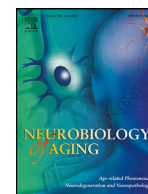




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CSF neurofilament light may predict progression from amnestic mild cognitive impairment to Alzheimer's disease dementia

Bryant Lim^{a,#}, Gøril Rolfseng Grøntvedt^{b,c,#}, Pradeepthi Bathala^d, Shraddha S. Kale^d, Christopher T. Campbell^d, Martin Stengelin^d, Sigrid Botne Sando^{b,c}, Ioannis Prassas^a, Eleftherios P. Diamandis^{a,e,f}, Geir Bråthen^{b,c,*}^a Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada^b Department of Neurology and Clinical Neurophysiology, University Hospital of Trondheim, Trondheim, Norway^c Department of Neuromedicine and Movement Science, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway^d Meso Scale Diagnostics, LLC., Rockville, MD, USA^e Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada^f Department of Clinical Biochemistry, University Health Network, Toronto, Canada

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ABSTRACT

Neurofilament light (NfL) is a promising biomarker of neurodegeneration in Alzheimer's disease (AD). In this study, cerebrospinal fluid (CSF) NfL was measured in a 24-month longitudinal cohort consisting of control (n = 52), amnestic mild cognitive impairment (aMCI) (n = 55), and probable AD dementia (n = 28) individuals. The cohort was reevaluated after 6–10 years. Baseline CSF NfL was significantly elevated in aMCI and probable AD dementia groups compared to controls ($p < 0.0001$). CSF NfL was significantly lower in stable aMCI patients compared to aMCI patients who converted to probable AD dementia within the 24-month period ($p = 0.004$). Substituting T-tau for NfL in the core AD biomarkers model (A β 42/P-tau/T-tau) did not improve ability to separate control and AD, or stable and converter aMCI patients. Our results support that elevated CSF NfL could predict progression in aMCI patients, but its utility cannot improve the core AD biomarkers.

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by extracellular aggregates of amyloid-beta (A β) plaques and intracellular neurofibrillary tangles. Established biomarkers of AD diagnosis include cerebrospinal fluid (CSF) A β 42, total tau (T-tau), and phosphorylated tau (P-tau). Imaging biomarkers are also available: structural magnetic resonance imaging (MRI), positron emission tomography (PET) imaging of brain metabolism, amyloid and tau (Blennow and Zetterberg, 2018; Jack et al., 2016). The "A/T/N" classification system divides these biomarkers into 3 categories based on the nature of their corresponding pathophysiology. "A" refers to the value

of a β -amyloid biomarker (amyloid PET or CSF A β 42), "T" to the value of a tau biomarker (CSF P-tau, or tau PET) and "N" to the value of biomarkers of neurodegeneration or neuronal injury ([¹⁸F]-fluorodeoxyglucose-PET, structural MRI, or CSF T-tau) (Jack et al., 2016).

Although existing cerebrospinal fluid (CSF) biomarkers are useful for identifying AD pathology and consequently for diagnosing AD, there is still a need for biomarkers that can predict disease progression and document treatment efficacy. Though levels of CSF amyloid and tau begin to alter pathologically in the preclinical stages of disease, the rate of change in these biomarker levels might be variable along the AD continuum (Dodge et al., 2014; Jack et al., 2013; Lleó et al., 2019). Furthermore, CSF amyloid and tau have been shown to plateau up to a decade before disease onset (Lewczuk et al., 2018b; Stomrud et al., 2015). These factors preclude the use of established core biomarkers to predict the rate of disease progression.

Neurofilament light chain (NfL) is increasingly recognized as a promising biomarker for neurodegeneration in AD. Residing pre-

* Corresponding author at: Dept. of Neurology and Clinical Neurophysiology, Trondheim University Hospital, Postboks 3250 Torgarden, 7006 Trondheim, Tel +47 922 90 661

E-mail address: geir.brathen@ntnu.no (G. Bråthen).

Co-first Author

dominantly within myelinated axons, NfL is a cytoskeletal protein that plays a role in maintaining neuronal structural integrity and axonal caliber (Yuan et al., 2012). Neuronal damage in neurodegenerative diseases releases NfL into the extracellular space and eventually into the CSF (Dhiman et al., 2020). Elevated CSF NfL has been found in AD and other neurodegenerative diseases such as frontotemporal dementia, motor neuron disease, dementia with Lewy bodies, and multiple sclerosis (Delaby et al., 2020; Varhaug et al., 2019), as well as in neuroinflammatory and neurovascular diseases (Gaetani et al., 2019). Along the Alzheimer's continuum, the difference in CSF NfL levels is particularly robust when comparing AD patients and cognitively unimpaired individuals (Forgrave et al., 2019). Elevated CSF NfL also relates to faster brain atrophy and cognitive decline in longitudinally-followed AD patients (Dhiman et al., 2020; Osborn et al., 2019; Zetterberg et al., 2016). Consequently, elevated levels of CSF NfL in early clinical stages of AD may predict faster cognitive decline and conversion to AD dementia (Zetterberg et al., 2016). Because of the strong association between NfL and neurodegeneration, it has been proposed to be a potential substitute of T-tau in the A/T/N classification system, though how it complements or improves existing diagnosis paradigms is not well studied (Jack et al., 2018).

