Original Investigation

# Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression

Henrik Zetterberg, MD, PhD; Tobias Skillbäck, MD; Niklas Mattsson, MD, PhD; John Q. Trojanowski, MD, PhD; Erik Portelius, PhD; Leslie M. Shaw, PhD; Michael W. Weiner, MD, PhD; Kaj Blennow, MD, PhD; for the Alzheimer's Disease Neuroimaging Initiative

**IMPORTANCE** The extent to which large-caliber axonal degeneration contributes to Alzheimer disease (AD) progression is unknown. Cerebrospinal fluid (CSF) neurofilament light (NFL) concentration is a general marker of damage to large-caliber myelinated axons.

**OBJECTIVE** To test whether CSF NFL concentration is associated with cognitive decline and imaging evidence of neurodegeneration and white matter change in AD.

**DESIGN, SETTING, AND PARTICIPANTS** A commercially available immunoassay was used to analyze CSF NFL concentration in a cohort of patients with AD (n = 95) or mild cognitive impairment (MCI) (n = 192) and in cognitively normal individuals (n = 110) from the Alzheimer's Disease Neuroimaging Initiative. The study dates were January 2005 to December 2007. The NFL analysis was performed in November 2014.

**MAIN OUTCOMES AND MEASURES** Correlation was investigated among baseline CSF NFL concentration and longitudinal cognitive impairment, white matter change, and regional brain atrophy within each diagnostic group.

**RESULTS** Cerebrospinal fluid NFL concentration (median [interquartile range]) was higher in the AD dementia group (1479 [1134-1842] pg/mL), stable MCI group (no progression to AD during follow-up; 1182 [923-1687] pg/mL), and progressive MCI group (MCI with progression to AD dementia during follow-up; 1336 [1061-1693] pg/mL) compared with control participants (1047 [809-1265] pg/mL) ($P < .001$ for all) and in the AD dementia group compared with the stable MCI group ($P = .01$). In the MCI group, a higher CSF NFL concentration was associated with faster brain atrophy over time as measured by changes in whole-brain volume ($\beta = -4177$, $P = .003$), ventricular volume ($\beta = 1835$, $P < .001$), and hippocampus volume ($\beta = -54.22$, $P < .001$); faster disease progression as reflected by decreased Mini-Mental State Examination scores ($\beta = -1.077$, $P < .001$) and increased Alzheimer Disease Assessment Scale cognitive subscale scores ($\beta = 2.30$, $P < .001$); and faster white matter intensity change ($\beta = 598.7$, $P < .001$).

**CONCLUSIONS AND RELEVANCE** Cerebrospinal fluid NFL concentration is increased by the early clinical stage of AD and is associated with cognitive deterioration and structural brain changes over time. This finding corroborates the contention that degeneration of large-caliber axons is an important feature of AD neurodegeneration.

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**Author Affiliations:** Author affiliations are listed at the end of this article.

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**Corresponding Author:** Henrik Zetterberg, MD, PhD, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, S-431, 80 Mölndal, Sweden (henrik.zetterberg@gu.se).

Alzheimer disease (AD) is a common neurodegenerative disorder characterized by distinct pathologic hallmarks, including neuronal degeneration and loss together with extracellular deposits of aggregated Aβ and intraneuronal accumulation of hyperphosphorylated tau proteins.[1] While AD is characterized by cortical and hippocampal neuronal loss and widespread gray matter atrophy, patients may also have progressive disconnection of cortical and subcortical regions due to white matter (WM) injury.[2] White matter pathologic conditions include loss of axons and myelin sheaths.[3,4] Patients with AD demonstrate significant WM atrophy[5-8] as well as a gradual decrease in the integrity of WM commissures, such as the corpus callosum, and key pathways, such as the cingulum and superior longitudinal fasciculus.[9-12] These tracts are composed of large-caliber myelinated axons that are particularly rich in neurofilaments.[13]

There are 3 different neurofilament subunits, including neurofilament light (NFL), neurofilament medium (NFM), and neurofilament heavy (NFH). A neurofilament is a structural component of the neural cytoskeleton, constituting one NFL and either NFM or NFH arranged head to tail.[14] Increased cerebrospinal fluid (CSF) concentrations of NFL correlate with WM lesions in multiple sclerosis,[15] subcortical vascular disease,[16] and AD[16] and are seen in other pathologic conditions, such as frontotemporal dementia,[17-20] idiopathic normal-pressure hydrocephalus,[21] amyotrophic lateral sclerosis,[22] progressive encephalopathies in children,[23] and various central nervous system infections.[24,25] A recent study[20] based on the Swedish Dementia Registry showed that a high CSF NFL concentration correlates with more severe cognitive impairment and shorter survival in several neurodegenerative diseases, including AD.

