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Plasma neurofilament light chain associated with impaired regional cerebral blood flow in healthy individuals

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Keywords
Neurofilament Light Chain; Alzheimer's Disease; Cognitive Dysfunction; Cerebrovascular Circulation

Abstract

Background: Recent findings suggest that the plasma axonal structural protein, neurofilament light (NFL) chain, may serve as a potential blood biomarker for early signs of neurodegenerative diseases, such as Alzheimer's disease (AD). Given the need for early detection of neurodegenerative disorders, the current study investigated the associations between regional cerebral blood flow (rCBF) in brain regions associated with neurodegenerative disorders and memory function with plasma NFL in AD, mild cognitive impairment (MCI), and healthy controls (HCs).

Methods: We recruited 29 AD, 76 MCI, and 39 HCs from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database in the current cross-sectional study. We used Pearson's correlation models adjusted for the effect of age, sex, and APOE genotype to investigate the association between plasma NFL and rCBF.

Results: We found non-significant differences in age ($F_{(2, 141)} = 1.304$; $P = 0.275$) and years of education ($F_{(2, 141)} = 0.013$; $P = 0.987$). Additionally, we found significant differences between groups in terms of MMSE scores ($F_{(2, 141)} = 100.953$; $P < 0.001$). Despite the observation of significantly reduced rCBF in AD and MCI groups versus HCs, we did not detect significant differences in plasma NFL between these groups. We found significant negative associations between plasma NFL and rCBF in various AD-related regions, these findings were only observed after analyses in all participants, and were observed in HCs alone and no significant associations were observed in the AD or MCI groups.

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Conclusion: These outcomes add to our current understanding surrounding the use of rCBF and plasma NFL biomarkers as tools for early detection and diagnosis of neurodegenerative diseases. A conclusion might be that the association between NFL and impaired rCBF exists before the clinical symptoms appear. Further longitudinal studies with a large sample size should be performed to examine the correlation between plasma NFL and rCBF in order to understand these complex relationships.

Introduction

Alzheimer's disease (AD) is a chronic, progressive neurological disorder marked by cognitive impairment, personality abnormalities, and memory loss.[1] Due to the exponential trajectory of new AD cases across the world, and the significant economic burden it has on global healthcare systems,[2] it is crucial to identify potential risk factors involved in the etiology of AD. Other milder forms of dementia, such as mild cognitive impairment (MCI), often precede the diagnosis of AD,[3-6] making it a prodromal sign for the development of AD, and thus, a potential target for early-stage identification and treatment. Therefore, it is vital to identify biomarkers associated with the etiology of neurodegenerative disorders, such as MCI and AD.

Given the importance of the early detection of AD for treatment, an emphasis has been placed on the use of biomarker assessments and therapy, especially for individuals in preclinical stages of dementia.[7] Several primary cerebrospinal fluid (CSF) candidate biomarkers have been used in these assessments, including total tau (T-tau), phosphorylated tau (P-tau), and amyloid beta-42 (Aβ), all of which have been shown to differentiate individuals with MCI and AD from healthy individuals.[8] Neurofilament light (NFL) chain, a structural protein reflecting neurodegenerative driven axonal damage,[9] has also been implicated as a CSF biomarker of AD.[10] However, unlike several CSF biomarkers, NFL can also be measured in blood plasma, with previous work observing increased levels of plasma NFL in individuals with neurodegenerative diseases, such as AD.[11] Plasma NFL levels can be used in combination with the CSF biomarkers of T-tau, P-tau, and Aβ to determine the specific stage of AD in patients.[12] As such, elevated levels of these CSF and plasma biomarkers often reflect neurodegeneration associated with AD.[13] In contrast to the invasive procedure of CSF collection, plasma biomarkers measurement is a less invasive procedure with lower costs, making it a suitable candidate for the identification of preclinical phases in neurodegenerative diseases.[14] Therefore, the use of plasma biomarkers in the identification of neurodegenerative diseases, such as AD and MCI, may hold considerable economic advantages over the more commonly used CSF biomarkers.

Thus, CSF and plasma molecular biomarkers yield robust indices of AD. Moreover, alterations in regional cerebral blood flow (rCBF), often used as an indicator of localized neuronal activity given its association with glucose metabolic activity within that specific region, has also recently been reported in individuals with late-onset AD.[15] Complementing these findings, Stomrud et al. have reported negative associations between rCBF and CSF levels of T-tau and P-tau in the medial frontal lobe, a region implicated in recognition memory function, and a positive association between CSF P-tau and rCBF in the left frontotemporal,[1] which encompasses structures involved in memory.[6] However, there was no significant association between the well-known AD biomarker, Aβ, and rCBF in their study.[1] These findings demonstrate an association between the rCBF and CSF biomarkers of AD, although the precise nature of this relationship appears to vary in specific brain regions. Nonetheless, these outcomes implicate rCBF changes as potential biomarkers for the detection and etiology of AD.