The primary aim of this study was to explore whether NfL levels in CSF could predict progression from aMCI to probable AD dementia in a clinical longitudinal cohort. The second aim was to determine whether NfL would give additional information on disease progression compared to the established core CSF AD biomarkers (A β 42, T-tau, and P-tau).

2. Methods

2.1. Sample source

Sample population is previously described (Grøntvedt et al., 2020). Study participants were part of a longitudinal study conducted at the Department of Neurology, University Hospital of Trondheim, consisting of 52 cognitively unimpaired individuals, 55 patients with aMCI, and 28 patients with probable AD dementia at inclusion. Cognitively unimpaired individuals were recruited from genetically unrelated caregivers or elderly volunteers from societies for retired people. All participants were recruited between 2009 and 2013, and initially monitored over a period of 2 years. In this period, 27 of the aMCI patients developed probable AD dementia (early converter aMCI). In 2019, an extended clinical follow-up of the cohort was conducted, with a median of 9 years (range of 6–10 years) from baseline. Within the extended follow-up, 4 of the control individuals developed aMCI or probable AD dementia and 9 of the remaining aMCI patients developed probable AD dementia (late converter aMCI). Detailed timeline of the cohort is shown in Fig. 1.

Neurological examination and cognitive tests including the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) were performed in all control individuals and patients during the initial study period (baseline, 12-months and 24-months). Additionally, patients with aMCI and probable AD dementia underwent lumbar puncture at baseline, after 12 and 24 months. For control individuals, lumbar puncture was only performed at baseline due to ethical restrictions (Berge et al., 2014, 2016,).

Clinical diagnoses for probable AD dementia and aMCI were given according to the NINCDS/ADRDA criteria (McKhann et al., 1984) and the International Working Group on Mild Cognitive Impairment criteria (Winblad et al., 2004), respectively, independent of biomarker evidence. The clinical diagnosis at the extended follow-up was based on clinical interviews and medical records

(Grøntvedt et al., 2020). APOE genotyping was done according to methods described elsewhere (Berge et al., 2014).

Concentrations of A β 42, T-tau, and P-tau in CSF were measured using commercially available enzyme-linked immunosorbent assay kits (ELISA) according to manufacturer protocols (Fujirebio Innogenetics). Cutoff values for core AD biomarkers in CSF were calculated by maximizing Youden index as described in a previous study (Grøntvedt et al., 2020). Accordingly, CSF A β 42 levels <630 pg/mL, T-tau levels >394 pg/mL, and P-tau levels >66 pg/mL were considered pathological. Informed consent was signed by all participating individuals, and in some cases also by their proxies. The study was approved by the Regional Committee for Medical Research Ethics for central Norway.

2.2. Measurement of NfL

CSF samples were blinded and measured for NfL concentration using an ultrasensitive NfL immunoassay from Meso Scale Diagnostics, LLC (Stengelin et al., 2019). The assay uses a next generation electrochemiluminescence detection technology which measures light signals emitted from SULFO-TAG labels that are stimulated electrochemically on the surfaces of the proprietary MULTI-ARRAY microplates (Glezer et al., 2014). The assay has a limit of blank (LOB) of 0.7 pg/mL and lower and upper limit of quantitation (LLOQ, ULOQ) of 3.4 and 1700 pg/mL respectively. LOB was determined by running 20 replicates of zero calibrator and calculating the concentration corresponding to the average zero calibrator signal plus 2.5 standard deviations. LLOQ and ULOQ were determined by running 6 plates on 2 days with 4 replicates per plate. LLOQ was defined as the lowest concentration with a total coefficient of variation (CV) of 20 % or less. ULOQ was defined as the highest concentration with a total CV of 20 % or less. 7 NfL calibrators with concentrations from 6.7 ng/mL to 0.05 pg/mL (7x serially diluted) plus zero calibrator as 8 level were used to establish a standard curve using a weighted logistic fit. Native purified neurofilament light from bovine spinal cord was used to calibrate the assay. Samples were run blinded in duplicates on 14 plates over 2 days. Each plate contained 4 quality control (QC) samples in duplicates: a diluent spiked with 3 NfL concentrations spanning the assay range. Most QC sample concentrations were within 20% of the expected value. Due to limited available sample volume, CSF samples were blinded and diluted 1:10. 25 μ L of diluted sample was used per measurement. Despite the fact that samples were diluted, all sample concentrations were measurable and at least one order of magnitude above the assay detection limit.

2.3. Statistical analysis

R statistical and graphical software (version 3.6.0) and Graphpad Prism (version 6.0e) were used for statistical analyses. A *p*-value of <0.05 was considered significant. The Kolmogorov-Smirnov test was used to determine whether the data was normally distributed. Data was normalized by transforming the data logarithmically and removing outliers identified by the Grubbs test for parametric analysis. Distribution of categorical demographic data was compared between groups using chi-squared test (overall and pairwise). Continuous demographic data was compared between groups using one-way ANOVA, and pairwise comparisons were analyzed using Tukey's Honest Significant Difference. For receiving operating characteristic (ROC) analyses, under the curve (AUC) estimates with 95 % confidence intervals (CI) were calculated using R. Optimal thresholds with specificity and sensitivity of CSF NfL were determined by maximizing the Youden Index (Youden, 1950). The DeLong test was used to compare the AUC between the ROC curves of different models (DeLong et al., 1988).