Herein, we performed a detailed analysis of associations between CSF NFL concentration and WM change and neuropsychological and neuroimaging measures of AD in a large cohort of longitudinally followed, cognitively normal (CN) control participants; individuals with mild cognitive impairment (MCI); and patients with AD. We tested the following 4 hypotheses: (1) CSF NFL concentration is increased in patients with AD compared with healthy controls, (2) high CSF NFL concentration predicts MCI conversion to AD dementia, (3) high CSF NFL concentration is associated with more rapid cognitive worsening in AD, and (4) high CSF NFL concentration is associated with WM change within the 4 diagnostic groups herein.

## Methods

Data used in this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the US Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations as a $60 million, 5-year public-private partnership. The primary objective of the ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure progression of MCI and early AD. The principal investigator of this initiative is one of us (M.W.W.). The ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and study participants have been recruited from more than 50 sites across the United States and Canada. The initial goal of the ADNI was to recruit 800 participants, but the initiative has been followed by the ADNI Grand Opportunities (GO) and the ADNI 2. To date, these 3 protocols have recruited more than 1500 adults (age range, 55-90 years) to participate in the research, including CN older individuals, persons with early or late MCI, and patients with early AD. The follow-up duration for each study group is specified in the protocols for the ADNI 1, ADNI 2, and ADNI GO. Participants originally recruited for the ADNI 1 and the ADNI GO had the option to be followed up in the ADNI 2. The most recent information on the ADNI is available online (http://www.adni-info.org).

### Participants

The study was conducted with prior institutional ethics approval from ADNI's 59 study sites (http://adni.loni.usc.edu/about/centers-cores/study-sites/). Written informed consent was obtained for all participants in the ADNI. Our study population consisted of all CN, MCI, and AD dementia group participants with available baseline CSF samples from the ADNI 1. Inclusion and exclusion criteria are described in detail online (http://www.adni-info.org). Briefly, all participants included in the ADNI 1 were between 55 and 90 years old, had completed at least 6 years of education, were fluent in Spanish or English, and were free of any significant neurological disease other than AD. The CN group had a Mini-Mental State Examination (MMSE) score of 24 or higher and a Clinical Dementia Rating of 0. The MCI group had an MMSE score of 24 or higher, objective memory loss based on delayed recall scores of the Wechsler Memory Scale logical memory II (>1 SD below the normal mean), a Clinical Dementia Rating of 0.5, preserved activities of daily living, and absence of dementia. The AD dementia group fulfilled the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria for probable AD, had an MMSE score between 20 and 26, and had a Clinical Dementia Rating of 0.5 or 1.0. For this analysis, the MCI group was stratified into stable MCI (sMCI), with no progression to AD dementia during at least 2 follow-up years, and progressive MCI (pMCI), with progression to AD dementia during at least 2 follow-up years. Therefore, the main analyses included the following 4 groups: CN, sMCI, pMCI, and AD dementia.

### CSF Measurements

Cerebrospinal fluid collection, processing, and storage procedures have been described previously.[26] Levels of Aβ42, total tau (t-tau), and phosphorylated tau (p-tau) were measured using an architectural platform (xMAP Multiplex; Luminex Corporation) and a kit (INNO-BIA AlzBio3; Fujirebio). Individuals were classified as Aβ42 positive or Aβ42 negative using a previously established cutoff (CSF Aβ42 level, <192 pg/mL) that maximized the delineation of autopsy-confirmed AD cases with pathologic Aβ from control subjects without pathologic Aβ.[26]

