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Research article

Plasma neurofilament light chain levels in Alzheimer's disease☆

Wenjun Zhou[a,1], Jie Zhang[b,*,1], Fanlong Ye[b], Guangzheng Xu[c], Hang Su[d], Yindan Su[e], Xiangyang Zhang[f,**], Alzheimer's Disease Neuroimaging Initiative

[a] Department of Respiratory Medicine, Zhongshan Hospital, Fudan University, Shanghai, China
[b] Department of Neurology, Zhongshan Hospital, Fudan University, Shanghai, China
[c] Xujiahui community health service center,Xuhui District, Shanghai, China
[d] Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[e] The Affiliated High School to Hangzhou Normal University, Hangzhou, China
[f] Beijing HuiLongGuan Hospital, Peking University, Beijing, China

HIGHLIGHTS

- We examined the utility of plasma NFL as a potential biomarker for MCI and AD.
- Plasma NFL levels were significantly different between diagnostic groups.
- The overlap of plasma NFL between groups limits its use as a diagnostic biomarker.

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ABSTRACT

Plasma neurofilament light (NFL) levels may be a marker of neuronal injury. We examined whether plasma NFL might be a potential biomarker for the prodromal and dementia stages of AD. Participants included 193 cognitively normal (CN), 198 amnestic mild cognitive impairment (aMCI) and 187 Alzheimer's disease (AD) individuals enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Plasma NFL levels were examined by the Single Molecule array (Simoa) technique. Our results showed significantly increased plasma NFL levels in both AD (50.9 pg/ml) and aMCI (43.0 pg/ml) groups compared to CN (34.7 pg/ml) group (both $p < 0.001$), but with substantial overlap between the groups. Plasma NFL levels in AD group was also markedly increased, compared with aMCI group ($p < 0.001$). Plasma NFL levels were positively associated with age ($r = 0.355$, $p < 0.001$) and negatively with global cognition ($r = -0.355$, $p < 0.001$) in all subjects. Our results suggest that plasma NFL levels may not be a useful biomarker for the diagnosis of prodromal and dementia stages of AD.

1. Introduction

Neurofilament light (NFL) is a putative marker of large-caliber axonal degeneration, and increased NFL levels in cerebrospinal fluid (CSF) appear to reflect neurodegeneration-related axonal injury [15]. Levels of CSF NFL were found to be abnormally higher in Alzheimer's disease (AD) [6,10,11,13,16], and also related to cognitive decline and hippocampal atrophy in subjects with mild cognitive impairment (MCI)[16], suggesting that CSF NFL could be a candidate biomarker for diagnosis and progression of MCI and early AD. However, the CSF NFL may not be practical or acceptable for some elderly patients. Therefore, blood-based measurement of NFL would be more desirable, since the collection of blood sample is relatively more applicable and less invasive.

A recent study revealed that serum NFL levels in AD, but not in MCI, were significant higher than those of cognitively normal (CN)

Abbreviations: MMSE, Mini-Mental State Examination; CN, cognitively normal; aMCI, amnestic mild cognitive impairment; AD, Alzheimer's disease; NFL, neurofilament light.

☆ Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

* Corresponding author at: Department of Neurology, Zhongshan Hospital, Fudan University, 180 Fenglin Rd., Shanghai 200032, China.
** Corresponding author at: Biological Psychiatry Center, Beijing Hui-Long-Guan hospital, Chang-Ping District, Beijing 100096, China
E-mail addresses: drzhangj@yahoo.com (J. Zhang), zhangxy9@gmail.com (X. Zhang).

[1] These authors contributed equally to this work.

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subjects [1], suggesting that NFL levels in serum may be a potential biomarker for AD diagnosis, but not for MCI. However, these findings should be interpreted with caution due to the small size of study sample (MCI, n = 33) and the heterogeneity of MCI individuals (included both amnestic MCI and non-amnestic MCI). In an effort to replicate the preliminary findings in previous study [1] and examine whether plasma NFL would be a potential diagnostic biomarker for prodromal and dementia stages of AD, we assessed plasma NFL levels in a large sample size of CN (n = 193), aMCI (n = 198) and AD (n = 187) individuals. We also investigated the correlations of plasma NFL with cognitive function and the clinical demographics in these subjects.

2. Methods

2.1. Alzheimer's disease neuroimaging initiative

Cross-sectional data used in this paper were extracted from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (*adni.loni.usc.edu*) in January 2017. The ADNI was initiated 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 private partnership. The primary aim of the ADNI has been to verify whether clinical and neuropsychological assessment, neuroimaging and other biomarkers can be integrated to measure the progression of MCI and early AD. Participants have been recruited from more than 50 sites in the USA and Canada. Further information about ADNI can be found at http://www.adni-info.org. The ADNI was conducted after institutional review board approval at each site. Written informed consent was obtained from all participants or their authorized representatives.

