

Original Article



Clinical Application of Plasma Neurofilament Light Chain in a Memory Clinic: A Pilot Study

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Conflict of Interest

The author has no financial conflicts of interest.

ABSTRACT

Background and Purpose: Neurofilament light chain (NfL) has been considered as a biomarker for neurodegenerative diseases including Alzheimer's disease (AD). We measured plasma NfL levels in older adults with cognitive complaints and evaluated their clinical usefulness in AD.

Methods: Plasma levels of NfL, measured by using the single molecule array method, were acquired in a total of 113 subjects consisting of subjective cognitive decline (SCD; n=14), mild cognitive impairment (MCI; n=37), or dementia of Alzheimer type (DAT; n=62). Plasma NfL level was compared among three groups, and its association with cognitive and functional status was also analyzed.

Results: After adjusting for age, plasma NfL level was higher in subjects with DAT (65.98±84.96 pg/mL), compared to in subjects with SCD (16.90±2.54 pg/mL) or MCI (25.53±10.42 pg/mL, $p=0.004$). NfL levels were correlated with scores of the mini-mental state examination ($r=-0.242$, $p=0.021$), clinical dementia rating (CDR) ($r=0.291$, $p=0.005$), or CDR-sum of boxes ($r=0.276$, $p=0.008$). Just for participants who performed amyloid positron emission tomography (PET), the levels were different between subjects with PET (-) (n=17, 25.95±13.25 pg/mL) and PET (+) (n=16, 63.65±81.90 pg/mL, $p=0.010$). Additionally, plasma NfL levels were different between vascular dementia and vascular MCI, and between Parkinson's disease- dementia and no dementia.

Conclusions: This pilot study shows that in subjects with DAT, plasma NfL levels increase. Plasma NfL level correlated with cognitive and functional status. Further longitudinal studies may help to apply the plasma NfL levels to AD, as a potential biomarker for the diagnosis and predicting progression.

Keywords: Alzheimer Disease; Neurofilament Proteins; Biomarkers; Plasma

INTRODUCTION

Alzheimer's disease (AD), which has senile amyloid plaques and neurofibrillary tangle as pathologic hallmarks, begins with amyloid β ($A\beta$) production and aggregation, and hyperphosphorylation of tau.¹ Currently, the validated core biomarkers of AD are based on imaging findings such as medial temporal atrophy on brain magnetic resonance imaging

(MRI),² glucose hypometabolism on positron emission tomography (PET), and uptake of A β or tau aggregates on PET,³ or cerebrospinal fluid (CSF) findings reflecting brain amyloidosis (A β 42), neurodegeneration (total tau), and tau pathology (phosphorylated tau).⁴ However, there has been a need for widely accessible, noninvasive, and inexpensive biomarkers to diagnose and monitor the AD progress. Blood tests may be considered good alternatives for AD neurodegeneration.

Neurofilament light chain (NfL), which is another axonal protein, is released into body fluids, such as the CSF or plasma when neurodegeneration-related axonal damage occurs.^{5,6} CSF NfL level showed abnormally increased in AD,⁷⁻⁹ and also correlated with cognitive impairment and hippocampal atrophy in patients with mild cognitive impairment (MCI),⁷ suggesting CSF NfL level may be a good candidate for the AD biomarker. Studies showed associations of neurodegenerative diseases with both levels of NfL in CSF and blood samples.^{6,10,13} MCI, AD, and other neurodegenerative disease causing dementia, including vascular dementia (VaD) and frontotemporal dementia (FTD), although nonspecifically, showed higher levels of NfL in plasma, as well as with that in CSF,^{14,15} suggesting that plasma NfL also is a promising biomarker for neurodegenerative diseases including AD.^{10,12} A recent report shows that plasma NfL could be considered as a neurodegeneration biomarker for the diagnosis of AD.¹² Additionally, higher levels of plasma NfL correlated with lower performance of cognitive assessment, suggesting the possibility of NfL for predicting the cognitive deterioration and AD.^{12,16}

We measured NfL levels in plasma samples of subjects with cognitive complaints, who meet the diagnostic criteria of MCI and dementia of Alzheimer type (DAT), as well as subjective cognitive decline (SCD). The present study, as a pilot study, will investigate the efficacy of plasma NfL levels for the early diagnosis and the quantification of the clinical severity of AD. In addition to the main cohort, plasma NfL levels were measured and analyzed also in some patients with cerebrovascular disease or Parkinson's disease (PD) who visited the memory clinic.

