $g < 0.5$) were observed for VaD and AD dementia (Fig. 2A). However, only small effect sizes existed in MCI groups (Hedges $g < 0.1$). When measuring the effect size of plasma NfL in the PD group (Fig. 2B), large effects sizes were observed for atypical parkinsonian disorders (CBS/PSP, Hedges $g = 2.0$; MSA, Hedges $g = 1.4$) but also large effect sizes for some cognitive impairment disorders (VaD, Hedges g = 1.88; FTD, Hedges $g = 1.4$; PDD/DLB, Hedges $g = 1.1$, AD dementia, Hedges $g = 1.0$). In contrast, only medium or small effect sizes where demonstrated when comparing AD dementia to other cognitive impairment disorders (Fig. 2C). A large effect size was observed when comparing plasma NfL in DSAD versus DS albeit not reaching statistical significance due to a small sample size (Hedges g = 1.7, $P = 0.085$).

Accuracy of plasma NfL in differentiating neurodegenerative disorders

Next, we investigated the diagnostic accuracies of plasma NfL in differentiating among neurodegenerative disorders and also from CU groups. AUC values for the KCL and Lund cohorts are displayed in Fig. 3A and 3B respectively. The 95% confidence intervals (CI) of AUC, sensitivity and specificity estimates can be found in Supplementary Table 4 and Supplementary Fig. 5.

ROC analyses for plasma NfL demonstrated low accuracy in separating CU Aβ- from CU Aβ+, SCD and MCI groups (AUC = 52–65%), but performed better for identifying AD dementia (KCL, AUC = 79%; Lund, AUC = 80%) with superior specificity (76–78%) than sensitivity (65–67%). High AUC (> 80%) were also found in distinguishing CU Aβ- from atypical parkinsonian in both cohorts and DS, DSAD, FTD, ALS and MS in the KCL cohort. Plasma NfL also performed well in identifying atypical parkinsonian disorders from PD patients with very high specificity in the KCL cohort (AUC = 86%; sensitivity = 56%; specificity = 89%) which was observed in the Lund cohort for both CBS/PSP (AUC = 95%; sensitivity = 51%; specificity = 100%) and MSA (AUC = 88%; sensitivity = 57%; specificity = 90%). Plasma NfL had a high accuracy in differentiating DS from DSAD (AUC = 91%; sensitivity = 100%; specificity = 71%). A moderate AUC in differentiating FTD from ALS (AUC = 72%) but higher for distinguishing FTD from depression (AUC = 85%) was observed. Low AUC's were observed for differentiating AD dementia from other cognitive impairment disorders (*e.g.*, VaD, PDD/DLB, FTD) and also PDD/DLB from atypical parkinsonian disorders.

Concentration cut-off points for neurodegeneration using plasma NfL

Three cut-off points of plasma NfL concentration for neurodegeneration were applied a) 90%, b) 95% and c) 99% CI of CU Aβ- participants. Additional methods for cut-offs for plasma NfL were also derived by two other approaches i) mean plus 2 standard deviations of the CU Aβ- participants and ii) Gaussian mixture modelling. The cut-offs were performed and generated in the KCL cohort and then tested in the Lund cohort. The cut-off concentrations for all methods are reported in the Supplementary Table 5.

The performance of concentration cut-offs based on CI of CU Aβ- participants of all ages is demonstrated in Fig. 4. This method, which was derived in the KCL cohort, calculated plasma NfL concentration cut-offs at 35.02 pg/mL, 38.04 pg/mL and 50.00 pg/mL for the 90%, 95% and 99% CI of the CU Aβ- participants, respectively. In both the KCL and Lund cohorts, a more stringent cut-off (99% CI) demonstrated relatively low false positives for all CU groups and also for depression, PD and EOAD (0–12%). A more moderate cut-off (CI 90–95%) demonstrated higher percentages of false positives in the same groups (0–25%). On the other hand, the 99% CI cut-off failed to identify neurodegeneration with a high degree of accuracy in disease groups, whereas a 90% CI accurately classified > 75% of participants with neurodegenerative disorders in the Lund cohort; VaD (77%), AD (79%), CBS/PSP (87%), FTD (88%) and MSA (89%). Similar findings were also observed in the KCL cohort, although the % abnormal for plasma NfL was lower for AD (68%) but higher for PDD/DLB (KCL = 78%; Lund = 68%). Concentration cut-offs of plasma NfL identified neurodegeneration in FTD (> 75%), CBS/PSP (> 80%), ALS (98–100%) and DSAD (100%) with very high accuracy. Plasma NfL cut-offs were then tested in ADNI participants (n = 870) to replicate the findings for AD dementia (Supplementary Fig. 5). Similar to the KCL and Lund cohorts, a 99% CI cut-off exhibited relatively low false positives in CU groups (< 10%), whereas for AD dementia, a 90% CI cut-off correctly classified > 75% of cases. Unlike the KCL and Lund cohorts, ADNI participants classified as MCI Aβ + had a significantly higher ($P < 0.001$) percentage of individuals with abnormal NfL above a 90% CI cut-off (61%) than MCI Aβ- (49%).