There is no evidence regarding the association between plasma NFL level as a newly emerged biomarker and rCBF changes which is an early pathological event in AD progress. Due to the growing promise of the use of plasma biomarkers, such as NFL, and rCBF as predictors of neurodegenerative diseases, we aimed to further evaluate their utility as early detection mechanisms of AD and MCI. In addition, we were interested in how alteration in rCBF within brain regions involved in cognitive functions was associated with plasma NFL. Importantly, we compared these issues between individuals with AD, individuals with MCI, and healthy controls (HCs). We isolated our analyses to regions of interest (ROIs) reported previously as being affected by AD.[16] We hypothesized that increased levels of plasma NFL would be associated with decreased rCBF in these brain regions. By identifying relationships between plasma NFL and rCBF, we aimed to expand the validity of using plasma NFL and rCBF as biomarkers for the detection of MCI and AD. Furthermore, our study

can improve our knowledge regarding the pathophysiological link between NFL and altered perfusion in AD progression and normal aging.

Materials and Methods

Data acquisition: The data used in this study were extracted from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI is a public-private partnership launched in 2003 led by Michael W. Weiner, MD. The primary goal of ADNI was to test whether the combination of serial magnetic resonance imaging (MRI), positron emission tomography (PET), biological markers, and clinical and neuropsychological assessments could be used to measure the progression of MCI and early AD. For up-to-date information see www.adni-info.org.

Participants: Data were obtained from participants in the ADNI cohort. Only participants with data containing plasma NFL levels, Mini-Mental State Exam (MMSE) scores, rCBF measures, and apolipoprotein E (Apo-E) genotype assessments outcome were included in the study. This resulted in a total of 144 (78 women; 71.1 ± 7.3) participants, divided into 3 groups: 29 diagnosed with AD, 76 with MCI, and 39 cognitively HCs. The AD participants were diagnosed according to the criteria for probable AD of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), MMSE score of 20-26 (inclusive), and Clinical Dementia Rating (CDR) score of 0.5 or 1.[17] All MCI participants were diagnosed as amnestic MCI based on the following criteria: MMSE score of 24-30, a memory complaint, objective memory loss based on education-adjusted scores measured by the Logical Memory II subtest of the Wechsler Memory Scale, a CDR score of 0.5, absence of significant impairment in other cognitive domains, and essentially preserved activities of daily living and absence of dementia.

Cognitive assessment: Participants' cognitive status was measured using the MMSE, which is a common assessment tool of cognitive functioning in aged individuals.[18] The MMSE assesses the language, memory, orientation, attention, and visual-spatial domains. Each patient's MMSE score was acquired from the ADNI Mini-Mental examination and used to determine group membership, as described above.

Plasma NFL measurement: Analyses of plasma NFL in the ADNI database were performed by Blennow and Zetterberg in the clinical neurochemistry laboratory of Gothenburg University, Sweden.[13] The single-molecule array (Simoa) technique, which incorporates a combination of purified bovine NFL and monoclonal antibodies as a calibrator was used.[19] All samples were measured in duplicate, except for one (due to technical reasons). The analytical sensitivity threshold was less than 1.0 pg/mL, and plasma NFL levels were not below the limit of detection in any sample.

APOE genotyping and CSF biomarkers assessment: Results concerning APOE genotyping of the ADNI database are publicly available (see http://adni.loni.usc.edu/methods/documents/). In our analyses, carriers with at least one ε4 allele were considered as positive for APOE ε4.

Measurement of rCBF: Quantitative maps of rCBF with regional analyses figures and perfusion-weighted images (PWI) were acquired from the ADNI database. Specifically, these were computed based on the Center for Imaging of Neurodegenerative Disease (CIND) processing pipeline for Arterial Spin Label (ASL) imaging. This pipeline includes the following critical steps: 1. motion correction of the ASL frames; 2. computation of PWIs by subtracting the mean of tagged from untagged ASL data sets; 3. adjustment of ASL and structural MRI data; 4. geometric distortion correction; 5. partial volume correction; and 6. CBF quantification in physical units by normalizing ASL to an estimated blood water density signal. The PWI and CBF images were corrected for EPI distortions in two representations. The first included native perfusion in MRI space, and the second involved subject-specific space of the corresponding structural MRI data. Further details concerning the processing pipeline and analyses for the ADNI database, including FSL tools, EPI nonlinear geometric distortion correction,[20] SPM8, Insight Toolkit (ITK),[21] Free Surfer, and in house MATLAB scripts, can be found online (see http://adni.loni.usc.edu/methods).