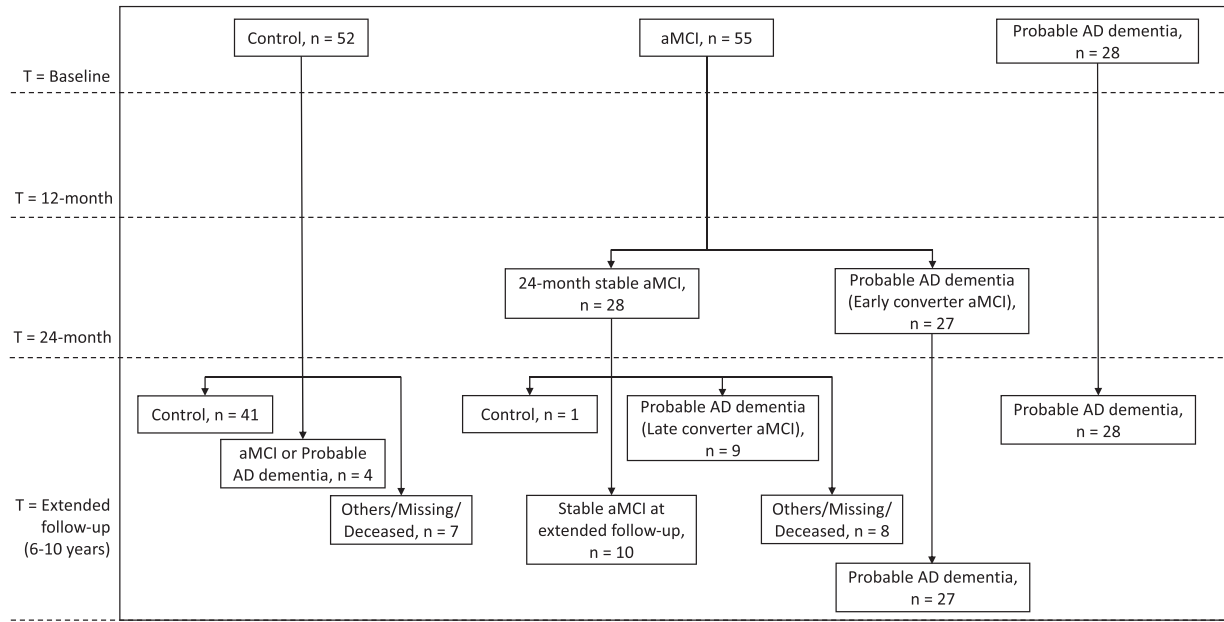


Fig. 1. Included individuals and diagnostic changes during follow-up.

A linear mixed model was implemented using the “lme4” package in R to estimate and compare annualized rate of change in NfL levels, adjusted for age, sex and patient individuals as a random effect. Pearson correlation was used to determine correlation between CSF NfL and the core AD biomarkers ($A\beta_{42}$, T-tau, and P-tau) and MMSE scores.

3. Results

Demographic and clinical details are shown in Table 1. There was no significant difference in the proportion of males and females in the cohort. Age was significantly different between groups, with the control individuals being on average older than aMCI ($p = 0.012$) and probable AD dementia patients ($p = 0.009$). As expected, MMSE score was significantly different between groups, being highest among the control individuals and progressively lower in cognitively impaired patients (for all comparisons $p < 0.0001$). The proportion of *APOE* $\epsilon 4$ carriers was also significantly lower in the control group than in the aMCI and probable AD dementia groups (for all comparisons $p < 0.03$).

For core AD biomarkers, one-way ANOVA showed significant differences in concentration of CSF $A\beta_{42}$ ($p < 0.0001$), CSF T-tau ($p < 0.0001$) and P-tau ($p = 0.0002$) between groups in the cohort. $A\beta_{42}$ concentration was significantly lower in the patient groups compared with the control group (for all comparison $p < 0.0001$), but did not differ between aMCI and probable AD dementia groups. In the multiple comparison analysis, both T-tau and P-tau concentrations were significantly lower in the control group compared with the probable AD dementia group ($p < 0.0003$) and aMCI group ($p < 0.03$). CSF T-tau was also higher in the probable AD dementia group compared with the aMCI group ($p = 0.049$).

4. CSF NfL concentration and cohort demographics

Baseline CSF NfL in the overall cohort was weakly but significantly correlated with age (Pearson's $r = 0.19$, $p = 0.035$) and was higher in males than in females ($p = 0.0001$). Baseline levels of CSF NfL were also compared between *APOE* $\epsilon 4$ carriers and non-carriers (Supplementary Table 1). In the comparison between all cohort individuals, CSF NfL was significantly higher in *APOE* $\epsilon 4$ carriers than

non-carriers ($p = 0.0001$). Significant differences were not found between carriers and non-carriers in control or patient subgroups.

5. CSF NfL concentration and cohort diagnosis at baseline

Baseline CSF NfL levels were analyzed for differences between control, aMCI, and probable AD dementia groups. After applying the Grubbs test for outliers, CSF NfL concentration in one control individual (45,830 pg/mL) was identified to be an outlier ($p < 0.05$) and thus removed. There were overall significant differences in baseline CSF NfL between diagnostic groups at baseline ($p < 0.0001$) (Fig. 2A). Compared with the control and aMCI groups, baseline CSF NfL levels were highest in the probable AD dementia group ($p \leq 0.034$ for both comparisons). CSF NfL was also higher in the aMCI group compared to the control group ($p = 0.008$).