MATERIALS AND METHODS

Patients and blood collection

After clinical evaluation, neurological examinations, laboratory tests, neuropsychological testing, and MRI, participants were categorized into three groups: SCD (n=14), MCI (n=37), and DAT (n=62). DAT patients in the present study were those who fulfilled the probable AD criteria proposed by the National Institute of Neurological and Communicative Disorders and the Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).¹⁷ The severity of dementia and general cognition were assessed using the clinical dementia rating (CDR) scale¹⁸ and the Mini-Mental State Examination (MMSE),¹⁹ irrespectively. MCI was diagnosed by Petersen's criteria. Patients with MCI had normal daily living activities but an objective memory impairment, which means less than 1.5 standard deviation from the norm in at least one memory test.^{20,21} Diagnosis of SCD was made when there was a complaint of memory decline without objective neuropsychological test results.^{22,23} Patients who had other conditions that might cause cognitive impairment, such as hypothyroidism, vitamin B12 deficiency, syphilis, or prior history of major psychiatric illness (e.g., major depression, bipolar disorder, or schizophrenia) were excluded. We also excluded those with MRI signal abnormalities such as brain tumors, radiation injury, hippocampal sclerosis, and multiple sclerosis.

In addition to the main study, blood samples of some, not many, patients with cerebrovascular disease or PD were also analyzed. Participants with cerebrovascular disease (n=10) were divided into vascular dementia (n=6) and vascular MCI (n=4) according to the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN).^{24,25} PD patients (n=21) were included with the diagnosis proposed by the UK Parkinson's Disease Society Brain Bank.²⁶ and further divided into PD-no dementia (n=11) and PD dementia (n=10). PD dementia was diagnosed with the criteria proposed by the Movement Disorder Society Task Force.²⁷

Blood samples were collected into standard polypropylene EDTA test tubes (Sarstedt, Nümbrecht, Germany), followed by centrifugation; the obtained plasma samples were approximately portioned into 500 μ L aliquots, frozen at -80°C , and kept frozen until the analyses.

The Institutional Review Board of the Catholic University of Korea, Eunpyeong St. Mary's Hospital approved the study, and all participants or their caregivers gave their written consent before blood sampling.

MRI

All participants underwent a 3.0 Tesla brain MRI (Magnetom Vida; Siemens Healthcare, Erlangen, Germany), including susceptibility-weighted imaging, fluid-attenuated inversion recovery imaging, and T1/T2-weighted imaging. The slice thickness was 5 mm without an interslice gap. For the severity of white matter hyperintensities (WMHs) on MRI, periventricular WMHs (PVHs) and deep WMHs (DWHs) were separately evaluated as proposed by the Clinical Research Center for Dementia of South Korea.^{28,29} Medial temporal atrophy (MTA) was rated in T1-weighted coronal images on a five-point scale (0–4 points) based on the width of the coronal fissure and the temporal horn and the height of the hippocampal formation.³⁰

Subjects with severe WMHs or multiple (more than 5) lacunar infarctions, which is compatible with the Erkinjuntti's brain imaging criteria for subcortical ischemic vascular dementia³¹ were excluded in the main study, comparisons among SCD, MCI and DAT. They had no previous stroke history and did not have focal neurological symptoms and signs.

PET acquisition and image analysis

The positron emission tomography/computed tomography (PET/CT) image was acquired for 30 minutes from 30 minutes after intravenous injection of 370 MBq (10 mCi) of F-18 florapronol (Alzavue; FutureChem Pharma, Seoul, Korea) using a PET/CT scanner (Siemens Biograph Vision 600; Siemens Healthcare), and was interpreted only by a trained expert. The competency was confirmed individually by reviewing a series of test images. Image interpretation was performed regardless of the clinical characteristic of the patients. A negative scan is defined as a case in which the border between the grey matter and the white matter is observed as the degree of uptake by the grey matter is lower than the degree of uptake by the white matter in all areas of cerebral cortex. A negative scan may have the following characteristics: The white matter tract connecting the frontal lobe and the parietal lobe or the occipital lobe and the temporal lobe is observed clearly. The shape of finger is observed due to the uptake of the whiter matter of the frontal lobe. A positive scan is defined as a case in which the uptake by one or more cerebral cortical grey matter is the same as or more than that of the adjacent white matter. A positive scan may have the following characteristics: It is difficult to observe the white matter tract connecting the frontal lobe and

the parietal lobe or the occipital lobe and the temporal lobe. The uptake of the grey matter by the medial parietal lobe (precuneus) has increased.