2.2. Participants

Inclusive and exclusive criteria are described in details at http://www.adni-info.org. In brief, all individuals recruited in the ADNI 1 were from 55 to 90 years old, having completed greater than or equal to 6 years of education, with fluent spoken English or Spanish, and were free of any severe neurological diseases except AD. The cognitively normal (CN) group had a score of at least 24 on the Mini-Mental State Examination (MMSE) and a score of 0 on the Clinical Dementia Rating (CDR). The aMCI group had a score of 24 or higher on the MMSE, a score of 0.5 on CDR, with objective memory impairment due to delayed recall scores of Logical Memory II (>1 SD below normal mean), essentially preserved activities of daily living, and being not demented. The AD group met the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria for probable AD, having a score of from 20 to 26 on the MMSE and a score of 0.5 or 1 on the CDR. Totally, 578 individuals (CN, n = 193; aMCI, n = 198 and AD, n = 187) were included in the present study, with available plasma NFL data from the ADNI 1.

2.3. Plasma NFL data

Plasma NFL was examined by the Single Molecule array (Simoa) technique. The assay uses a combination of monoclonal antibodies, and purified bovine NFL as a calibrator. All samples were measured in duplicate. Analytical sensitivity was <1.0 pg/ml. Values are presented as pg/mL. Further information about the methods can be found at adni.loni.usc.edu.

Table 1
Demographic and clinical data.

Characteristics	CN (n = 193)	aMCI (n = 198)	AD (n = 187)	P value
Age, years	75.7 (4.9)	74.5 (7.4)	75.5 (7.4)	0.4
Female sex, %	45.1	32.8	48.1	0.005
Education, years	16.0 (2.8)	15.8 (3.0)	14.7 (3.1)	<0.001
MMSE, score	29.1 (0.99)	26.9 (1.8)	23.3 (2.1)	<0.001
Plasma NFL, pg/ml	34.7 (21.4)	43.0 (29.1)	50.9 (26.8)	<0.001

2.4. Statistics analysis

Demographic and plasma NFL data were compared between diagnostic groups using nonparametric tests (Kruskal-Wallis and Mann-Whitney) for continuous variables (age, education, MMSE score, plasma NFL) and Pearson's X^2 test for dichotomous variable (gender). Spearman correlation analysis was applied to examine the relationships between plasma NFL and age, education, MMSE score in the whole sample and within each diagnostic group.

3. Results

3.1. Sample characteristics

Table 1 shows the demographic and clinical data of the subjects included in the study. No group difference in age was detected. The aMCI group had fewer females than the other two study groups. Participants with AD dementia had lower educational levels than the other two groups. As expected, a significant difference in MMSE scores was found across the three groups (AD < aMCI < CN, 23.3 vs. 26.9 vs. 29.1, $p < 0.001$). Continuous data are summarized as mean (standard deviation).

P values tested by Pearson's X^2 test and Kruskal-Wallis test.

3.2. Plasma NFL levels in different diagnostic groups

NFL levels in plasma were significantly elevated in AD subjects compared with both CN and aMCI subjects (Table 1 and Fig. 1). We also found that plasma NFL levels were higher in aMCI subjects than in CN subjects (Table 1 and Fig. 1).

3.3. Plasma NFL levels and clinical characteristics

To examine the sex difference in plasma NFL, NFL levels were compared between males and females in the three groups. However, no sex difference in plasma NFL was found in the all sample or within any diagnostic group (Fig. 2).

To confirm the effect of age on plasma NFL levels, plasma NFL levels were compared between participants who were younger than 75.3 years and those who were 75.3 years or older (dichotomized based on median; <75.3 years or ≥75.3 years) within CN, aMCI and AD groups. Plasma NFL levels were increased in participants who were 75.3 years or older than those who were younger than 75.3 years in each diagnostic group (all $p < 0.001$, Fig. 3).

In addition, spearman correlation analyses were performed to examine the relationships between plasma NFL levels and clinical characteristics. Table 2 summarizes the relationships between plasma NFL levels and age, education or MMSE score in all the sample and within each diagnostic group. A positive correlation between plasma NFL levels and age was found in the entire sample and in each diagnostic group (all $p < 0.001$). No relationships between plasma NFL levels and education were found in the entire sample or in any diagnostic group (all $p > 0.05$). An inverse correlation between plasma NFL levels and MMSE scores was found in