Table. Demographic and Clinical Characteristics of Study Participants at Baseline

| Characteristic | Group | | | |
|---|---|---|---|---|
| | CN (n = 110) | AD Dementia (n = 95) | pMCI (n = 101) | sMCI (n = 91) |
| Sex, No. | | | | |
| Female | 55 | 42 | 37 | 26 |
| Male | 55 | 53 | 64 | 65 |
| **Clinical Characteristics, Median (Interquartile Range)** | | | | |
| Age at lumbar puncture, y | 76 (72-78) | 76 (69-80) | 74 (69-80) | 74 (71-80) |
| MMSE score | 29 (29-30) | 24 (22-25)[a] | 26 (25-28)[a] | 28 (26-29)[a] |
| ADAS-cog score | 9.7 (6.3-12.7) | 28.7 (22.3-34.0)[a] | 20.7 (17.0-24.7)[a] | 16.7 (11.7-21.0)[a] |
| White matter change volume, $mm^3$ | 3956 (2769-6272) | 5180 (3781-9101)[b] | 4533 (2942-7606) | 4031 (2883-6301) |
| Hippocampus volume, $mm^3$ | 7336 (6730-7703) | 5521 (4833-6501)[a] | 5988 (5459-6731)[a] | 6834 (6025-7534) |
| Ventricular volume, $mm^3$ | 32 593 (20 399-42 098) | 42 875 (29 497-60 923)[c] | 40 331 (28 929-55 946)[b] | 37 777 (24 512-49 416) |
| Whole-brain volume, $mm^3$ | 1 006 650 (925 721-1 057 460) | 937 168 (868 796-1 042 700) | 981 244 (920 902-1 074 200) | 1 030 925 (962 926-1 103 060) |
| CSF NFL concentration, pg/mL | 1047 (809-1265) | 1479 (1134-1842)[a] | 1336 (1061-1693)[a] | 1182 (923-1687)[c] |

Abbreviations: AD, Alzheimer disease; ADAS-cog, Alzheimer Disease Assessment Scale cognitive subscale; CN, cognitively normal; CSF NFL, cerebrospinal fluid neurofilament light; MMSE, Mini-Mental State Examination; pMCI, progressive mild cognitive impairment; sMCI, stable mild cognitive impairment.

[a] $P < .001$ vs CN group.

[b] $P < .05$ vs CN group.

[c] $P < .01$ vs CN group.

Cerebrospinal fluid NFL concentration was measured using a commercially available enzyme-linked immunosorbent assay (NF-light; Uman Diagnostics) as described by the manufacturer. The measurements were performed by board-certified laboratory technicians, who were masked to clinical data using 1 batch of reagents. Intrabatch coefficients of variation were below 10%.

## Brain Structure

Structural MRI brain scans were acquired using 1.5-T imaging systems (at ≤7 time points, including screening and at 6, 12, 18, 24, 36, and 48 months) with a standardized protocol that included T1-weighted images using a sagittal, volumetric, magnetization-prepared rapid acquisition with gradient echo sequence.[27] In brief, automated volume measures were obtained with a software package (FreeSurfer; http://surfer.nmr.mgh.harvard.edu/fswiki).[28,29] For this study, we used averaged volume measurements for the right and left hippocampi and combined volumes for the ventricles. Code ST128SV (volume of hypointensities) in FreeSurfer was used for a measure of WM change.

## Cognition

Overall cognition was assessed by MMSE and Alzheimer Disease Assessment Scale cognitive subscale (ADAS-cog) 13 scores. Data were acquired at up to 7 time points, including screening and at 6, 12, 18, 24, 36, and 48 months.