Plasma NfL analysis using Quanterix single molecule array (SIMOA) technology

Samples from the stored aliquots were shipped to prismCDX Co., Ltd. (Hwaseong, Korea) for the analysis of phosphorylated NfL (pNfL) levels using a Simoa NF-light Advantage Kit (Quanterix, Billerica, MA, USA) with a Simoa HD-1 Analyzer (Quanterix), according to the manufacturer's instructions (<https://www.quanterix.com/resources/publications-posters>). For calibration, a combination of monoclonal antibodies and purified bovine NfL was used. Concentrations of pNfL were measured, in units of pg/mL, while demographic and clinical data were blinded. Analytical sensitivity was <1.0 pg/mL. All samples tested were within the assay's dynamic range, with the coefficient of variation ranging from 4.2% to 7.4% for measurement. The lower limit of quantification was 10.0 pg/mL, and the upper limit of quantification was 498.0 pg/mL. All samples were measured within the range spanned by the limits of quantification. The reliability of quantification was high ($R^2=0.999$ of calibration curve).

Statistical analysis

Demographics and plasma NfL levels were compared between three diagnostic groups using the analysis of covariance (ANCOVA), adjusted for age, and followed by the Dunnett *post hoc* tests for multiple comparisons. The Shapiro-Wilk test was used to check for normality. The Mann-Whitney *U* test was performed to compare the NfL levels between Vascular MCI and VaD, between PD-no dementia and PD dementia, and between SCD participants with amyloid PET (+) and amyloid PET (-). The relationship between plasma NfL levels and age in the entire sample was evaluated by using Spearman correlation analysis. And then, a partial correlation was used to estimate the correlation coefficients and their significances for the relationships between plasma NfL levels, MMSE, CDR scores, and MRI findings, adjusted for age.

A $p<0.05$ (two-tailed) was considered statistically significant, and all the analysis was performed using the Statistical Package for the Social Sciences version 28.0 software program (IBM Corp., Armonk, NY, USA).

RESULTS

Plasma NfL levels in 3 diagnostic groups

Table 1 shows the clinical and demographic characteristics of the study participants. The mean age was 64.70 ± 19.43 years in the SCD group, 77.14 ± 6.09 years in the MCI group, and 79.39 ± 6.66 years in the DAT group ($p<0.001$). Although not significant, the MCI ($n=31$, 83.8%) and DAT ($n=45$, 72.6%) groups had more females, compared to the SCD group, which had the same sex ratio (7 males and 7 females) ($p=0.053$). After adjusting for age, the duration of education in participants with SCD, MCI and DAT were 9.29 ± 3.31 years, 7.46 ± 4.93 years, and 5.92 ± 4.76 years respectively ($p=0.339$). As expected, significant differences in MMSE, CDR, and CDR-sum of boxes (CDR-SOB) scores were found across the three groups (all, $p<0.001$). Plasma NfL levels were increased in participants with DAT (65.98 ± 84.96 pg/mL) compared to both subjects, with SCD NfL levels being 16.90 ± 2.54 pg/mL and MCI NfL levels being 25.53 ± 10.42 pg/mL, even after adjusting for age ($p=0.004$) (**Fig. 1A**). Vascular risk factors such as hypertension, diabetes, and dyslipidemia, and presence of the apolipoprotein (APO) E4 genotype were not different across groups. However, amyloid PET positivity was

Table 1. Characteristics of study participants with cognitive complaints, from SCD through MCI to DAT