All rCBF analyses were isolated to ROIs identified in previous work as being implicated by AD.[16] Specifically, these ROIs include: the left and right hippocampus, cingulate, insula, entorhinal cortex, precuneus, fusiform, frontal poles, superior and inferior frontal gyri, the middle frontal cortex (including caudate-middle frontal cortex), pre- and post-central cortices, middle temporal gyrus, inferior parietal and temporal gyri, and the temporal cortices.

All statistical analyses were carried out using SPSS software (version 16, SPSS Inc., Chicago, IL, USA). First, we examined differences in demographics, clinical characteristics, plasma levels of NFL, and rCBF of each region of interest (ROI) between groups using separate one-way ANOVA and Kruskal-Wallis for parametric and non-parametric tests, respectively. Additional parametric group comparisons were carried out using t-test, and non-parametric group comparisons were conducted using Mann-Whitney U test. Finally, to assess the relationship between plasma NFL and rCBF in AD-related regions which were mentioned earlier, we conducted multiple Pearson's correlation models adjusted for the effect of age, sex, and APOE genotype once in each group, and then, among all participants. We defined covariates based on previous studies. We applied bootstrapping methods to correct for multiple comparisons in our correlation models.[22,23]

Results

Sample characteristics: Preliminary analyses were conducted to examine differences in demographic variables between the study groups (Table 1). Using two one-way ANOVAs, we found non-significant differences in age ($F_{(2, 141)}$ = 1.304; P = 0.275) and years of education ($F_{(2, 141)}$ = 0.013; P = 0.987). An additional one-way ANOVA revealed significant differences between groups in terms of MMSE scores ($F_{(2, 141)}$ = 100.953; P < 0.001), with Bonferroni post-hoc comparisons demonstrating non-significant differences in MMSE scores between the HC and MCI group (P = 0.314), but significantly lower MMSE scores in the AD versus HC (P < 0.001) and MCI groups (P < 0.001). The results of Kruskal–Wallis test illustrated significant differences in APOE ε4 allele count between groups ($F_{(2, 141)}$ = 7.908; P = 0.001). Follow-up Mann–Whitney U-tests demonstrated that the AD group contained significantly more APOE ε4 carriers than the MCI (P = 0.002) and HC (P = 0.001) groups, while the MCI and HC groups did not significantly differ in terms of APOE ε4 carriers (P = 0.482). There was no significant difference in plasma NFL between the groups ($F_{(2, 141)}$ = 0.736; P = 0.481).

rCBF differences between AD, MCI, and HC groups: Using one-way ANOVA analysis, we found significant differences in rCBF in the left entorhinal area ($F_{(2, 141)}$ = 3.617; P = 0.029), left ($F_{(2, 141)}$ = 5.967; P = 0.003) and right hippocampus ($F_{(2, 141)}$ = 3.900; P = 0.022), left middle temporal gyrus ($F_{(2, 130)}$ = 4.195; P = 0.017), and left inferior parietal lobule ($F_{(2, 141)}$ = 4.911; P = 0.009) between the groups (See Figure 1 for overview of all regions showing differences between groups). Post-hoc Bonferroni corrections found that subjects in the AD group demonstrated lower levels of rCBF in these regions compared to the MCI and HC groups, with the MCI also demonstrating lower levels of rCBF than the HC group (Table 2).

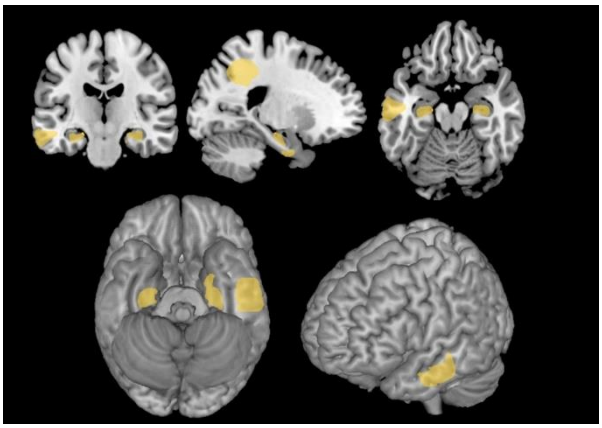

Figure 1. Significant difference of regional cerebral blood flow (rCBF) in regions of interest between groups

Table 1. Participant demographic and health variables

Variable	HCs (n = 39)	MCI (n = 76)	AD (n = 29)	P
Sex (M/F)	15/24	37/39	14/15	0.562
Age (years) (mean ± SD)	71.6 ± 6.9	70.2 ± 7.6	72.6 ± 6.8	0.275
Education (years) (mean ± SD)	16.3 ± 2.4	16.4 ± 2.7	16.4 ± 2.2	0.987
Plasma NFL (pg/ml) (mean ± SD)	38.8 ± 41.9	35.8 ± 16.9	42.5 ± 16.2	0.481
MMSE (mean ± SD)	29.0 ± 1.4	28.4 ± 1.6	23.7 ± 1.9	< 0.001*
APOE genotype				0.001*
Without ε4	27	49	9	
One ε4	11	20	13	
Two ε4	1	7	7	