Due to the strong relationship between age and NfL, age-related cut-offs were also determined (Supplementary Table 5). Firstly, we tested 65 + year cut-off, combining the KCL and Lund cohorts (n = 1646, Fig. 5A). As expected, the cut-off derived from CU participants aged 65 + yielded marginally higher plasma NfL cut-offs than previously described for 90%, 95% and 99% CI based approaches (37.02, 46.00, 79.20 pg/mL). While no major differences were observed from Fig. 4, lower percentages of abnormal plasma NfL were observed for Aβ- CU and SCD were lower (6%) as well as the PD group (7%) for the 99% CI cut-off as compared to the cut-off derived from all ages.

Concentration cut-offs in CU participants aged < 65 were substantially lower; 19.37 pg/mL, 21.50 pg/mL and 30.01 pg/mL, respectively and were tested in participants in the KCL and Lund cohorts combined (n = 653, Fig. 5B). Firstly, with this age-related cut-off, abnormal levels of NfL were found in 100% of patients with MSA, ALS and DSAD regardless of % CI employed. Secondly, identifying abnormal NfL vastly improved in FTD (> 90%), CBS/PSP (> 90%), PDD/DLB (84%) and MCI groups (40–80%). While these improvements were seen for disorder groups, false positives for abnormal plasma NfL remained low for Aβ- controls (CU and SCD), depression and PD (0–7%). Interestingly, higher rates of abnormal plasma NfL were now detected in controls that were Aβ+ (> 22% in 90% CI; >60% in 99% CI). Simialrly, greater rates of abnormal plasma NfL were also observed in MCI Aβ + compared with MCI Aβ-. Finally, improved rates of abnormal plasma NfL were observed in EOAD when utilizing an age-related cut-off (77%, 90% CI) which was comparable to abnormal NfL in AD using the > 65-year cut-off. Interestingly, a small percentage (12%) of depression participants demonstrated abnormal plasma NfL when using an age appropriate cut-off for this diagnostic group.

Discussion

This study, to the best of our knowledge, includes the largest and most diverse investigation for plasma NfL comprising 2311 participants from CU individuals and fifteen neurodegenerative disorders and depression. Firstly, our findings corroborate, on a large scale, the globally increased plasma NfL concentration in major neurodegenerative disorders. Secondly, while these increases are seemingly not disease-specific, we demonstrate that plasma NfL is clinically useful in differentiating atypical parkinsonian disorders from PD, in identifying dementia in Down Syndrome, distinguishing neurodegenerative disorders from depression in older adults and, potentially, identifying frontotemporal dementia in patients with cognitive impairment. However, NfL provides limited information in separating specific disorders of cognitive impairment (*e.g.*, FTD vs AD) or preclinical conditions (*e.g.* CU Aβ- vs CU Aβ+). Lastly, we derived data-driven and age-related concentration cut-offs that give relatively low false positives of abnormal plasma NfL but also indicate neurodegeneration in cortical neurodegenerative disorders, parkinsonian and other neurogenerative disorders depending on the cut-off strategy employed. The importance of age-related cut-offs was clearly demonstrated in disorders with a younger age of onset (*e.g*, EOAD and FTD).

A recent meta-analysis on more than 10,000 individuals demonstrated that individuals with human immunodeficiency virus (HIV), FTD, ALS and Huntington's disease (HD) presented with CSF NfL concentrations averaging 21-fold, 11-fold, 8-fold and 6-fold higher than CU controls, respectively [47]. In comparison, in the same study, CSF NfL was 1.9-fold higher in AD dementia patients. This is in-line with the present plasma study, which also showed that individuals with ALS and FTD presented with the highest concentrations of plasma NfL and among the largest effect sizes against CU individuals, albeit less dramatic than what has been reported for CSF. Although HIV and HD groups were not examined in this study, we were able to determine that DSAD and atypical parkinsonian disorders have the largest increases and effect sizes of plasma NfL as compared to individuals without cognitive impairment. The AD dementia population in this study was on average 1.8-fold higher than CU, mirroring the observations reported in CSF studies.