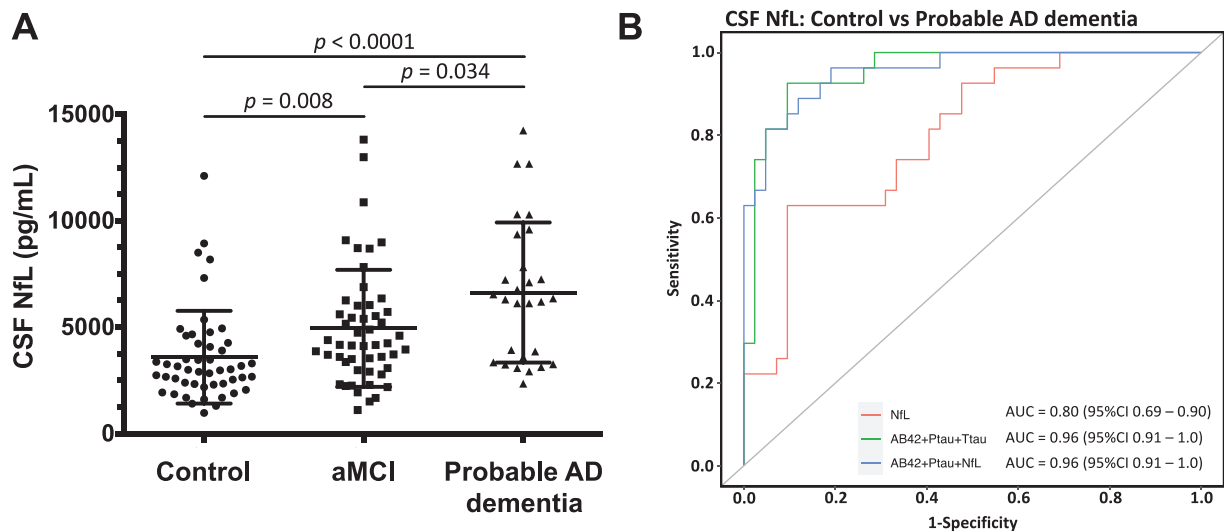
Using ROC curve analysis, we determined the AUC for separating control and probable AD dementia using baseline CSF NfL levels alone. Additionally, AUC was calculated for 2 multiparametric models using the A/T/N criteria: 1) with levels of core AD biomarkers ($A\beta_{42}$, P-tau, and T-tau) in CSF; and 2) with levels of $A\beta_{42}$, P-tau, and NfL in CSF. Since NfL is considered a general biomarker for neurodegeneration, the second multiparametric model substituted T-tau for NfL as a proxy of the neurodegeneration biomarker. Comparing between the control group and probable AD group, the ROC curve analysis for CSF NfL alone, $A\beta_{42}$ /P-tau/T-tau, and $A\beta_{42}$ /P-tau/NfL showed AUCs of 0.80 (95% confidence interval (CI) 0.69–0.90), 0.96 (95%CI 0.91–1.00), and 0.96 (95%CI 0.91–1.00) respectively (Fig. 2B). Threshold cutoff of 5352 pg/mL CSF NfL (Youden Index = 0.56, Specificity = 0.90, Sensitivity = 0.63) for differentiating probable AD dementia patients from controls was determined by maximizing the Youden Index. DeLong test showed that the AUC for CSF NfL alone was significantly different from $A\beta_{42}$ /P-tau/T-tau ($Z = -2.9$; $p = 0.004$). There was no significant difference between the 2 multiparametric models ($Z = 0$; $p = 1$).

6. CSF NfL concentration is correlated with MMSE score and core AD CSF biomarkers

In addition to the separation between control and the clinically defined groups, baseline CSF NfL correlated significantly and nega-

Table 1
Demographic and clinical data at baseline

	Control	aMCI	Probable AD dementia	p-value ^a
Sample size (n)	52	55	28	
Sex-female, n (%) ^b	33 (63.5)	28 (50.9)	14 (50.0)	0.39
Age at baseline ^c	68.0 ± 5.4	64.9 ± 5.5	64.2 ± 6.0	0.003
MMSE ^{c,d}	29.4 ± 0.7	27.2 ± 1.75	22.5 ± 3.2	< 0.0001
APOE ε4 carrier, n (%)	20 (39.2)	34 (61.8) ^f	23 (82.1)	0.001
Biomarkers ^{c,e}				
CSF Aβ42 ^g	1015 ± 267	578 ± 240	499 ± 184	< 0.0001
CSF T-tau	286 ± 99	549 ± 425	700 ± 386	< 0.0001
CSF P-tau	56.1 ± 15.9	73.9 ± 33.6	88.9 ± 36.0	0.0002
CSF NFL	4454 ± 5969 ^h	4944 ± 2748	6630 ± 3290	< 0.0001
A/T/N Classification				
A+T+N+	2 (3.8%)	24 (43.6%)	18 (64.3%)	
A+T-N-	8 (15.4%)	10 (18.2%)	5 (17.9%)	
A+T+N-	0 (0%)	2 (3.6%)	2 (7.1%)	
A+T-N+	0 (0%)	2 (3.6%)	0 (0%)	
A-T+N+	4 (7.7%)	4 (7.3%)	2 (7.1%)	
A-T+N-	0 (0%)	1 (1.8%)	1 (3.6%)	
A-T-N+	6 (11.5%)	1 (1.8%)	0 (0%)	
A-T-N-	32 (61.5%)	11 (20%)	0 (0%)	
Change in diagnosis ⁱ				
24-months follow-up	None	27 AD dementia (Early converters; 49%)	None	
6-10 years follow-up	2 aMCI (4%), 2 AD dementia (4%)	9 AD dementia (Late converters; 16%)	None	