Characteristics	SCD ^a (n=14)	MCI ^b (n=37)	DAT ^c (n=62)	Total (n=113)	p-value*	Post hoc
Age (yr)	64.70±19.43	77.14±6.09	79.39±6.66	76.83±10.10	<0.001	a<c
Sex (female)	7 (50.0)	31 (83.8)	45 (72.6)	83 (73.5)	0.053	
Education (yr)	9.29±3.31	7.46±4.93	5.92±4.76	6.85±4.77	0.339	
MMSE	26.40±2.68	21.86±3.61	14.94±5.51	18.34±6.25	<0.001	a, b<c
CDR (median, IQR)	0.00±0.00	0.50±0.00	1.26±0.57 (1, 1-2)	0.92±0.62 (1, 0.5-1)	<0.001	a, b<c
CDR-SOB (median, IQR)	0.07±0.19 (0, 0.0-0.0)	1.85±0.63 (2, 1.5-2.5)	7.93±3.18 (7.0, 6.0-10.0)	5.66±4.05 (5.0, 2.5-8.0)	<0.001	a, b<c
NfL (pg/mL)	16.90±2.54	25.53±10.42	65.98±84.96	46.92±66.81	0.004	a, b<c
APOE4 genotype	4/13 (30.8)	11/34 (32.4)	13/46 (28.3)	28/93 (30.1)	0.827	
MRI findings						
MTA-R (minimum–maximum range)	0.92±1.08 (0-3)	1.41±0.93 (0-3)	2.44±1.04 (0-4)	1.91±1.16 (0-4)	<0.001	a, b<c
MTA-L (minimum–maximum range)	1.25±1.06 (0-3)	1.60±1.95 (0-3)	2.54±0.93 (0-4)	2.06±1.08 (0-4)	<0.001	a, b<c
PVH (minimum–maximum range)	1.46±0.88 (0-3)	2.00±0.72 (1-3)	2.30±0.76 (1-3)	2.09±0.80 (0-3)	0.027	a<c
DWH (minimum–maximum range)	1.23±0.60 (0-2)	1.50±0.81 (0-3)	1.70±0.82 (0-3)	1.58±0.80 (0-3)	0.571	
Amyloid PET (+)	1/5 (20.0)	5/15 (33.3)	10/13 (76.9)	16/33 (48.5)	0.056	
Vascular risk factors						
Hypertension	6 (42.9)	24 (64.9)	44 (71.0)	74 (65.5)	NS	
Diabetes mellitus	2 (14.3)	10 (27.0)	22 (35.5)	34 (30.1)	NS	
Dyslipidemia	8 (57.1)	22 (59.5)	27 (43.5)	57 (50.4)	NS	
Ischemic heart disease	1 (7.1)	3 (8.1)	6 (9.7)	10 (8.8)	NS	

Data are presented as mean±standard deviation (or median, interquartile range, minimum–maximum range), or number (%) values. SCD: subjective cognitive decline, MCI: mild cognitive impairment, DAT: dementia of Alzheimer type, MMSE: mini-mental state examination, CDR: clinical dementia rating, IQR: interquartile range, CDR-SOB: clinical dementia rating-sum of boxes, NfL: neurofilament light chain, APOE4: apolipoprotein E4, MRI: magnetic resonance imaging, MTA-R: medial temporal atrophy-right, MTA-L: medial temporal atrophy-left, PVH: periventricular hyperintensity, DWH: deep white matter hyperintensity, PET: positron emission tomography. *p-value using the analysis of covariance for continuous data (or the χ^2 test for noncontinuous data), adjusted for age, and followed by the Dunnett *post hoc* tests for multiple comparisons.