We tested the accuracy, sensitivity and specificity of plasma NfL in differentiating neurodegenerative disorders. Although the majority of comparisons would not be a realistic diagnostic challenge in a clinical setting, high performance of plasma NfL was seen in predicting atypical parkinsonian disorders from PD. While plasma NfL data from atypical parkinsonian patients in the Lund cohort has been previously reported [24,25] it is congruent with novel data included from the KCL cohort. In both cohorts, atypical parkinsonian disorders (*e.g.*, CBS, PSP, MSA) had substantial increases in plasma NfL as compared to PD with very high diagnostic accuracies (KCL AUC > 86%; Lund AUC > 95%) and large effect sizes. Therefore, a presentation of parkinsonism with high levels of plasma NfL is highly suggestive of an atypical parkinsonian disorder and this finding is likely due to the degree of axonal damage being more severe in atypical parkinsonian disorders than in PD. Furthermore, although not typically a diagnostic challenge, plasma NfL level was able to distinguish ALS from controls in > 90 percent of cases. In this study, we show the highest NfL levels of the fifteen neurodegenerative diseases that have been compared were observed in ALS and FTD. This may be indicative of the intensity of neurodegeneration or level of axonal damage and/or the extent of the degenerated axons. Substantial evidence supports that neuronal and axon damage in ALS and FTD results in the release of neurofilament proteins into the CSF and plasma [48,49]. Separately high levels of plasma NfL in ALS and FTD have also been linked to disease severity, as shown by NfL levels correlating with survival and disease progression in ALS and FTD [48,50,51]. Interestingly ALS and FTD might be phenotypic extremes on a spectrum disorder, which is called motor neuron disease–FTD continuum, and up to 15% of all incident in ALS cases are associated with FTD [52]. Yet, the diagnosis of FTD and especially the behavioral variant (bvFTD) subtype is often challenging, as the heterogeneous clinical manifestation may overlap not only with other neurodegenerative diseases but also with psychiatric disorders. A further novel contribution of this study is we demonstrate the normal plasma NfL concentrations of individuals with moderate and severe depression, and that high AUC (85%) existed when comparing depressed patients with those with an FTD diagnosis. Therefore, this study shows promise in plasma NfL discriminating between FTD and psychiatric disorders when the significant clinical overlap does exist [26]. Our data is also consistent with previous studies on plasma NfL in DS [53–55] where an increase of plasma NfL levels were substantially higher in the DSAD group. Using our defined concentration cut-offs, we were able differentiate DSAD from DS in the KCL cohort (AUC = 91%) and demonstrate that all DSAD patients exhibited abnormal plasma NfL when applying cut-offs.

We derived and tested concentration cut-offs to identify neurodegeneration ranging from high specificity (99% CI) to a cut-off favoring greater sensitivity (90% CI) which could be used as a guide in primary care assessment. We confirmed that NfL is abnormally elevated in multiple disorders but overlapping concentrations among disorders limit plasma NfL as a disease-specific marker. When a more sensitive cut-off was applied, abnormal NfL levels were consistently observed in the majority of neurodegenerative disorders. This also included AD dementia where plasma NfL is seen to be only mildly elevated as compared to other neurodegenerative disorders. In contrast, a plasma NfL cut-off set using the 99% CI demonstrated very the ability to give reliability low false positives in cognitively unimpaired, subjective complaints, depression and PD groups were absent axonal damage is expected. These cut-offs produced similar results when applied independently in ADNI.

In addition to the diagnostic capabilities of plasma NfL, this study highlights other key factors which should be detailed. Multiple lines of evidence have reported age and CSF NfL as having strong relationships with plasma NfL. While these statements are without-a-doubt true, based on the findings presented herein one cannot simply apply this generalized rule to all age groups and conditions. Firstly, plasma NfL is unequivocally influenced by age but this association is stronger in younger individuals (*e.g.*, < 65 years) and, to some degree, is minimized in older individuals (*e.g.*, > 65 years, Supplementary Table 2). This is due to older individuals being more likely to have developed a neurodegenerative condition and these disorders have a different relationship with age; that is, neurodegenerative disorders that typically exhibit higher concentrations of plasma NfL have weaker correlations with age (*e.g.*, FTD). Furthermore, plasma NfL is likely to increase in response to pathologies that manifest in later life (*e.g.*, limbic-predominant age-related TDP-43 Encephalopathy, LATE). In our study, the influence of age on NfL is shown in multiple aspects, but most prominently by EOAD patients seemingly being no different from CU adults if an age adjustment is not taken into consideration. Our < 65-year plasma NfL cut-offs (19.4 pg/mL, 21.5 pg/mL, 30.0 pg/mL) were substantially lower as to compared older cut-offs (38.0 pg/mL, 46.0 pg/mL, 54.8 pg/mL) and when this was applied, EOAD patients had the equivalent rate of abnormal plasma NfL as typical AD dementia – consistent with the reported literature on familial AD [56,57]. We also observed that age-related cut-offs may be more sensitive to neurodegeneration related to Aβ deposition, although it is clear that recent developments in plasma p-tau181 or p-tau217 would be a superior measure of Aβ and tau pathologies [10,11,14,15,51,58]. In individuals < 65 years, rates of abnormal plasma NfL were 3-fold higher in Aβ + controls as compared to Aβ- controls and also higher in MCI Aβ + than MCI Aβ-. The influence of Aβ-positivity on plasma NfL has been previously described [12,13,59] however, in our study, this was far