^a One-way ANOVA^b Expressed in female, n (%)^c Expressed in mean ± SD.^d Mini mental status examination.^e Expressed in pg/mL.^f APOE ε4 genotype missing for three patients in the aMCI group.^g CSF stands for cerebrospinal fluid^h NFL concentration removed as outlier for one individual in the control groupⁱ Expressed in n condition (%)**Fig. 2.** (A) Distribution (horizontal lines show average, standard deviation error bars) of baseline CSF NFL levels in clinical groups (One-way ANOVA). (B) ROC curves comparing between control and probable AD dementia for models consisting of NFL alone (red), Aβ42/P-tau/T-tau (green), and Aβ42/P-tau/NfL (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tively with MMSE score, suggesting higher CSF NFL levels in more severely cognitively impaired individuals (Pearson's $r = -0.35$, $p < 0.0001$). There was also a significant negative correlation with CSF Aβ42 (Pearson's $r = -0.39$, $p < 0.0001$) (Fig. 3A). A significant positive correlation between CSF NFL and T-tau (Pearson's $r = 0.58$, $p < 0.0001$) and P-tau (Pearson's $r = 0.53$, $p < 0.0001$) was also observed (Fig. 3B AND C).

7. CSF NFL predicts AD conversion among aMCI patients

Participant diagnoses were evaluated at baseline, 24-months, and extended follow-up (median of 9 years) (Fig. 1). At the 24-month period, all control individuals remained cognitively unimpaired. Among the 55 aMCI patients, 28 patients remained as aMCI (24-month stable aMCI) and 27 patients converted to probable AD

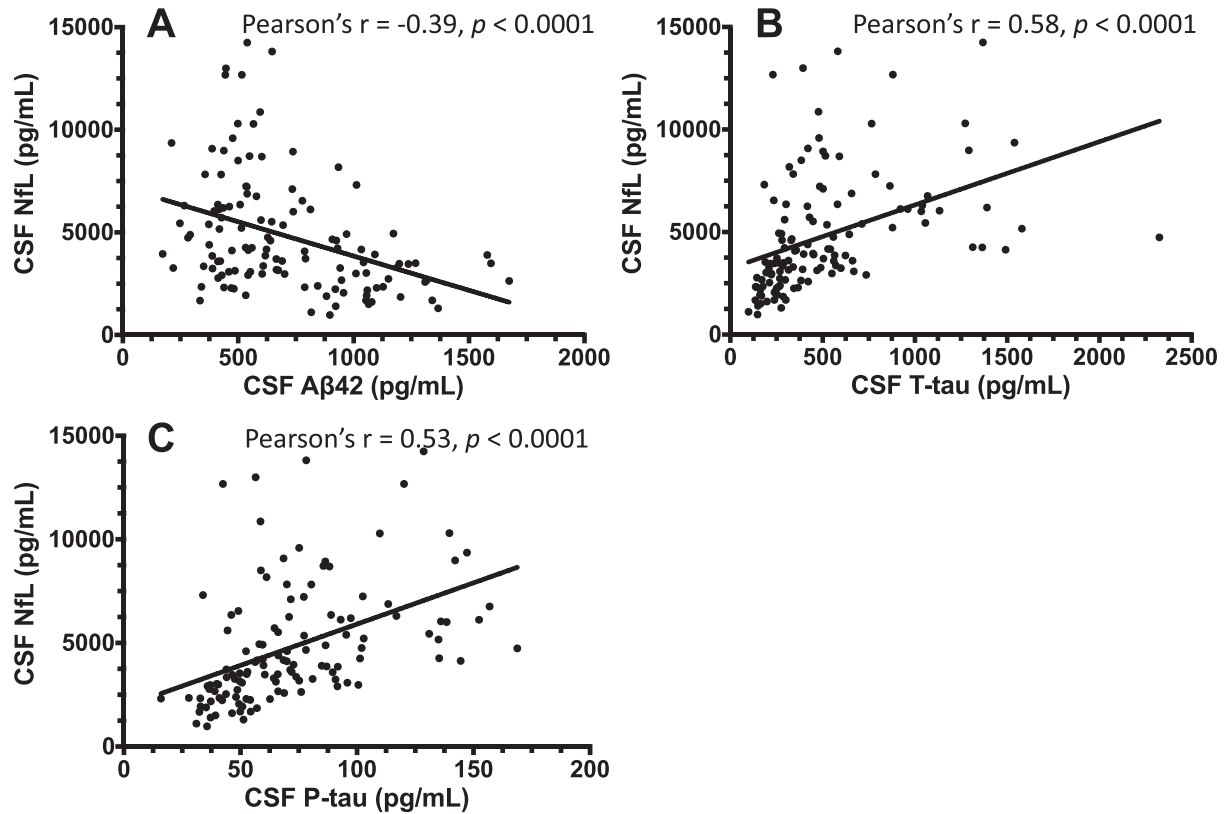


Fig. 3. Scatter plots and line of best fit for correlational analysis between baseline CSF NfL concentrations and CSF (A) A β 42, (B) T-tau, and (C) P-tau.