Mean values with standard deviation (i.e., ± SD) are shown for each variable. APOE genotypes include total subject count for each group.
NFL: Neurofilament light
*Significant level

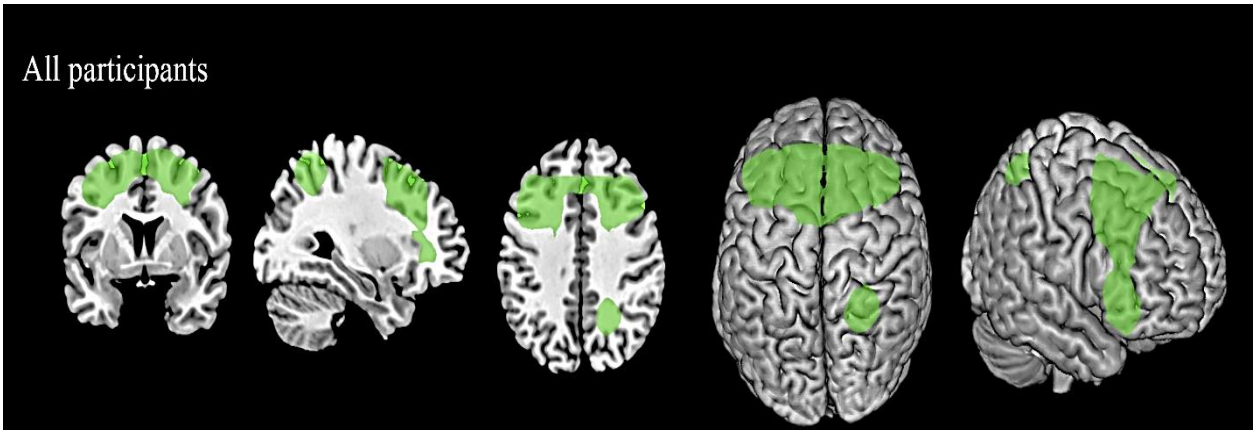

Figure 2. Significant correlation between plasma NFL and regional cerebral blood flow (rCBF) in regions of interest among all participants

Table 2. Significant differences in regional cerebral blood flow (rCBF) between groups

ROI	df	F	P
Left entorhinal area	2, 141	3.617	0.029
Left hippocampus	2, 141	5.967	0.003
Gray matter of left middle temporal gyrus	2, 130	4.195	0.017
Right hippocampus	2, 141	3.900	0.022
Gray matter of left inferior parietal lobule	2, 141	4.911	0.009

Representation of significant ROI form one-way ANOVA analysis.
ROI: Region of interest; df: Degree of freedom

Correlation between biomarkers and rCBF among all participants: We observed significant negative correlations between plasma levels of NFL and rCBF in the various brain regions, including inferior parietal lobule, right middle temporal gyrus, rostral and caudal part of the left and right middle frontal gyrus, triangular and orbital part of the right inferior frontal gyrus, and right and left superior frontal gyrus, in the three groups (Table 3). An overview of all regions' rCBF associated with plasma NFL can be seen in figure 2.

Correlation between biomarkers and rCBF within each group: In our within-group analyses, we found significant negative correlations between plasma levels of NFL and rCBF in the right middle and superior temporal gyrus, triangular part of the right inferior frontal gyrus, right and left posterior cingulate gyrus, caudal part of the left and right middle frontal and rostral part of the right middle frontal, and left superior frontal gyrus in the HC group (Table 4).

An overview of rCBF and plasma NFL associations in the HC group can be seen in figure 3. However, we did not find any significant correlations between rCBF and plasma levels of NFL in the MCI and AD group.

Table 3. Significant partial correlation outcomes of regional cerebral blood flow (rCBF) and plasma neurofilament light (NFL) across all participants

ROI	Coefficient	P
Left inferior parietal lobule	-0.197*	0.019
Rostral part of left middle frontal gyrus	-0.179*	0.036
Right superior temporal gyrus	-0.194	0.027
Right middle temporal gyrus	-0.194*	0.027
Orbital part of right inferior frontal gyrus	-0.204*	0.015
Triangular part of right inferior frontal gyrus	-0.200*	0.017
Caudal part of left middle frontal gyrus	-0.224**	0.009
Left superior frontal gyrus	-0.172*	0.044
Caudal part of right middle frontal gyrus	-0.217*	0.011
Rostral part of right middle frontal gyrus	-0.202*	0.018
Right superior frontal gyrus	-0.190*	0.026