more apparent in the younger age groups. It is not guaranteed that Aβ deposition leads to cognitive decline; however, when coupled to age-dependent abnormal levels of NfL (a proxy for on-going axonal damage), this may indicate those at a far greater risk. This is further supported by the very low rate of false positives of plasma NfL in Aβ- controls but also in patients with depression and PD which are likely to be Aβ-. Neurodegenerative disorders with a typically younger age of onset also demonstrated higher rates of abnormal NfL if a < 65-year cut-off was applied (*e.g.*, FTD). We have also demonstrated that the plasma-to-CSF relationship of NfL is dependent on condition. While the majority of cognitive impairment disorders and parkinsonian disorders display a strong relationship between plasma and CSF NfL, VaD and CBS/PSP have a non-significant and weak relationship. This is an important consideration when using plasma NfL to infer CSF NfL levels.

Our study has limitations. Although this study was done in 2311 individuals, in certain diagnostic categories and comparisons, it was underpowered. Several neurodegenerative diseases included in this study, such as DS and atypical parkinsonian disorders have a relatively small number of participants. However, although our sample size was small in these groups, we were able to show with excellent accuracy and effect sizes the differentiation between controls and disorders but also within neurodegenerative disorders which maybe a clinically challenging. Unlike many putative plasma biomarkers that have preceded it measurements of plasma NfL are robust and widely reported finding. In this study, we have technically demonstrated very high correlation in the measurements of plasma NfL using two different assays on the Simoa platform, which were performed in independent laboratories. However, it must be noted that absolute concentrations of plasma NfL differed between assays and therefore platform dependent cut-offs would need to be calculated in the likelihood of multiple methodologies to measure NfL in blood in the future. Despite being a multicenter study, this has not influenced our results. This has been shown by i) the very high level of replication between the two cohorts, even when applying a concentration derived in KCL and tested in Lund and ii) CU participants provided by multiple centers having similar concentrations of plasma NfL despite varying preanalytical procedures which have been fully outlined.

In conclusion, in two large independent datasets, we have detailed the meaningful strengths and weaknesses of utilizing plasma NfL as a biomarker for neurodegeneration that could be useful in a primary care setting. Plasma NfL concentrations are increased across multiple neurodegenerative disorders but are highest in samples from individuals with ALS, FTD and DSAD. Though plasma NfL cannot differentiate between different cognitive impairment disorders, in patients with parkinsonism, high plasma NfL values indicate atypical parkinsonian disorders and in patients with DS, high plasma NfL differentiates between those with and without dementia, suggesting it may be useful in both clinical and research settings in these patients. Data-driven age-related concentration cut-offs demonstrated that plasma NfL is suitable to identify neurodegeneration in many neurodegenerative disorders, though false positives rates were low when using an age appropriate cut-off set using the 99% CI of Aβ- CU.

Declarations

Author Contributions

Concept and design: Ashton, Janelidze, Zetterberg, Blennow, Hye, Hansson

Acquisition, analysis, or interpretation of data: All authors

Drafting of the manuscript: Ashton, Janelidze, Zetterberg, Blennow, Hye, Hansson

Statistical analysis: Ashton, Janelidze, Al Khleifat, Leuzy

Obtained funding: Hye, Hansson

Conflicts of Interest

JL has received travel support and/or lecture honoraria from Biogen, Novartis, Merck, Roche and SanofiGenzyme; has served on scientific advisory boards for Biogen, Novartis, Merck, Alexion, Roche and SanofiGenzyme; serves on the editorial board of the Acta Neurologica Scandinavica; has received unconditional research grants from Biogen and Novartis. AS has been a consultant for AC-Immune and is a member of the scientific advisory board of ProMIS Neurosciences. PS has received speaker fees for Shire/Takeda and Sanofi Genzyme. HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg**.** KB has served as a consultant or at advisory boards for Alector, Alzheon, CogRx, Biogen, Lilly, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper. OH has acquired research support (for the institution) from Roche, GE Healthcare, Biogen, AVID Radiopharmaceuticals and Euroimmun. In the past 2 years, he has received consultancy/speaker fees (paid to the institution) from Biogen and Roche.