dementia (early converter aMCI). 25 of the 27 early converter aMCI patients had abnormal AD biomarker levels (A+T+N+, A+T-N-, or A-T+N+). For the control group at extended follow-up, 41 remained cognitively unimpaired, 2 converted to aMCI, 2 converted to probable AD dementia, 1 developed other neurological disorder, 2 deceased, and 4 were lost to follow-up. The 2 control individuals who converted to probable AD dementia had abnormal AD biomarker levels (A-T+N+ and A+T+N+). 1 of the 2 control individuals who converted to aMCI was biomarker positive (A-T+N+). Among the 24-month stable aMCI patients ($n = 28$) at extended follow-up, 10 remained aMCI, 9 converted to probable AD dementia (late converter aMCI), 1 recovered to cognitively unimpaired, 2 developed other neurological disorders, 2 deceased, and 3 were lost to follow-up. 8 of the 9 late converter aMCI patients had abnormal AD biomarker levels (A+T+N+, A+T-N-, or A-T+N+). The early converter aMCI and probable AD dementia groups were still diagnosed as probable AD dementia. Baseline demographic and clinical data for the stable at extended follow-up, early, and late converter aMCI are detailed in Supplementary Table 2.

The 4 control individuals who converted to either aMCI or probable AD dementia during the extended follow-up had baseline CSF NfL concentration in the top 25th percentile of the group (8936 pg/mL, 4262 pg/mL, and 5352 pg/mL; 1 individual measurement missing). NfL in the overall control group was 2994 (2314, 4244) (median (25th, 75th percentile): pg/mL). These results suggest that elevated CSF NfL may be indicative of increased neurodegenerative risk even in control individuals. However, the small sample size did not allow statistical analyses.

Baseline CSF NfL was compared between aMCI patients who were stable and those who converted to probable AD dementia during follow-up. At the 24-month time point, aMCI patients who remained as aMCI had significantly lower levels of CSF NfL compared to those who converted to probable AD dementia

($p = 0.004$) (Fig. 4A). ROC curve analysis showed that NfL alone produced an AUC of 0.73 (95%CI 0.59 – 0.88), with a threshold of 3608 pg/mL (Youden Index = 0.47, Specificity = 0.58, Sensitivity = 0.85) (Fig. 4B). The A β 42/P-tau/T-tau model produced an AUC of 0.78 (95%CI 0.65 – 0.90) and the A β 42/P-tau/NfL model produced an AUC of 0.81 (95%CI 0.69 – 0.93). There were no significant differences between the NfL alone and A β 42/P-tau/T-tau models ($Z = -0.61$, $p = 0.54$) or between the A β 42/P-tau/T-tau and A β 42/P-tau/NfL models ($Z = 0.82$, $p = 0.41$).

Among the 24-month stable aMCI patients ($n = 28$), 9 converted to probable AD dementia (late converter aMCI) and 10 remained stable aMCI at the extended follow-up (extended follow-up stable aMCI). Baseline CSF NfL was compared between these 2 groups to determine if CSF NfL could predict aMCI conversion to probable AD dementia at the later time point. The differences in baseline CSF NfL concentrations between the extended follow-up stable aMCI and late converter aMCI groups trended to significance ($p = 0.08$) (Fig. 4C). The ROC curve analysis for NfL alone produced an AUC of 0.75 (95%CI 0.43 – 1.0) (Fig. 4D). The multiparametric model with A β 42/P-tau/T-tau produced an AUC of 0.86 (95%CI 0.61 – 1.0), while the A β 42/P-tau/NfL model produced an AUC of 0.84 (95%CI 0.61 – 1.0). There were no significant differences between the AUC of all 3 models ($p > 0.3$).

8. CSF NfL significantly increased longitudinally in 24-month stable aMCI, early converter aMCI, and probable AD dementia groups

CSF NfL concentration was only available at baseline, 12-month, and 24-month for aMCI and probable AD dementia groups. Since CSF NfL concentration in 24-month stable aMCI was significantly lower than in early converter aMCI, the aMCI group was separated into 24-month stable aMCI and early converter aMCI groups for