Representation of significant ROI form one-way ANOVA analysis
Analysis adjusted for the effect of age, sex, and APOE genotype
NFL: Neurofilament light; ROI: Region of interest
*P < 0.05, **P < 0.01

Table 4. Significant partial correlation outcomes of regional cerebral blood flow (rCBF) and plasma neurofilament light (NFL) in the healthy control group

ROI	Coefficient	P
Right middle temporal gyrus	-0.360*	0.036
Triangular part of right inferior frontal gyrus	-0.381*	0.022
Right posterior cingulate gyrus	-0.483**	0.003
Right superior temporal gyrus	-0.392*	0.022
Caudal part of right middle frontal gyrus	-0.360	0.031
Caudal part of left middle frontal gyrus	-0.369*	0.027
Left superior frontal gyrus	-0.339*	0.043
Rostral part of right middle frontal gyrus	-0.380*	0.022
Left posterior cingulate gyrus	-0.497**	0.002

Representation of significant ROI form one-way ANOVA analysis
Analysis adjusted for the effect of age, sex, and APOE genotype
NFL: Neurofilament light; ROI: Region of interest
*P < 0.05, **P < 0.01

Discussion

Reduced rCBF is seen in patients with AD as one of the earliest events.[24,25] Previous reports suggested that reduced rCBF affects Na/K pump functionality, electrochemical maintenance of resting potential, and glutamate reuptake.[26] These alterations induce several negative outcomes, including an imbalance in protein synthesis and degradation.[27] Reduced rCBF is frequently found in AD patients prior to grey matter loss[28] and Aβ accumulation,[29] making it a potential candidate for the early detection and diagnosis of AD. Previous work has also found that increased plasma NFL levels correlate with future brain tissue loss, reduced brain metabolism, and cognitive impairment.[30] Thus, the current study examined the ADNI database to investigate the associations between rCBF in brain regions associated with AD and plasma levels of NFL within AD, MCI, and HC groups.

Consistent with our first hypothesis, we found significant differences in rCBF between groups in regions commonly implicated in AD.[22] Specifically, these regions included the left entorhinal cortex, left and right hippocampi, left middle temporal gyrus, left inferior parietal lobule, and right parahippocampal gyrus. HCs displayed greater rCBF in these regions compared to the MCI and AD groups, while the AD group showed less rCBF than the MCI group. Others have also found lower rCBF in the hippocampus and temporal brain regions in AD patients compared to HCs.[31] Alterations in rCBF have been proposed as one of the earliest detectable biomarkers contributing to the development of AD. While previous work has identified decreased rCBF in AD pathology, others have found increases in rCBF, particularly in the hippocampus, in patients with AD.[32,33] Here, we found decreased rCBF in regions involved in cognitive function including the hippocampus, and middle temporal, entorhinal, and inferior parietal lobule in the MCI and AD groups compared to HCs. Importantly, rCBF disruptions in these regions yield reduced vascular clearance, and the formation of Aβ and neurofibrillary tangles, which can lead to atrophy and neurodegeneration.[33]

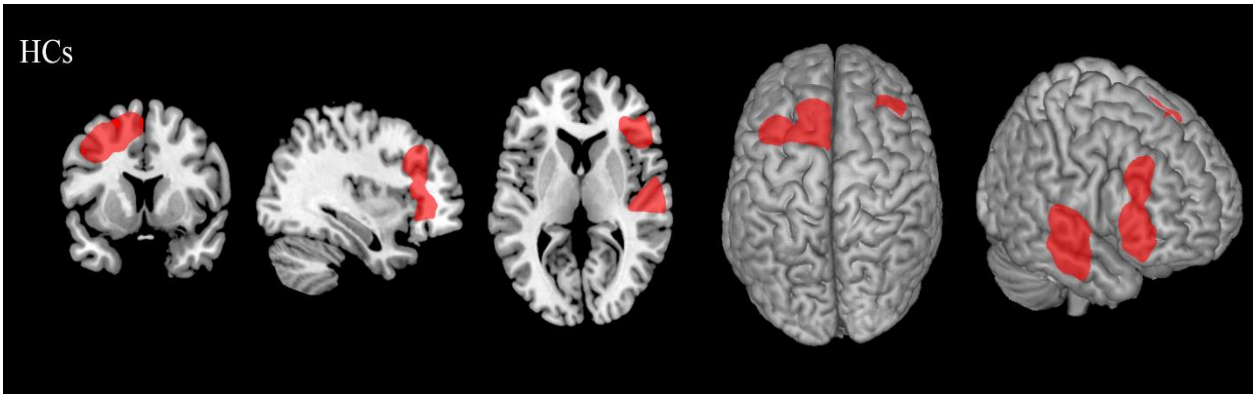

Figure 3. Significant correlation between plasma NFL and regional cerebral blood flow (rCBF) in regions of interest in healthy controls (HCs)