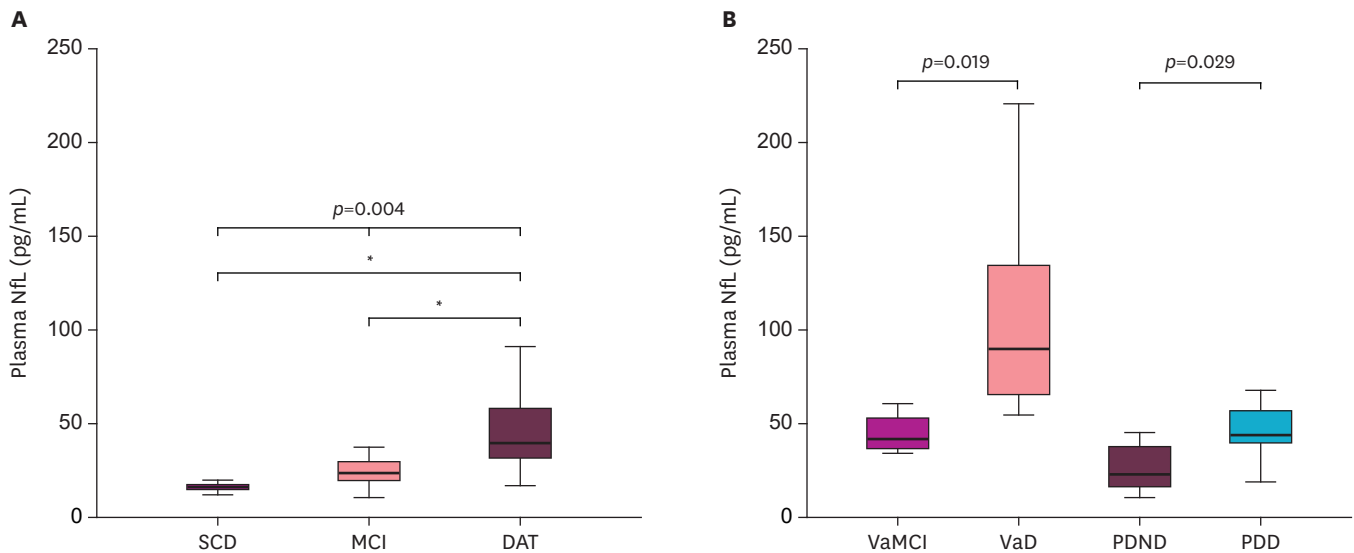


Fig. 1. Comparison of plasma NfL levels in three groups (A), SCD, MCI, and DAT, and in 2 groups (B) between VaMCI and VaD, and between PDND and PDD. (A) NfL levels in plasma were significantly elevated in participants with DAT (65.98±84.96 pg/mL) compared to both groups with SCD (16.90±2.54 pg/mL) and MCI (25.53±10.42 pg/mL), even after adjusting for age ($p=0.004$). (B) By using the Mann-Whitney *U* test, plasma NfL levels were different between VaMCI (45.05±11.51 pg/mL) and VaD (109.54±62.14 pg/mL) ($p=0.019$), and between PDND (29.80±20.46 pg/mL) and PDD (46.16±13.56 pg/mL) ($p=0.029$). NfL: neurofilament light chain, SCD: subjective cognitive decline, MCI: mild cognitive impairment, DAT: dementia of Alzheimer type, VaMCI: vascular mild cognitive impairment, VaD: vascular dementia, PDND: Parkinson’s disease no dementia, PDD: Parkinson’s disease dementia. *Indicates significant difference between them ($p<0.05$) by Dunnett *post hoc* tests.

different between the three groups, with a borderline significance, 25.0% in SCD, 33.3% in MCI, and 76.9% in DAT ($p=0.056$). Plasma NfL levels were different between amyloid PET (-) and (+) groups. This difference was seen in the entire sample (PET [-], $n=17$, 25.95±13.25