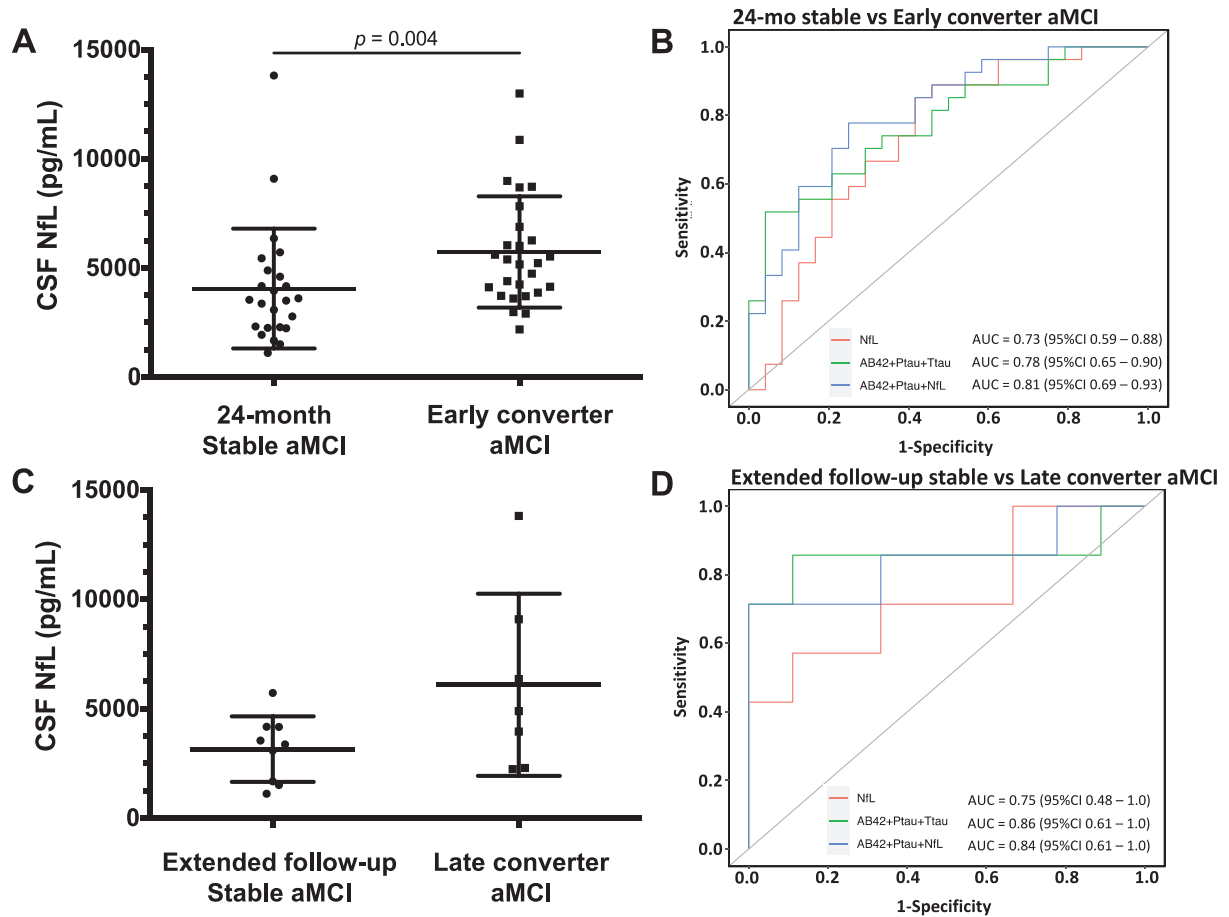


Fig. 4. (A) Distribution (horizontal lines show average, standard deviation error bars) of baseline CSF NfL in aMCI patients who remained as aMCI (24-month stable aMCI) and those who converted to probable AD dementia within the 24-month (early converter aMCI) follow up period. (B) ROC curves comparing between 24-month stable and early converter aMCI for models consisting of NfL alone (red), A β 42/P-tau/T-tau (green), and A β 42/P-tau/NfL (blue). (C) Distribution (horizontal lines show average, standard deviation error bars) of baseline CSF NfL in aMCI patients who remained as aMCI (extended follow-up stable aMCI) and those who converted to probable AD dementia at extended follow-up (late converter aMCI). (D) ROC curves comparing between extended follow-up stable and late converter aMCI for models consisting of NfL alone (red), A β 42/P-tau/T-tau (green), and A β 42/P-tau/NfL (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the longitudinal analysis. Dissecting raw within-individual trend-lines of CSF NfL revealed an increase in concentration over time in the majority of patients in all 3 patient groups (Supplementary Figure 1A). Linear mixed model was used to estimate grouped annual linear change adjusted for age, sex, and patient individuals as a random factor, as described in methods. The linear mixed model showed that CSF NfL significantly increased over the 24-month period in all 3 clinically defined groups (Supplementary Figure 1B). The annual rates of change were +14.1% (95%CI 6.1%, 22.7%; $p = 0.0004$) for the 24-month stable aMCI group, +19.8% (95%CI 12.6%, 27.4%; $p < 0.0001$) for the early converter aMCI group, and +15.8% (95%CI 8.7%, 23.3%; $p < 0.0001$) for the AD dementia group. However, the rate of change was not significantly different between groups ($p = 0.56$).

9. Discussion

In this study, we investigated if CSF NfL could be used as a predictor of progression from aMCI to probable AD dementia and whether CSF NfL could give additional information on disease progression compared to core CSF AD biomarkers.

While a majority of individuals with MCI remain stable even after 10 years of follow-up, 5–15% of all patients with MCI as a whole and over 50% of patients with aMCI develop AD dementia

(McGuinness et al., 2015; Michaud et al., 2017; Mitchell and Shiri-Feshki, 2009; Rountree et al., 2007). Early identification of individuals at high risk of developing AD dementia enables early and targeted interventions. This prediction remains a challenge in the clinic, as neurodegeneration and cognitive impairments evolve at different paces. Recent studies have indicated that elevated CSF NfL concentrations in MCI individuals are associated with increased risk of developing AD dementia (Ou et al., 2019; Zetterberg et al., 2016), while others have found contradicting results (Lin et al., 2018).