Together, these findings provide additional evidence that rCBF decreases in cortical and subcortical regions, such as the hippocampus, parahippocampal gyrus, middle temporal gyrus, parietal lobule, and entorhinal cortex, all of which are major areas for hypometabolism and atrophy, and are involved in the development of neurodegenerative diseases such as AD.[34-36]

Somewhat consistent with our hypotheses concerning plasma NFL and rCBF, we observed significant negative correlations between these measures in several ROIs, including the left inferior parietal lobule, the rostral part of the left and right middle frontal gyri, right superior temporal gyrus, right middle temporal gyrus, orbital part of the right inferior frontal gyrus, triangular part of the right inferior frontal gyrus, the caudal part of the left and right middle frontal gyri, left and right superior frontal gyri, and the rostral part of the right middle frontal. Interestingly, these outcomes were observed across all participants, regardless of being classified into groups. Contrary to our group-specific predictions regarding rCBF and plasma NFL, we did not observe significant relationships between these variables in our AD and MCI groups. However, we found negative associations between rCBF and plasma NFL in the HC group in the right middle temporal gyrus, triangular part of the right and left inferior frontal gyri, right posterior cingulate gyrus, right superior temporal gyrus, caudal part of the right and left middle frontal gyri, the rostral part of the right middle frontal gyrus, and the left posterior cingulate gyrus.

The pathogenic roles of the above reported ROIs, and the networks they constitute, have previously been established.[31,37,38] For instance, previous studies found hypoperfusion in the cingulate cortex[39] and reduced blood supply in temporal brain regions to be predictors of AD in patients with MCI and patients with AD,[40] respectively. Our findings indicated a significant negative correlation between plasma NFL and rCBF in the middle temporal gyrus. The middle temporal gyrus is involved in different tasks such as visual perception, and language and semantic memory processing.[41] Moreover, we detected an association between plasma NFL and several parts of the frontal gyrus which has important roles in executive function, attention, memory, and language.[42] Moreover, we assumed that a similar result for the posterior cingulate based on our study improves the diagnostic role of plasma NFL as posterior cingulate is believed to have a central role in cognition direction within the brain and is a key node in the default mode network.[43] However, we did not find significant correlations between plasma NFL levels and rCBF changes in either AD or MCI groups. Maybe the association between NFL and altered rCBF is limited to the normal aging process, and the beginning of pathological neurodegenerative mechanisms are involved in rCBF decline in AD and MCI patients. Although the association of NFL and rCBF is possible in AD development, other mechanisms related to neurodegeneration cannot be excluded. Therefore, future research should further examine such associations to evaluate the relation of rCBF with NFL considering the other pathological factors leading to MCI and AD.

NFL chain is an intermediate filament protein of the subcortical axons involved in the maintenance and assembly of the neuronal cytoskeleton.[44] As a result of neuronal injury, plasma NFL is released into the CSF, and then, into the blood.[45] An elevated level of NFL has been reported in various neurodegenerative diseases such as frontotemporal dementia (FTD), vascular dementia (VaD), and AD.[46] However, the 2018 Research Framework of the National Institute on Aging and Alzheimer's Association (NIA-AA) demonstrated that NFL is not a specific biomarker for AD neurodegeneration, but it is a reflection of synaptic loss which is strongly correlated with AD symptoms.[47] Furthermore, previous studies found that CSF NFL is associated with survival and disease severity in AD.[48] Recently, NFL levels in the blood have been used to assess stages of neurodegeneration in AD patients.[30] In comparison to more invasive procedures, plasma biomarkers are less expensive and more easily available.[49]

A study by Mayeli et al. revealed that plasma and CSF NFL are associated with hypometabolism in AD signature regions among MCI patients.[50] They found that higher levels of plasma NFL correlated with hypometabolism in bilateral parahippocampal and middle temporal gyri. In addition, another study reported that NFL in both CSF and plasma is related to lower metabolism, hippocampal atrophy, and poor cognitive function.[51] There is no evidence regarding the association of CSF NFL and rCBF to date.

Regarding our plasma NFL predictions, we failed to identify significant differences in this plasma biomarker between the study groups. Previous studies using a similar dataset found a significant difference in this plasma biomarker in contrast to

our results.[52,53] A reason for this can be the lower number of subjects in our study due to enrolling patients with available ASL-MRI results, which considerably limited our sample size. Moreover, maybe the lack of association between rCBF and plasma NFL in the MCI and AD subjects of our study is the result of the small sample size. Finding significant results in all participants regardless of their group may also be driven by HCs.