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Figures

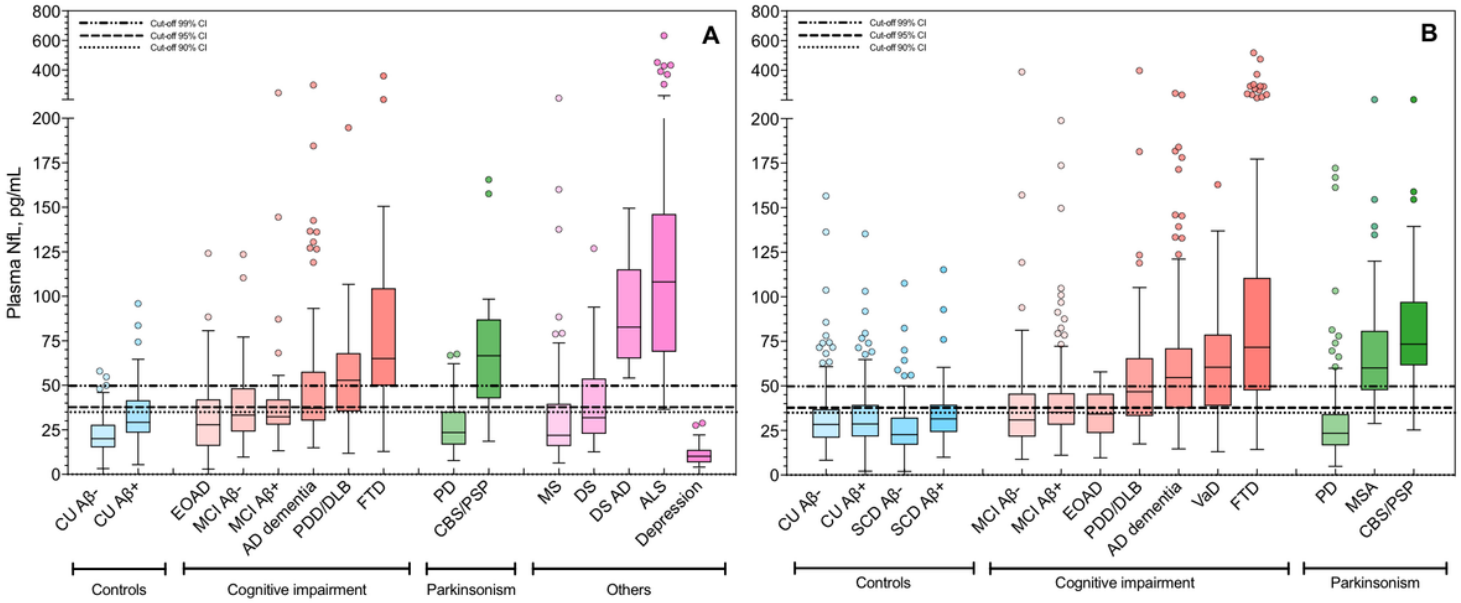

Figure 1

Plasma neurofilament light (NfL) in different diagnostic groups; KCL (A) and Lund (B) cohorts. For each plot, the horizontal bar shows the median, and the upper and lower boundaries show the 25th and 75th percentiles, respectively. AD = Alzheimer's disease, ALS = amyotrophic lateral sclerosis, CBS = cortical basal syndrome, DLB = dementia with Lewy bodies, DS = Down syndrome, DSAD = Down syndrome Alzheimer's disease, EOAD = Early onset Alzheimer's

disease, FTD = frontotemporal dementia. MCI = mild cognitive impairment, MS = multiple sclerosis, MSA = multiple system atrophy, PD = Parkinson's disease, PDD = Parkinson's disease dementia, PSP = progressive supranuclear palsy, SCD = subjective cognitive decline, VaD = vascular dementia.