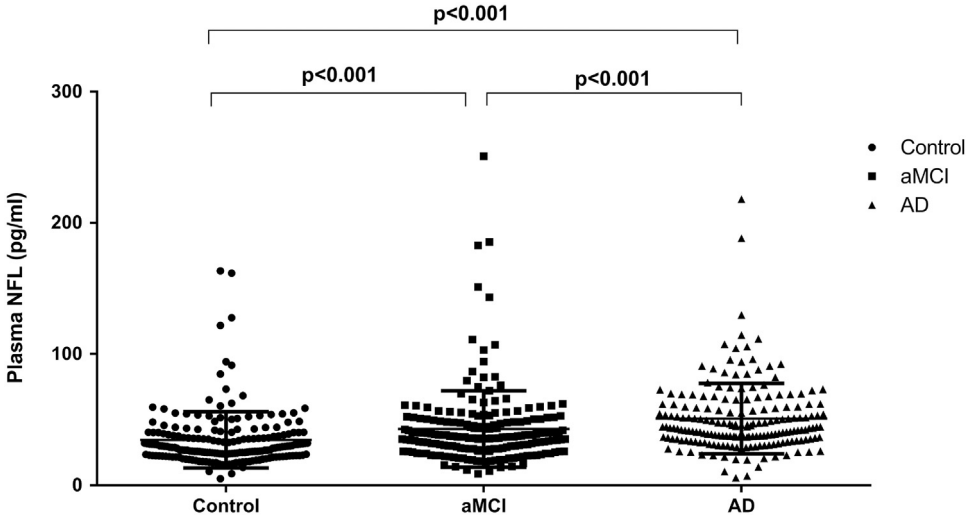

Fig. 1. Plasma NFL levels in different diagnostic groups. A significant difference in plasma NFL levels was found across the three groups (AD > aMCI > CN, 50.9 vs. 43.0 vs 34.7 pg/ml, all p < 0.001). Values are expressed in pg/ml. aMCI = amnestic mild cognitive impairment; AD = Alzheimer's disease; NFL = neurofilament light. P values tested by Mann-Whitney test.

Table 2
Correlations between Plasma NFL and clinical variables.

Group	All subjects (n = 578)		CN (n = 193)		aMCI (n = 198)		AD (n = 187)	
	R	p	R	p	R	p	R	p
Age	0.355	<0.001	0.317	<0.001	0.436	<0.001	0.389	<0.001
Education	−0.022	0.603	0.126	0.08	−0.015	0.838	−0.007	0.928
MMSE	−0.355	<0.001	0.07	0.334	−0.189	0.008	−0.219	0.003

Abbreviations: MMSE, Mini-Mental State Examination.
P values tested by Spearman correlation analyses.

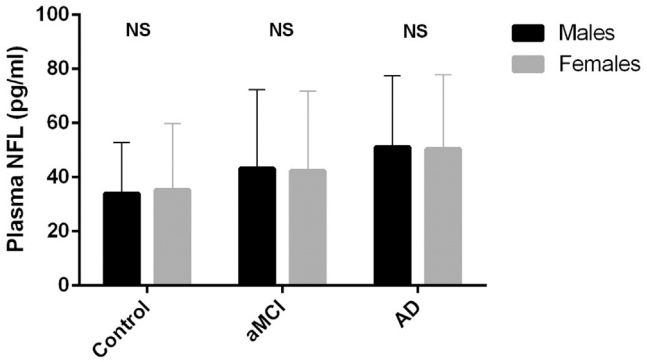

Fig. 2. Comparison of plasma NFL in males and females with cognitively normal, aMCI and AD. No sex difference in plasma NFL was found within any diagnostic group (all p > 0.05). Values are expressed in pg/ml. aMCI = amnestic mild cognitive impairment; AD = Alzheimer's disease; NFL = neurofilament light. NS = not significant (p > 0.05). P values tested by Mann-Whitney test. The error bars represent standard deviation.

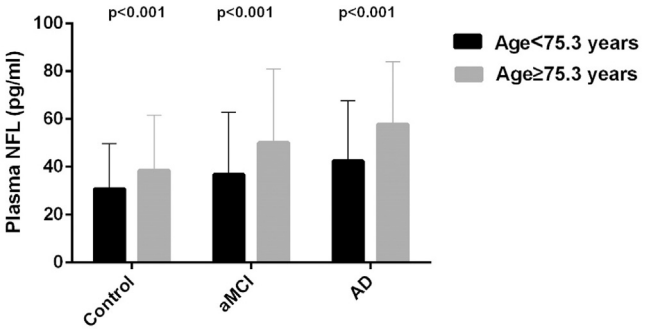

Fig. 3. Comparison of plasma NFL in individuals who were younger than 75.3 years and those who were 75.3 years or older with cognitively normal, aMCI and AD. Plasma NFL levels were increased in participants who were 75.3 years or older than those who were younger than 75.3 years in each diagnostic group (all p < 0.001). Values are expressed in pg/ml. aMCI = amnestic mild cognitive impairment; AD = Alzheimer's disease; NFL = neurofilament light. NS = not significant (p > 0.05). P values tested by Mann-Whitney test. The error bars represent standard deviation.

the entire sample, aMCI and AD groups (all p < 0.05), but not in CN group (p > 0.05).