Our study has some limitations. We had a small sample size due to the limited number of participants with available rCBF data in the ADNI cohort. Moreover, only a few subjects had longitudinal ASL-MRI in the ADNI. Therefore, we conducted our study with a cross-sectional design which did not allow us to examine longitudinal changes in rCBF in relation to plasma level NFL. We did not include the CSF NFL level of participants as few subjects had the data.

Conclusion

The current study complements prior work showing reduced rCBF in regions commonly associated with cognitive function in individuals with AD and MCI compared to HCs. Furthermore, we add to the evidence supporting a relationship between plasma NFL and altered rCBF in AD-related regions. However, these findings appeared to be isolated to HCs alone. A conclusion might be that the association between NFL and impaired rCBF exists before the clinical symptoms appear. These outcomes add to our current understanding surrounding the use of rCBF and plasma NFL biomarkers as tools for early detection and diagnosis of neurodegenerative diseases. Further longitudinal studies with a large sample size should be performed to examine the correlation between plasma NFL and rCBF in order to understand these complex relationships.

Conflict of Interests

The authors declare no conflict of interest in this study.

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Since the data in this paper were obtained from the ADNI database (adni.loni.usc.edu), it does not include any research involving human or animal subjects.

References

1. Stomrud E, Forsberg A, Hagerstrom D, Ryding E, Blennow K, Zetterberg H, et al. CSF biomarkers correlate with cerebral blood flow on SPECT in healthy elderly. Dement Geriatr Cogn Disord 2012; 33(2-3): 156-63.
2. Meek PD, McKeithan K, Schumock GT. Economic considerations in Alzheimer's disease. Pharmacotherapy 1998; 18(2 Pt 2): 68-73.
3. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7(3): 270-9.
4. Kelley BJ, Petersen RC. Alzheimer's disease and mild cognitive impairment. Neurol Clin 2007; 25(3): 577-609, v.
5. Mimura M, Yano M. Memory impairment and awareness of memory deficits in early-stage Alzheimer's disease. Rev Neurosci 2006; 17(1-2): 253-66.
6. Pennanen C, Kivipelto M, Tuomainen S, Hartikainen P, Hanninen T, Laakso MP, et al. Hippocampus and entorhinal cortex in mild cognitive impairment and early AD.