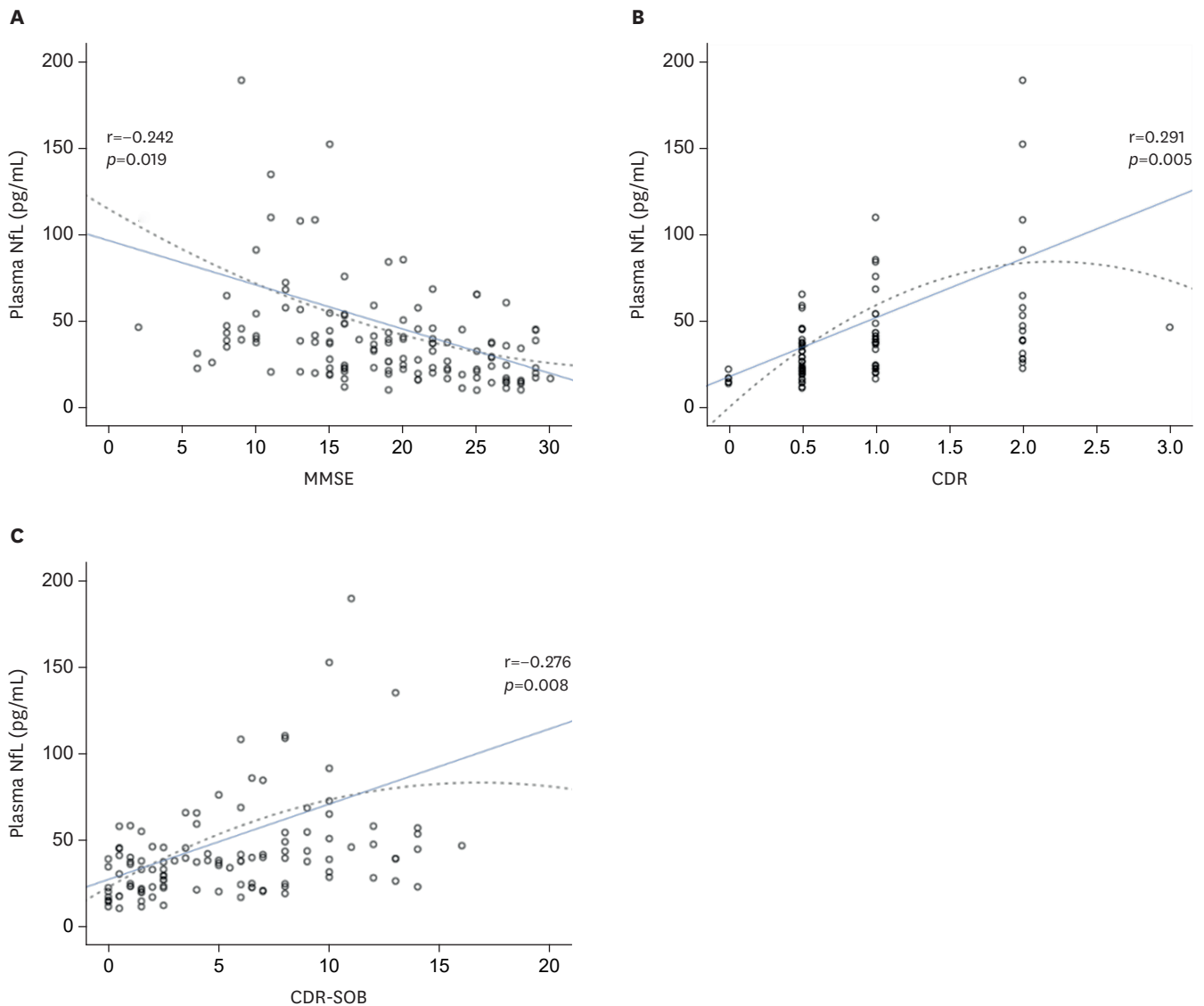


Fig. 2. Scatter plots and trend lines of the levels of plasma NfL according to MMSE and CDR, CDR-SOB of the study participants with cognitive complaints, SCD, MCI, and DAT. Partial correlations were used to estimate the correlation coefficients and their significances for the relationships, adjusted for age. Fit lines were presented using linear (full line) and quadratic (dotted line) methods. Plasma NfL levels showed the cognition- or function-related changes. NfL: neurofilament light chain, MMSE: mini-mental state examination, CDR: clinical dementia rating, CDR-SOB: clinical dementia rating-sum of boxes, SCD: subjective cognitive decline, MCI: mild cognitive impairment, DAT: dementia of Alzheimer type.

pg/mL vs. PET [+], $n=16$, 63.65 ± 81.90 pg/mL, $p=0.010$) and in the DAT group (PET [-], $n=3$, 27.73 ± 9.32 pg/mL vs. PET [+], $n=10$, 87.74 ± 97.12 pg/mL, $p=0.028$).

Plasma NfL levels and clinical characteristics: association with MMSE and CDR association of plasma NfL levels with MMSE and CDR-SOB

Plasma NfL levels correlated with age ($r=0.387$, $p<0.001$). Even after adjusting for age, plasma NfL levels were related with MMSE ($r=-0.242$, $p=0.021$), CDR ($r=0.291$, $p=0.005$), and CDR-SOB ($r=0.276$, $p=0.008$) scores for all the samples (Fig. 2). A positive correlation of plasma NfL levels with MRI findings, MTA, was found in the entire sample ($r=0.227$, $p=0.023$). Association of NfLs with PVHs by means of Spearman correlation ($r=0.268$, $p=0.005$) changed into insignificant after adjusting for age ($r=0.092$, $p=0.350$).