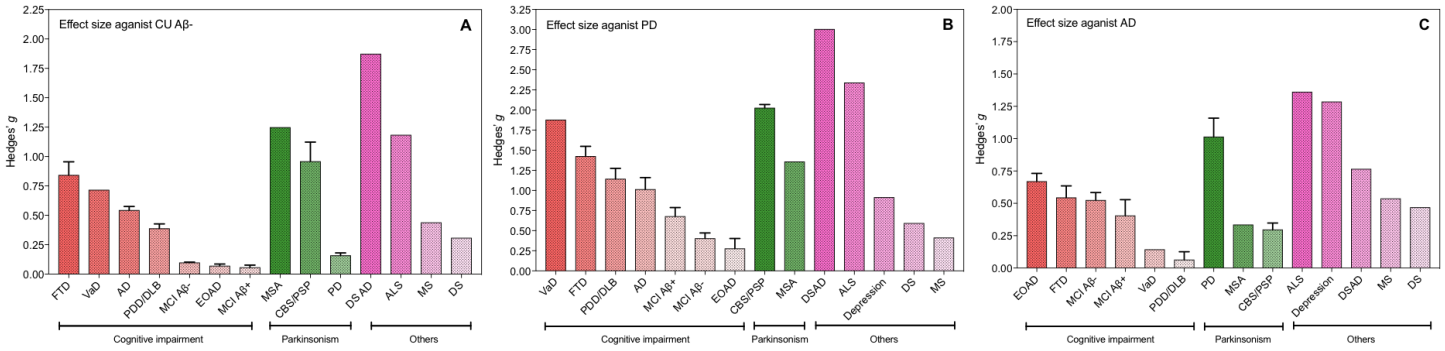

Figure 2

Effect sizes (Hedges's g) of different neurodegenerative disorders as compared to amyloid-negative cognitively unimpaired controls (A), Parkinson's disease (B) and Alzheimer's disease (C). AD = Alzheimer's disease, ALS = amyotrophic lateral sclerosis, CBS = cortical basal syndrome, DLB = dementia with Lewy bodies, DS = Down syndrome, DS = Down syndrome, DSAD = Down syndrome Alzheimer's disease, EOAD = Early onset Alzheimer's disease, FTD = frontotemporal dementia. MCI = mild cognitive impairment, MS = multiple sclerosis, MSA = multiple system atrophy, PD = Parkinson's disease, PDD = Parkinson's disease dementia, PSP = progressive supranuclear palsy, SCD = subjective cognitive decline, VaD = vascular dementia.