## Statistical Analysis

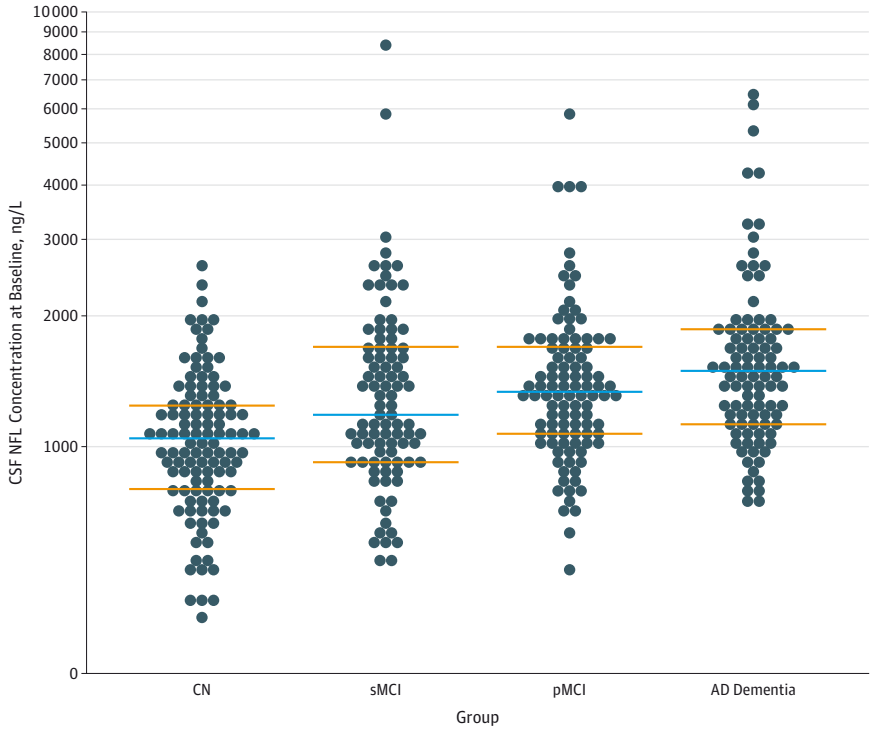
To evaluate potentially confounding factors, we tested associations between CSF NFL concentration and demographic factors (age, sex, apolipoprotein E [*APOE*] ε4 genotype, and educational level) using the Mann-Whitney test and the Spearman rank correlation test. Associations between CSF NFL concentration and the diagnostic groups were tested in an analysis of covariance model, adjusted for age and sex (coded as 0 or 1). Correlation of Aβ42, t-tau, and p-tau levels with NFL concentration was tested with linear regression. Within the diagnostic groups, association between CSF NFL concentration and Aβ42 positivity (CSF Aβ42 level, <192 pg/mL) was analyzed by the nonparametric Kruskal-Wallis test. Associations between baseline CSF NFL concentration and subsequent disease progression (as measured by MMSE and ADAS-cog scores, hippocampus volume, ventricular volume, whole-brain volume, and WM change) were tested with linear mixed-effects models, adjusted for age and sex (and educational level for cognitive measurements and intracranial volume for volume measurements). Associations were further demonstrated using Loess regression trend lines, with participants divided into quartiles according to their CSF NFL concentration. All tests were 2 sided, and significance was set at $P < .05$. Statistical analyses were performed using a software program (SPSS, version 20; IBM or R, version 3.0.1; The R Foundation for Statistical Computing).

# Results

## CSF NFL Concentration and Demographic Factors

Demographic and biomarker characteristics of the study participants are shown in the **Table** and in **Figure 1**. Cerebrospinal fluid NFL concentration had a moderately strong correlation with age ($r = 0.35$, $P < .001$), and men had a significantly

Figure 1. Cerebrospinal Fluid Neurofilament Light (CSF NFL) Concentration in the Diagnostic Groups




Cerebrospinal fluid NFL concentrations in the diagnostic groups are shown as scatterplots. Horizontal blue lines indicate the median, and horizontal orange lines indicate the interquartile range. Cerebrospinal fluid NFL concentration was higher in the Alzheimer disease (AD) dementia, progressive mild cognitive impairment (pMCI), and stable mild cognitive impairment (sMCI) groups compared with the cognitively normal (CN) group ($P < .001$, $P < .001$, and $P = .001$, respectively). Higher CSF NFL concentration was also found in the AD dementia group compared with the sMCI group ($P = .01$).

higher NFL concentration than women ($P < .001$). Patient educational level in years correlated weakly with baseline NFL concentration (Spearman $\rho = 0.113$, $P = .02$). There were no significant differences in NFL concentration between patients stratified according to *APOE* ε4 genotype.

## CSF NFL Concentration in the Diagnostic Groups

Cerebrospinal NFL concentration was higher in the AD, pMCI, and sMCI groups compared with the CN group ($P < .001$, $P < .001$, and $P = .001$, respectively) (Figure 1). Higher CSF NFL concentration was also found in the AD dementia group compared with the sMCI group ($P = .01$).