In this study, aMCI individuals who developed probable AD dementia within 24 months had higher CSF NfL levels at baseline compared with aMCI individuals who remained stable during the same period. However, aMCI individuals who developed probable AD dementia during the extended follow-up (9 years median) did not have significantly higher levels of CSF NfL than those who remained stable as aMCI during the same period. This comparison trended to significance, possibly explained by the small sample. We also found that 3 of 4 control individuals (one missing) who converted to either aMCI or probable AD dementia at a later time point had CSF NfL concentrations in the top quartile of the group. These findings support the role of CSF NfL as a potential biomarker of neurodegeneration, predicting disease progression along the AD continuum.

We also aimed to determine if CSF NfL alone or in combination with other AD biomarkers gave additional information to the established core CSF AD biomarkers. The ability to differentiate between stable aMCI and converter aMCI at both the 24-month and extended follow-up periods did not differ between a model with CSF NfL alone and an A/T/N multiparametric model of core CSF AD biomarkers. In a recent publication, Jack et al suggested that NfL could be incorporated into the A/T/N classification scheme as a neurodegenerative marker (Jack et al., 2018). Substituting NfL for T-tau in the A β 42/P-tau/T-tau multiparametric model did not change model performance significantly. These results suggest that NfL does not give additional information to the core AD biomarkers for predicting conversion to AD dementia. Nevertheless, NfL could be a possible substitution for the neurodegeneration criteria in the A/T/N classification scheme (Jack et al., 2018).

CSF NfL differed between clinical diagnostic groups and had moderate negative correlation with MMSE score, suggesting NfL's association with cognitive decline. We observed progressively higher levels of CSF NfL in patients with more severe cognitive impairment. This is in concordance with other cross-sectional comparisons of CSF NfL in AD studies (Forgrave et al., 2019; Mattsson et al., 2017; Zetterberg et al., 2016). ROC curve analyses revealed that CSF NfL could differentiate AD dementia from control individuals with reasonable discriminatory capability. However, the AUC of CSF NfL alone was significantly lower than that of the core CSF AD biomarker model of A β 42/P-tau/T-tau. Substituting NfL for T-tau in the multiparametric model did not make a significant difference in the AUC of the model.

In the longitudinal analysis of the initial 24-month follow-up, we found CSF NfL to increase in patients with 24-month stable aMCI, early converter aMCI, and AD dementia at an annual rate of 14.1%, 19.8%, and 15.8% respectively. However, the rate of change did not differ between groups. Though another study similarly found increase in CSF NfL irrespective of stage in AD (Lleó et al., 2019), others have found that CSF NfL increases significantly faster in converter MCI and AD dementia patients compared with stable MCI patients (Kester et al., 2012; Mielke et al., 2019; Ou et al., 2019; Palmqvist et al., 2019). The reason for this inconsistency may be due to small sample size in the present study. Despite controlling for age and sex in the linear mixed model, the lack of longitudinal data in the control group is a major limitation that prevents making strong conclusions on whether the uniform increase in CSF NfL is pathological or associated with factors that are unrelated to the disease, such as natural variation.

Future studies should investigate and improve the clinical utility of NfL. Increasing evidence suggests that serum NfL could be a clinically useful non-invasive biomarker of AD, with multiple studies citing high correlation of NfL in CSF and serum (Alagaratnam et al., 2021). This could offer advantages to current AD biomarkers given the unmet need for noninvasive blood-based methods to evaluate and track neurodegeneration in AD, and the unclear association between blood and CSF levels of amyloid and tau (Fossati et al., 2019; Teunissen et al., 2018).

In general, NfL levels increase with increasing age. Consequently, the clinical utility of NfL may also require age-dependent cutoff values. More research is needed to investigate the potential of plasma NfL as a non-invasive biomarker for diagnosis, progression and monitoring during the different stages of the AD continuum (Lewczuk et al., 2018a; Mattsson et al., 2017).

10. Conclusions

Taken together, our study confirms CSF NfL as a biomarker of neurodegeneration along the AD continuum. NfL also separates early and late converter aMCI. CSF NfL was increased, both within-

individual and between-individual, with increasing cognitive impairment. However, in the presence of core CSF AD biomarkers, NfL does not give significant additional information on disease progression.

Original data and manuscript

This data has not been submitted or published elsewhere. It will not be submitted elsewhere while under consideration at *Neurobiology of Aging*.

Ethics approval and consent to participate

The Institutional Review Boards of Mount Sinai Hospital (MSH), University Health Network (UHN) and the Regional Committee for Medical Research Ethics for central Norway approved all of our protocols, including collection of cerebrospinal fluid samples. Regarding patient cerebrospinal fluid and clinical information, written informed consent has been obtained from all participants, or their proxies.

Disclosure statement

PB, SSK, CTC, and MS are employed by Meso Scale Discovery.

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Supplementary materials

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CRedit authorship contribution statement

Bryant Lim: Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Gøril Rolfseng Grøntvedt:** Investigation, Data curation, Writing – original draft. **Pradeepthi Bathala:** Data curation, Investigation, Resources. **Shraddha S. Kale:** Data curation, Investigation, Resources. **Christopher T. Campbell:** Data curation, Investigation, Resources. **Martin Stengelin:** Data curation, Investigation, Resources. **Sigrid Botne Sando:** Resources, Writing – review & editing. **Ioannis Prassas:** Writing – review & editing. **Eleftherios P. Diamandis:** Conceptualization, Writing – review & editing, Project administration, Supervision. **Geir Bråthen:** Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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