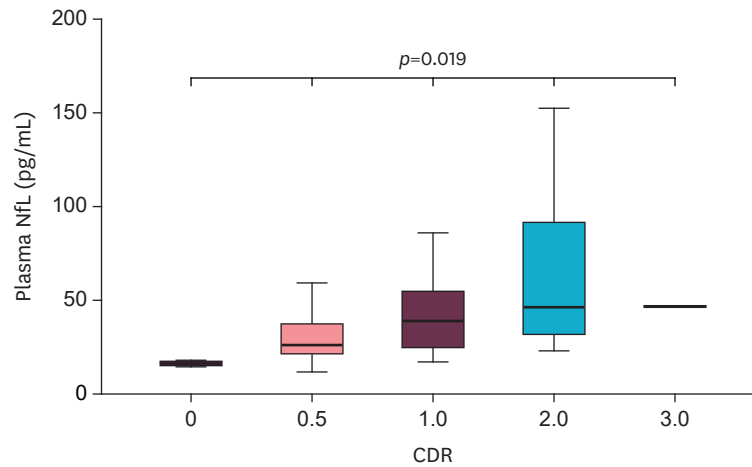


Fig. 3. Mean plasma NfL levels according to CDR scores. Plasma NfL levels were 17.08±3.00 pg/mL in CDR 0 (n=6), 29.48±12.68 pg/mL in CDR 0.5 (n=42), 61.36±79.79 pg/mL in CDR 1 (n=34), 86.99±112.51 pg/mL in CDR 2 (n=18), 46.83 pg/mL in CDR 3 (n=1), by using the Mann-Whitney U test (p=0.019). NfL: neurofilament light chain, CDR: clinical dementia rating.

According to CDR ratings, mean plasma NfL levels were different (p=0.019). Plasma NfL levels were 17.08±3.00 pg/mL in CDR 0 (n=6), 29.48±12.68 pg/mL in CDR 0.5 (n=42), 61.36±79.79 pg/mL in CDR 1 (n=34), 86.99±112.51 pg/mL in CDR 2 (n=18), 46.83 pg/mL in CDR 3 (n=1) (Fig. 3). Correlation of NfL levels with CDR was significant in the entire sample and in participants with CDR-SOB <8 (n=84, r=0.322, p=0.010), but not in participants with CDR-SOB ≥8 (n=29, r=0.176, p=0.179).

Plasma NFL levels in other groups

Additionally, in a total of 31 subjects (10 with cerebrovascular disease and 21 with PD) who had cognitive assessments, plasma NfL levels were also measured. Clinically, subjects were sub-diagnosed as Vascular MCI (n=4), VaD (n=6), PD-no dementia (n=11), PD dementia (n=10). Plasma NfL levels were different between Vascular MCI (45.05±11.51 pg/mL) and VaD (109.54±62.14 pg/mL) groups (p=0.019), and between PD-no dementia (29.80±20.46 pg/mL) and PD dementia groups (46.16±13.56 pg/mL) (p=0.029) (Fig. 1B).

DISCUSSION

This pilot study showed differences in plasma NfL levels between three diagnostic groups of AD, suggesting that plasma NfL may be a useful diagnostic biomarker for the AD dementia stage. Plasma NfL levels inversely correlated with the global cognitive status measured by MMSE scores and directly correlated with the functional state, or the dementia severity measured by the CDR. Similar to previous studies and meta-analyses,^{10,14,32} we observed increased levels of plasma NfL in patients with DAT, compared to nondemented, SCD subjects. However, we did not see a difference in plasma NfL levels between SCD and MCI subjects at the post hoc analysis. In addition, there were also differences in plasma NfL levels between demented and not-demented states for patients with cerebrovascular disease and PD. Although the sample sizes were small, there were differences between VaMCI and VaD, and between PD-no dementia and PD dementia.