## CSF NFL Concentration in Relation to Core AD Biomarkers

At baseline, high NFL concentration correlated with low Aβ42 level ($P = .01$, $\beta = -0.127$). However, there were no significant differences in NFL concentration between Aβ42-positive (CSF Aβ42 level, <192 pg/mL) and Aβ42-negative individuals in any of the diagnostic groups (CN, sMCI, pMCI, or AD dementia). Both t-tau level ($P < .001$, $\beta = 0.213$) and p-tau level ($P = .02$, $\beta = 0.118$) correlated with NFL concentration at baseline.

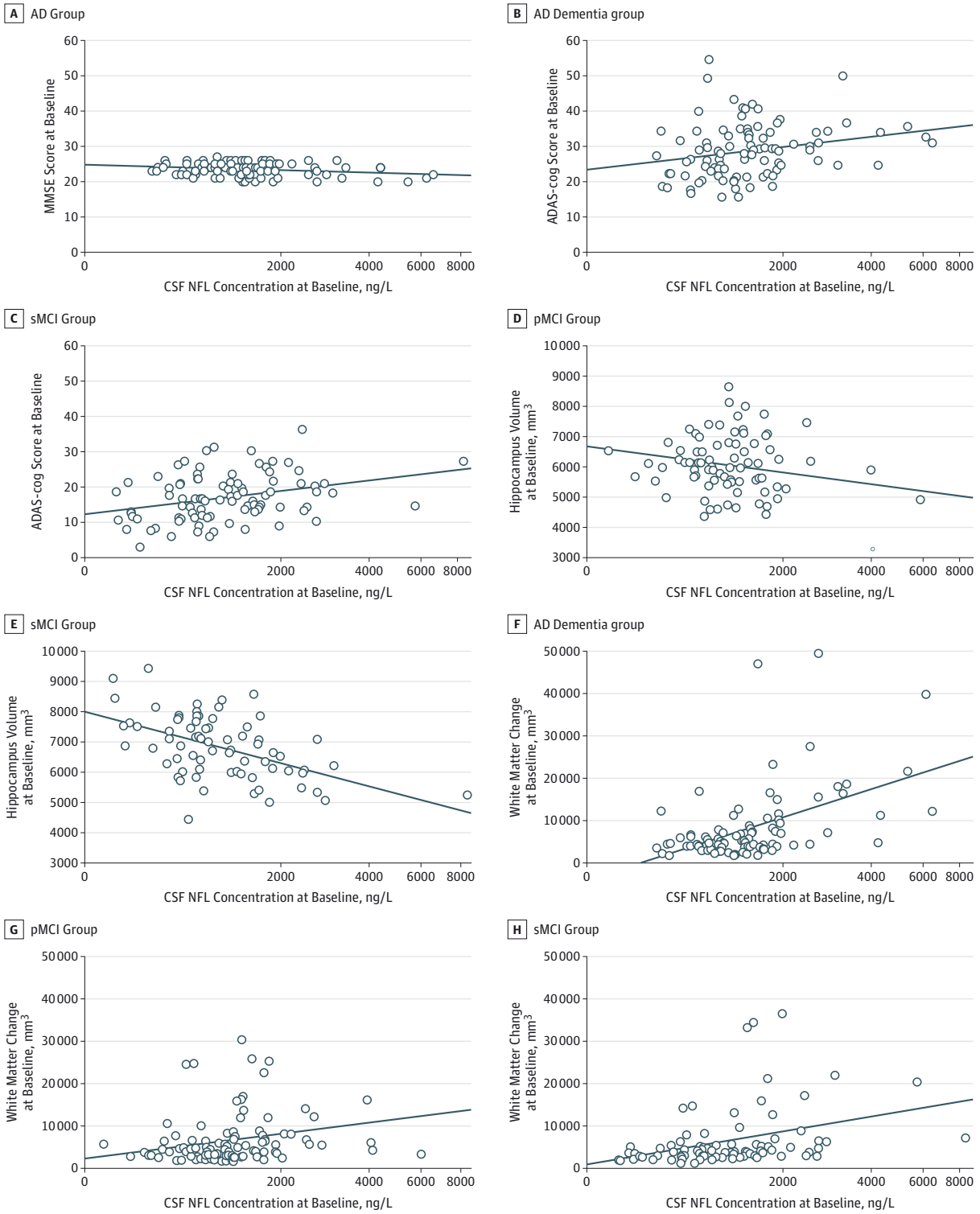
## CSF NFL Concentration in Relation to Baseline Measures of Cognition, Brain Structure, and WM Change