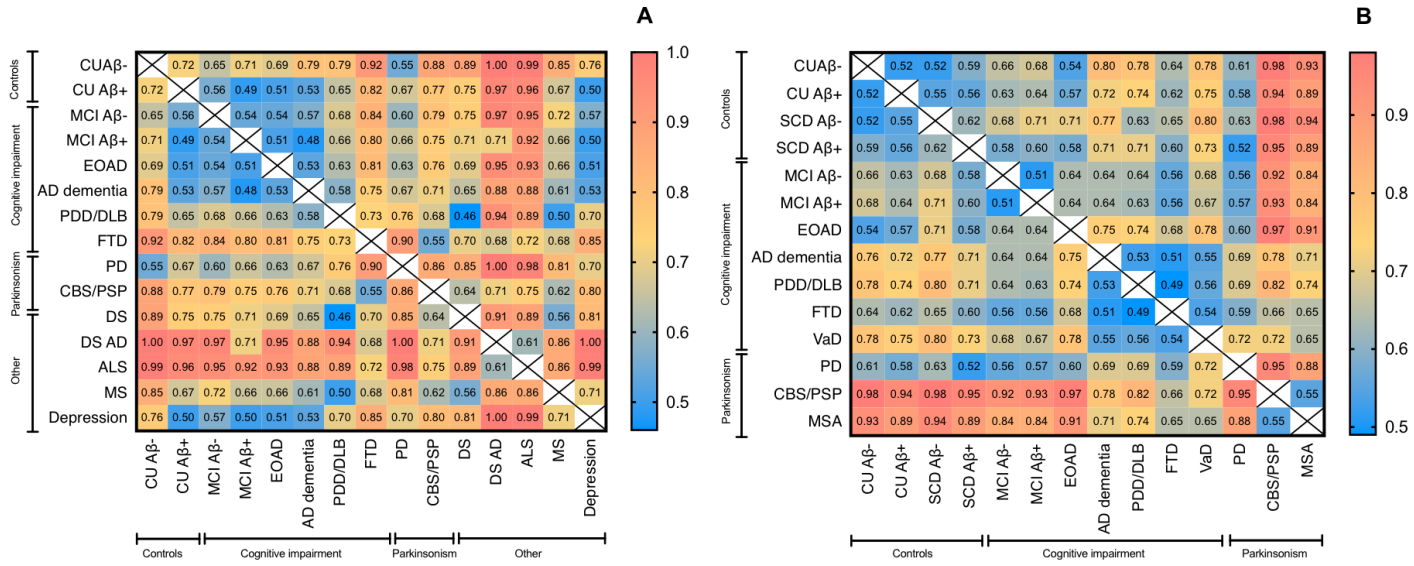

Figure 3

Effect sizes (Hedges's g) of different neurodegenerative disorders as compared to amyloid-negative cognitively unimpaired controls (A), Parkinson's disease (B) and Alzheimer's disease (C). AD = Alzheimer's disease, ALS = amyotrophic lateral sclerosis, CBS = cortical basal syndrome, DLB = dementia with Lewy bodies, DS = Down syndrome, DS = Down syndrome, DSAD = Down syndrome Alzheimer's disease, EOAD = Early onset Alzheimer's disease, FTD = frontotemporal dementia. MCI = mild cognitive impairment, MS = multiple sclerosis, MSA = multiple system atrophy, PD = Parkinson's disease, PDD = Parkinson's disease dementia, PSP = progressive supranuclear palsy, SCD = subjective cognitive decline, VaD = vascular dementia.

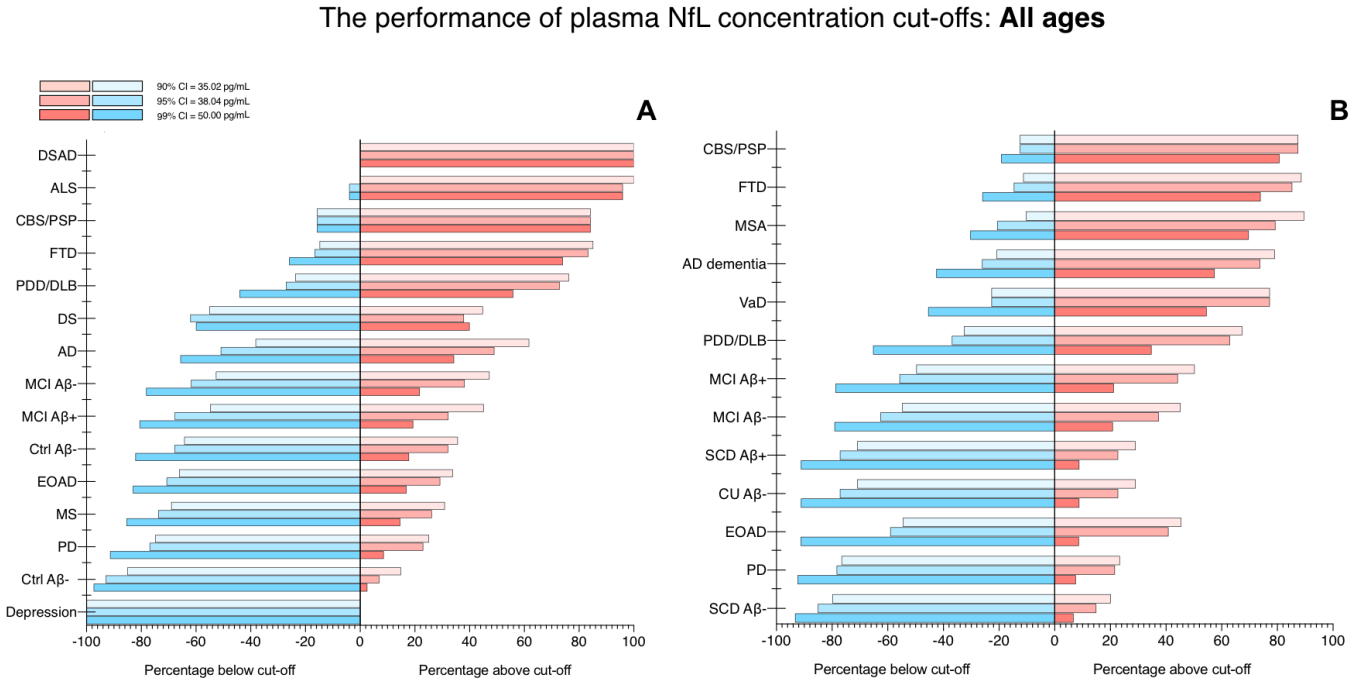

Figure 4

The performance of plasma neurofilament light (NfL) concentration cut-offs to identify neurodegenerative disorders in KCL (A) and Lund (B). AD = Alzheimer's disease, ALS = amyotrophic lateral sclerosis, CBS = cortical basal syndrome, DLB = dementia with Lewy bodies, DS = Down syndrome, DS = Down syndrome, DSAD = Down syndrome Alzheimer's disease, EOAD = Early onset Alzheimer's disease, FTD = frontotemporal dementia. MCI = mild cognitive impairment, MS = multiple sclerosis, MSA = multiple system atrophy, PD = Parkinson's disease, PDD = Parkinson's disease dementia, PSP = progressive supranuclear palsy, SCD = subjective cognitive decline, VaD = vascular dementia.