Increased NfL level correlated with axonal damage in the brain, especially in white matter and subcortical regions.³³⁻³⁵ Therefore, high levels of blood NfL were found not only in AD but also in other neurological disorders, such as amyotrophic lateral sclerosis,¹⁰ Guillain-Barré syndrome,¹⁰ multiple sclerosis,³⁶ FTD,³⁷ spinal cord injury,³⁸ and others, suggesting that NfL may be nonspecific. Increased blood levels of NfL were furthermore reported in neurodegenerative and neuroinflammatory disorders.³⁹⁻⁴¹ Although PD patients already had higher levels of plasma NfLs compared to normal controls,⁴² the present study showed patients with PD dementia showed higher levels of NfL than PD-no dementia, which has been also reported previously.⁴³ Considering that NfL levels may be a marker for white matter damage,⁴⁴ patients with PD dementia are expected to show increased plasma NfL levels than PD-no dementia. Reduction of the gray matter volume, especially frontotemporal areas, might also increase NfL levels in patients with PD dementia.^{45,46} Patients with VaD may have multiple cerebral small vessel changes in the white matter.⁴⁷⁻⁴⁹ NfL levels, with some associations with PVHs in the present study, showed to be higher in VaD compared to AD in a previous meta-analysis.⁹ Those findings were observed also in both CSF and blood samples, and therefore, NfL levels could be used to differentiate patients with AD, VaD, and other neurodegenerative diseases causing dementia from healthy controls, which is suggestive of the usefulness of NfL for the diagnosis of many dementia diseases.^{8,9} Although NfL levels showed the high sensitivity for the diagnosis of AD and many different neurodegenerative dementia diseases (as well as neurodegenerative diseases themselves),^{8,9} the specificity for the AD diagnosis is lower than that of other validated biomarkers (e.g., A β 42, t-tau).⁸

Plasma NfL level correlated negatively with MMSE score, and such an association has also been reported previously.⁵⁰ In addition to MMSE score, NfL concentrations are found to correlate negatively with other assessment tools such as the Recognition Memory Test⁵¹ and the cognitive subscale of the AD assessment scale.^{11,32,43,52} The present study showed a correlation with CDR, CDR-SOB, as well as MMSE. However, the correlation was more significant in CDR 0.5, CDR 1, and CDR 2 than in CDR 3, implying that the plasma NfL might not reflect subtle severity changes in advanced dementia with CDR 3. Severe dementia patients might not have reflected linear (dose-responsive) changes in plasma NfL levels, because their brains had already undergone severe axonal degeneration approaching plateau. However, these findings might be refuted or strengthened through further studies with more patients with CDR 3.

Bos et al. identified NfL as a strong predictor of cognitive decline across the clinical AD spectrum.⁵³ Plasma NfL can be used as a noninvasive biomarker to track neurodegeneration and to predict the progression of AD. The NfL level is a dynamic biomarker that changes throughout the course of AD and is sensitive to progressive neurodegeneration.³² We also found that plasma NfL levels in participants with amyloid PET (+) were higher than those in amyloid PET (-) participants. Higher NfL level, assuming amyloid pathology, was different according to the cognitive stages, which result was concordant with the previous study showing the relationship of NfL in Mexican Americans with normal cognition, MCI, and dementia without biomarker confirmation.⁵⁴

The present pilot study needs some improvements to overcome limitations. As mentioned earlier, DAT and MCI were diagnosed by using only the clinical criteria, without the confirmation of amyloid- or tau-related biomarkers. However, we tried to exclude patients who have other causes causing cognitive impairments than AD throughout careful history taking, neurological examination, blood tests, MRI, and so on. Moreover, there was also

a difference in NfL levels between amyloid PET (+) and (-), although not all participants performed amyloid PET. This pilot study, showing the difference of NfL levels across the clinical, cognitive spectrum, not in the biomarker-proven AD-continuum, would be augmented by further studies with biomarker confirmation. Second, the small sample size of each diagnostic group is another concern. We compared among three diagnostic groups of SCD, MCI, and DAT, and these results may serve as supplemental evidence for future clinical use of plasma NfL as a diagnostic biomarker. However, future studies with a big sample size including cognitively normal older adults will be beneficial. Although there were also differences in plasma NfL levels between Vascular MCI and VaD and between PD-no dementia and PD dementia in this study, we could not compare plasma NfL levels between more precisely subdivided groups such as MCI, DAT, Vascular MCI, VaD, PD-MCI, PD dementia, and all other neurodegenerative diseases. Third, the present study is cross-sectional, but was not longitudinally followed up. Higher NfL level caused by increased neuronal damage could predict AD progression with further longitudinal studies, which can confirm the utility of plasma NfL as a prognostic biomarker for AD neurodegeneration.

Patient with higher NfL level can receive prompt treatment, because NfL levels might be an important biomarker to detect AD, other neurodegenerative dementia diseases, and VaD. However, its expression is detected among numerous neurodegenerative diseases. Rather than as a diagnostic biomarker, plasma NfL levels may be a potentially useful monitoring or prognostic biomarker for detecting changes in response to treatment.

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