Among the 4 major diagnostic groups, we found significant correlation between CSF NFL concentration and MMSE ($P = .006$, $\beta = -0.026$) and ADAS-cog ($P = .008$, $\beta = 0.006$) scores in the AD dementia group (**Figure 2**A and B). Significant correlation between CSF NFL concentration and ADAS-cog score was also found in the sMCI group ($P = .01$, $\beta = 0.007$) (Figure 2C). Furthermore, we found significant correlation between CSF NFL concentration and hippocampus volume in the pMCI group ($P = .01$, $\beta = -5.88e\text{-}5$) (Figure 2D) and the sMCI group ($P = .049$, $\beta = -4.94e\text{-}5$) (Figure 2E). White matter change correlated significantly with CSF NFL concentration in the AD dementia group ($P < .001$, $\beta = 0.048$) (Figure 2F), pMCI group ($P = .02$, $\beta = 0.058$) (Figure 2G), and sMCI group ($P = .04$, $\beta = 0.034$) (Figure 2H). No significant correlation was found between CSF NFL concentration and whole-brain or ventricular volume.

## CSF NFL Concentration and Longitudinal Change in Cognition and Brain Structure

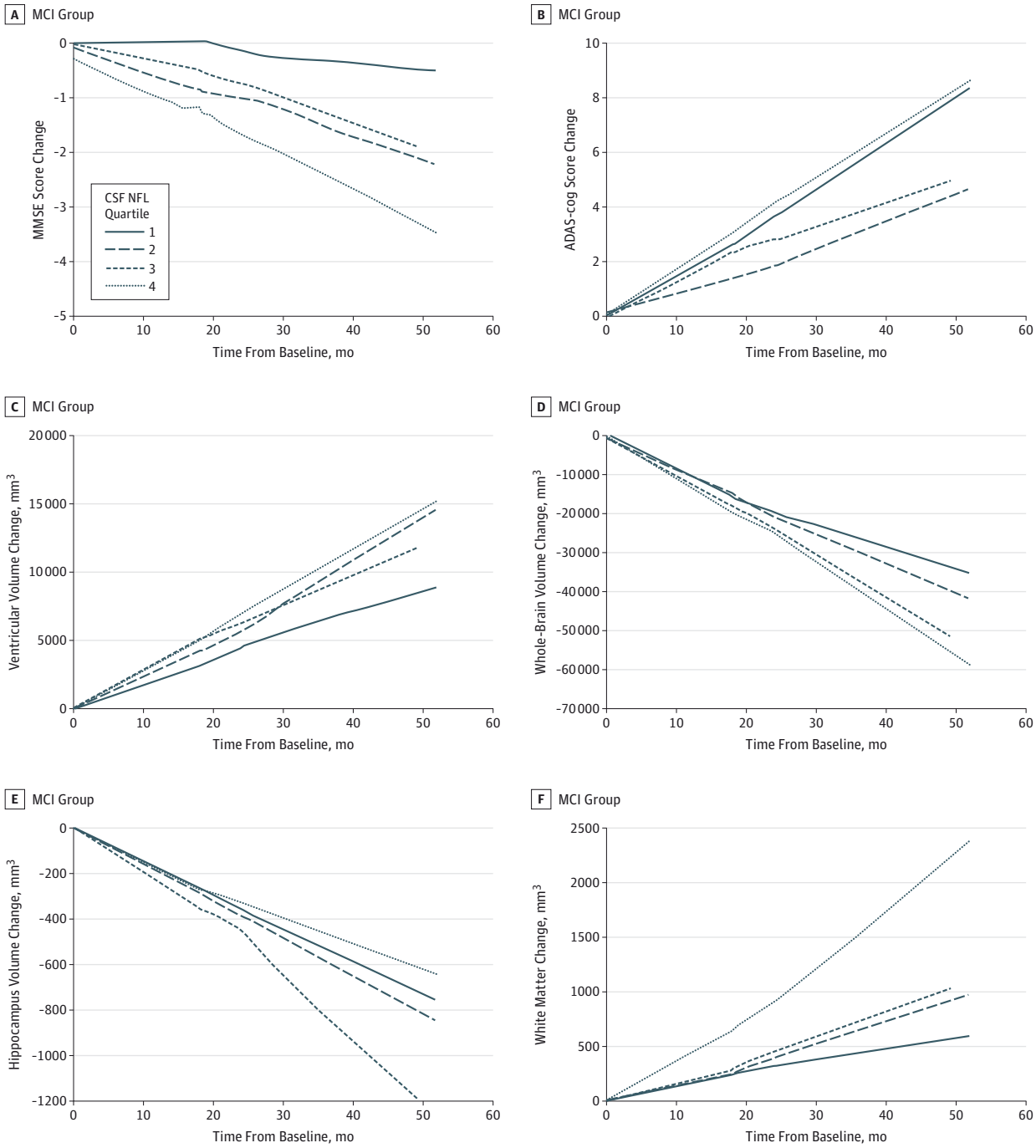
Using linear mixed-effects models, we tested associations between baseline CSF NFL concentration and subsequent disease progression in MCI as measured by MMSE and ADAS-cog scores, hippocampus volume, ventricular volume, whole-brain volume, and WM change, adjusted for age and sex (and educational level for cognitive measurements and intracranial volume for volume measurements). The interaction analyses showed that higher CSF NFL concentration was associated with longitudinal deterioration in all 6 parameters ($\beta = -1.077$, $P < .001$ for MMSE score; $\beta = 2.30$, $P < .001$ for ADAS-cog score; $\beta = 1835$, $P < .001$ for ventricular volume; $\beta = -4177$, $P = .003$ for whole-brain volume; $\beta = -54.22$, $P < .001$ for hippocampus volume; and $\beta = 598.7$, $P < .001$ for WM change). For these analyses, we used continuous (log-transformed) NFL concentration data, but the results were essentially the same when using CSF NFL concentration quartiles as a categorical predictor. In