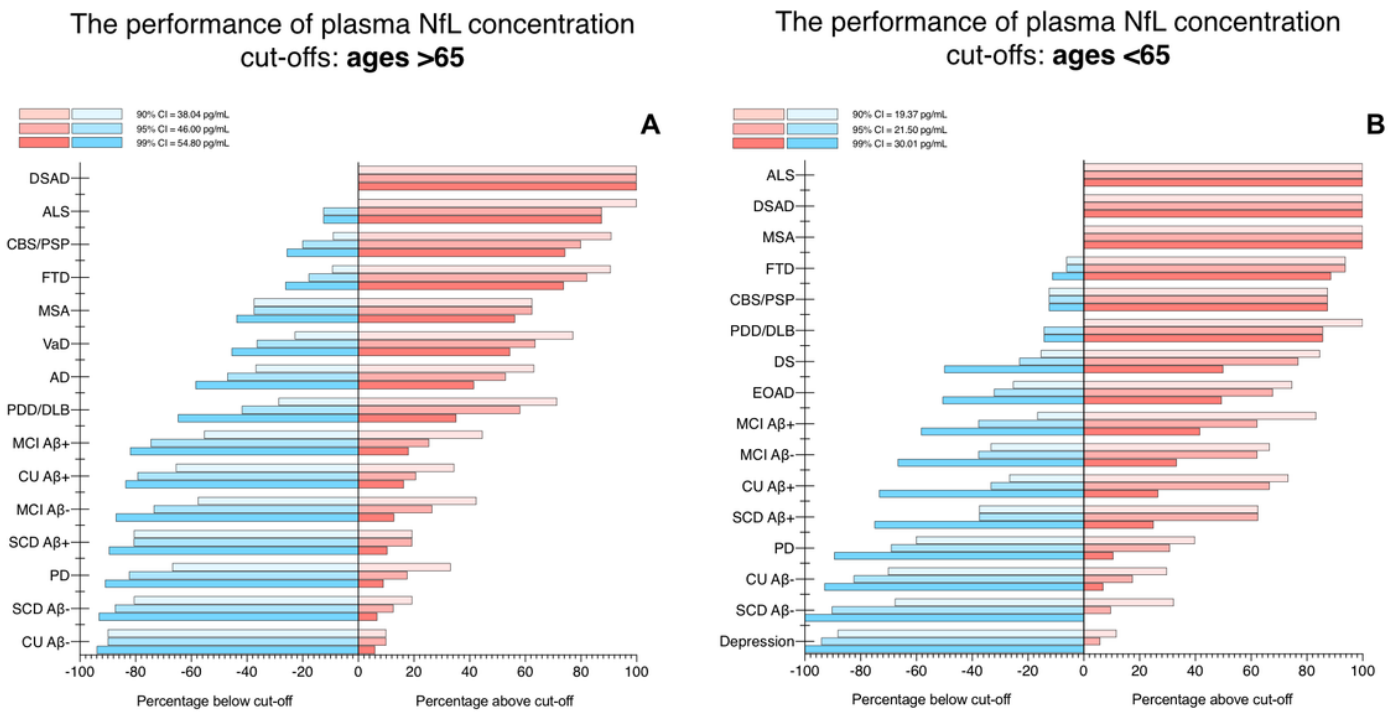

Figure 5

The performance of plasma neurofilament light (NfL) concentration cut-offs to identify neurodegenerative disorders in >65 (A) and <65 (B). The KCL and Lund cohorts are combined for this analysis. AD = Alzheimer's disease, ALS = amyotrophic lateral sclerosis, CBS = cortical basal syndrome, DLB = dementia with Lewy bodies, DS = Down syndrome, DS = Down syndrome, DSAD = Down syndrome Alzheimer's disease, EOAD = Early onset Alzheimer's disease, FTD = frontotemporal dementia. MCI = mild cognitive impairment, MS = multiple sclerosis, MSA = multiple system atrophy, PD = Parkinson's disease, PDD = Parkinson's disease dementia, PSP = progressive supranuclear palsy, SCD = subjective cognitive decline, VaD = vascular dementia.

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