4. Discussion

In the present study, we examined the utility of plasma NFL as a peripheral biomarker in the prodromal and dementia stages of AD. Our findings showed significantly different plasma NFL levels between diagnostic groups (AD > aMCI > CN) but with substantial overlap between the groups, suggesting that plasma NFL levels may not be a useful biomarker for the diagnosis of prodromal and dementia stages of AD.

To the best of our knowledge, there have been only two studies examining levels of blood NFL in AD patients, with inconsistent results. The first study reported no significant differences in serum NFL levels between AD patients and healthy controls after age was adjusted [3]. More recently, Bacioglu et al. examined serum NFL levels among subjects clinically diagnosed as CN, MCI and AD. They found that serum NFL levels were significantly increased in AD, but not in MCI, compared with CN participants [1]. Additionally, there was significant overlap in serum NFL levels between the groups. Notably, the Bacioglu's study had a small sample size including 35 CN, 33 MCI and 34 AD and heterogeneous MCI participants includ-

ing both amnestic MCI and non-amnestic MCI. In the current study, we sought to replicate the previous findings in a larger sample, and also to differentiate amnestic from non-amnestic individuals with MCI. Our results showed significantly different plasma NFL levels between diagnostic groups (AD > aMCI > CN) but with substantial overlap between the groups.

Our findings have several critical implications. First, this overlap between the groups reduces the possibility of utilizing plasma NFL as a diagnostic test. However, further investigations are needed to examine whether plasma NFL may be used as a preliminary screening test (for instance, perhaps together with other biomarkers in a biomarker panel). Second, the higher NFL levels could be caused by the increased neuronal damage, rather than a diagnostic biomarker. Further longitudinal studies are needed to confirm the utility of plasma NFL as a neuropathological or prognostic biomarker for AD. It also should be noted that abnormal NFL levels in blood were not only found in AD but also in other neurological disorders, such as multiple sclerosis [8], clinically isolated syndrome [2], amyotrophic lateral sclerosis [3], frontotemporal dementia [12], Guillain Barré syndrome [3], spinal cord injury [9], and others, suggesting that blood NFL levels may not be disease-specific. Finally, the increase could be related with the aggravated neuronal damages associated with cognitive impairment severity, rather than a diagnostic biomarker. Plasma NFL levels may be a potentially useful biomarker for detecting changes in response to treatment, rather than a biomarker for diagnosis because of its expression detected among numerous neurodegenerative diseases. Further investigations are needed to clarify this matter.

In this study, we also found that plasma NFL was positively associated with age independent of diagnostic status. In agreement with our findings, previous studies suggested that age was correlated with NFL levels in CSF [4,7,14], which were closely related with NFL levels in blood [3]. However, the mechanisms underlying this age-NFL association were largely unknown. Perhaps it could be interpreted that the process of aging may affect the metabolism of NFL and axonal degeneration. Additionally, cerebrovascular pathologies may also be involved due to the fact that cerebrovascular changes were associated with both higher age and NFL levels [5]. Further investigations are warranted to clarify this association between age and NFL.

In our present study, plasma NFL was found to be associated with MMSE score in the aMCI and AD, but not in CN subjects. A ceiling effect of MMSE in cognitively normal elderly may explain this negative result. However, further studies with more sensitive or comprehensive neuropsychological tests will be needed to elucidate the exact relationship between plasma NFL levels and cognition in cognitively normal elderly.

A few limitations should be noted. First, it would be better to provide the standard curve by using standard samples in our current report, which may prove the reliability of the Single Molecule array (Simoa) technique in detecting plasma NFL. Unfortunately, the biomarker data in the ADNI datasets are from the different labs, which have only provided the simple assay methods and the results, without the exact experimental procedures. Therefore, the plasma NFL levels displayed in our present report may be inaccurate, which warrants further investigation to confirm our current findings. Second, plasma NFL levels were compared between diagnostic groups with nonparametric tests (Kruskal-Wallis and Mann-Whitney) because of the non-normal distribution of plasma NFL data. Therefore, potential covariates, such as age, were not adjusted. However, no significant difference in age was found between diagnostic groups in our study (Table 1), indicating that age was unlikely to confound our results.

In conclusion, our findings showed substantially different plasma NFL levels between diagnostic groups (AD > aMCI > CN) but with significant overlap between the groups, indicating that plasma NFL levels may not be a useful biomarker for the diagnosis of prodromal and dementia stages of AD.

Conflicts of interest

The authors declare that they have no conflict of interest.

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