Figure 2. Cerebrospinal Fluid Neurofilament Light (CSF NFL) Concentration in Relation to Baseline Measures of Cognition, Brain Structure, and White Matter Change




Linear regression trend lines are shown in blue. These regression lines are unadjusted, while the corresponding analysis in the text is adjusted for age and sex. Cerebrospinal fluid NFL concentration on the x-axes is logarithmic. Cerebrospinal fluid NFL concentration correlated significantly with Mini-Mental State Examination (MMSE) score (A) and Alzheimer Disease Assessment Scale cognitive subscale (ADAS-cog) score (B) in the Alzheimer disease (AD) dementia group, with ADAS-cog score in the stable mild cognitive impairment (sMCI) group (C), with hippocampus volume in the progressive mild cognitive impairment (pMCI) group (D) and sMCI group (E), and with white matter (WM) change in the AD (F), pMCI (G), and sMCI (H) groups.

Figure 3. Cerebrospinal Fluid Neurofilament Light (CSF NFL) Concentration and Disease Progression in Mild Cognitive Impairment (MCI)




Associations between baseline CSF NFL concentration and subsequent disease progression in the MCI group (n = 192) as measured by Mini-Mental State Examination (MMSE) score (A), Alzheimer Disease Assessment Scale cognitive subscale (ADAS-cog) score (B), ventricular volume (C), whole-brain volume (D), hippocampus volume (E), and white matter (WM) change (F) were all significant in linear mixed-effects models. Associations are shown using Loess regression trend lines, with all participants classified into quartile groups according to their baseline CSF NFL concentration. The regression trend lines confirm the pattern of association between high CSF NFL concentration and worse outcome for all parameters except ADAS-cog score (B) and hippocampus volume (E).

**Figure 3**, all study participants with an MCI diagnosis at baseline were classified into quartile groups according to their baseline CSF NFL concentration, and change in each parameter (defined as the difference between baseline and each time point) was demonstrated using Loess regression trend lines. These regression trend lines confirmed that the pattern of high CSF NFL concentration was associated with worse outcome for all parameters except ADAS-cog score and hippocampus volume.

## Discussion

The main findings of this study were that CSF NFL concentration was elevated in patients with AD dementia compared with CN controls and participants with sMCI and that CSF NFL concentration correlated with accelerated cognitive decline, WM change, and increased brain atrophy in patients with MCI. Taken together, these findings support the use of CSF NFL concentration as a progression marker in MCI and AD and indicate that degeneration of large-caliber axons is an important element of disease progression in AD. The diagnostic usefulness of CSF NFL concentration might be limited because of overlap with other neurodegenerative conditions, but high concentrations in AD or MCI suggest that rapid disease progression is to be expected.

As a highly expressed structural protein in myelinated tracts, NFL interconnects cortical and subcortical brain regions.[13] Expression of NFL is also found in neurites in the cerebral and cerebellar cortices and in the hypothalamus, as well as in the spinal cord.[13] Cerebrospinal NFL concentration is increased in a broad range of neurological disorders and is thus not disease specific, which also means that it could be useful as a disease intensity marker not only in AD but also in several other neurodegenerative and neuroinflammatory diseases, as well as in traumatic brain injury. High CSF NFL concentration correlates with short survival in amyotrophic lateral sclerosis,[22] and similar results have been obtained in frontotemporal dementia, subcortical vascular dementia, and AD.[20] Although we detected positive correlation of CSF NFL concentration with CSF tau protein level, associations of CSF NFL concentration with increased ventricular volume and whole-brain atrophy over time suggest that the marker contributes information on neurodegeneration that is at least in part different from CSF tau (a protein predominantly expressed in cortical brain regions, with CSF tau level being more strongly associated with hippocampal and cortical atrophy[30]). Similar CSF NFL concentration in Aβ42-positive and Aβ42-negative individuals, as determined by CSF Aβ42 level, indicate that CSF NFL concentration changes are not driven by pathologic Aβ42. The findings that CSF NFL concentration correlated with baseline MMSE and ADAS-cog scores, as well as change in MMSE score over time (the correlation with longitudinal change in ADAS-cog score seen in linear mixed-effects models could not be visualized using Loess regression trend lines and may thus be considered less robust), suggest that elevated CSF NFL concentration provides clinically meaningful information. In contrast to our results, a recent study[31] failed to find an association between CSF NFL concentration and baseline cognition in AD, but the patients with AD in that study were younger and more cognitively impaired than the patients in this study.

One limitation of our study is that patients with MCI were considered to have sMCI if they remained cognitively stable during 2 follow-up years, which may be regarded as too short. Hence, the sMCI group may have contained some individuals who eventually would develop progressive neurodegenerative disease, which could explain why CSF NFL concentration was somewhat higher in this group compared with the CN group.

## Conclusions

Our findings support the use of CSF NFL as a progression marker in AD and extend earlier results[20] by showing an association between this marker and longitudinal imaging data of neurodegeneration and WM change. Together with MRI, CSF NFL concentration may track non-Aβ42 and non-tau aspects of AD neurodegeneration and may help to identify individuals with extensive involvement of large-caliber axons in the disease process. Additional research is needed to determine whether these findings should have an influence on inclusion criteria in clinical trials of novel disease-modifying drugs against AD.

ARTICLE INFORMATION

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**Author Affiliations:** Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Mölndal, Sweden (Zetterberg, Skillbäck, Portelius, Blennow); Department of Molecular Neuroscience, University College London Institute of Neurology, London, England (Zetterberg); Department of Veterans Affairs Medical Center, University of California, San Francisco (Mattsson, Weiner); Center for Imaging of Neurodegenerative Diseases, University of California, San Francisco (Mattsson); Department of Radiology and Biomedical Imaging, University of California, San Francisco (Mattsson); Clinical Memory Research Unit, Faculty of Medicine, Lund University, Lund, Sweden (Mattsson); Department of Pathology and Laboratory Medicine, Center for Neurodegenerative Disease Research, Institute on Aging, University of Pennsylvania School of Medicine, Philadelphia (Trojanowski, Shaw).

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*Study concept and design:* Zetterberg, Blennow.
*Acquisition, analysis, or interpretation of data:* All authors.
*Drafting of the manuscript:* Zetterberg, Skillbäck.
*Critical revision of the manuscript for important intellectual content:* Mattsson, Trojanowski, Portelius, Shaw, Weiner, Blennow.
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