Neurobiol Aging 2004; 25(3): 303-10.
7. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. J Neuropathol Exp Neurol 2012; 71(4): 266-73.
8. Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis. Lancet Neurol 2016; 15(7): 673-84.
9. Khalil M, Pirpamer L, Hofer E, Voortman MM, Barro C, Leppert D, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat Commun 2020; 11(1): 812.
10. Forgrave LM, Ma M, Best JR, DeMarco ML. The diagnostic performance of neurofilament light chain in CSF and blood for Alzheimer's disease, frontotemporal dementia, and amyotrophic lateral sclerosis: A systematic review and meta-analysis. Alzheimers Dement (Amst) 2019; 11: 730-43.
11. Ashton NJ, Leuzy A, Lim YM, Troakes C, Hortobagyi T, Hoglund K, et al. Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. Acta Neuropathol Commun 2019; 7(1): 5.
12. de Wolf F, Ghanbari M, Licher S, McRae-McKee K, Gras L, Weverling GJ, et al. Plasma tau, neurofilament light chain and amyloid-beta levels and risk of dementia; a population-based cohort study. Brain 2020; 143(4): 1220-32.
13. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: Current status and prospects for the future. J Intern Med 2018; 284(6): 643-63.
14. Altuna-Azkargorta M, Mendioroz-Iriarte M. Blood biomarkers in Alzheimer's disease. Neurologia (Engl Ed) 2021; 36(9): 704-10.
15. Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Perez JM, Evans AC. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. Nat Commun 2016; 7: 11934.
16. Pini L, Pievani M, Bocchetta M, Altomare D, Bosco P, Cavedo E, et al. Brain atrophy in Alzheimer's Disease and aging. Ageing Res Rev 2016; 30: 25-48.
17. Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, Harvey DJ, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): Clinical characterization. Neurology 2010; 74(3): 201-9.
18. Arevalo-Rodriguez I, Smailagic N, Roque-Figuls M, Ciapponi A, Sanchez-Perez E, Giannakou A, et al. Mini-Mental State Examination (MMSE) for the early detection of dementia in people with mild cognitive impairment (MCI). Cochrane Database Syst Rev 2021; 7(7): CD010783.
19. Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, et al. Plasma tau in Alzheimer disease. Neurology 2016; 87(17): 1827-35.
20. Tao R, Fletcher PT, Gerber S, Whitaker RT. A variational image-based approach to the correction of susceptibility artifacts in the alignment of diffusion weighted and structural MRI. Inf Process Med Imaging 2009; 21: 664-75.
21. Yoo TS, Ackerman MJ, Lorensen WE, Schroeder W, Chalana V, Aylward S, et al. Engineering and algorithm design for an image processing Api: A technical report on ITK--the Insight Toolkit. Stud Health Technol Inform 2002; 85: 586-92.
22. Wang FK, Yang SW. Applying Bootstrap method to the types III errors in the measurement system. Qual Reliab Engng Int 2008; 24(1): 83-97.
23. Parra-Frutos I. Controlling the Type I error rate by using the nonparametric bootstrap when comparing means. Br J Math Stat Psychol 2013; 67(1): 117-32.
24. Asllani I, Habeck C, Scarmeas N, Borogovac A, Brown TR, Stern Y. Multivariate and univariate analysis of continuous arterial spin labeling perfusion MRI in Alzheimer's disease. J Cereb Blood Flow Metab 2008; 28(4): 725-36.
25. Korte N, Nortley R, Attwell D. Cerebral blood flow decrease as an early pathological mechanism in Alzheimer's disease. Acta Neuropathol 2020; 140(6): 793-810.
26. Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 2001; 21(10): 1133-45.
27. Thiebaut AM, Hedou E, Marciniak SJ, Vivien D, Roussel BD. Proteostasis During Cerebral Ischemia. Front Neurosci 2019; 13: 637.
28. Mattsson N, Tosun D, Insel PS, Simonson A, Jack CR, Beckett LA, et al. Association of brain amyloid-beta with cerebral perfusion and structure in Alzheimer's disease and mild cognitive impairment. Brain 2014; 137(Pt 5): 1550-61.
29. Wierenga CE, Hays CC, Zlatar ZZ. Cerebral blood flow measured by arterial spin labeling MRI as a preclinical marker of Alzheimer's disease. J Alzheimers Dis 2014; 42 Suppl 4(Suppl 4): S411-9.
30. Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. JAMA Neurol 2019; 76(7): 791-9.
31. Zheng W, Cui B, Han Y, Song H, Li K, He Y, et al. Disrupted regional cerebral blood flow, functional activity and connectivity in Alzheimer's disease: A combined ASL perfusion and resting state fMRI Study. Front Neurosci 2019; 13: 738.
32. Alsop DC, Casement M, de Bazelaire C, Fong T, Press DZ. Hippocampal hyperperfusion in Alzheimer's disease. Neuroimage 2008; 42(4): 1267-74.
33. Zhang N, Gordon ML, Goldberg TE. Cerebral blood flow measured by arterial spin labeling MRI at resting state in normal aging and Alzheimer's disease. Neurosci Biobehav Rev 2017; 72: 168-75.
34. Park KW, Yoon HJ, Kang DY, Kim BC, Kim S, Kim JW. Regional cerebral blood flow differences in patients with mild cognitive impairment between those who did and did not develop Alzheimer's disease. Psychiatry Res 2012; 203(2-3): 201-6.
35. Rondina JM, Ferreira LK, de Souza Duran FL, Kubo R, Ono CR, Leite CC, et al. Selecting the most relevant brain regions to discriminate Alzheimer's disease patients from healthy controls using multiple kernel learning: A comparison across functional and structural imaging modalities and atlases. Neuroimage Clin 2018; 17: 628-41.
36. Horie K, Barthelemy NR, Sato C, Bateman RJ. CSF tau microtubule binding region identifies tau tangle and clinical stages of Alzheimer's disease. Brain 2021; 144(2): 515-27.
37. Chen B. Abnormal cortical regions and subsystems in whole brain functional connectivity of mild cognitive impairment and Alzheimer's disease: A preliminary study. Aging Clin Exp Res 2021; 33(2): 367-81.
38. Yu E, Liao Z, Mao D, Zhang Q, Ji G, Li Y, et al. Directed functional connectivity of posterior cingulate cortex and whole brain in alzheimer's disease and mild cognitive impairment. Curr Alzheimer Res 2017; 14(6): 628-35.
39. Huang C, Wahlund LO, Svensson L, Winblad B, Julin P. Cingulate cortex hypoperfusion predicts Alzheimer's disease in mild cognitive impairment. BMC Neurol 2002; 2: 9.
40. Eberling JL, Jagust WJ, Reed BR, Baker MG. Reduced temporal lobe blood flow in Alzheimer's disease. Neurobiol Aging 1992; 13(4): 483-91.
41. Onitsuka T, Shenton ME, Salisbury DF, Dickey CC, Kasai K, Toner SK, et al. Middle and inferior temporal gyrus gray matter volume abnormalities in chronic schizophrenia: An MRI study. Am J Psychiatry 2004; 161(9): 1603-11.
42. Chayer C, Freedman M. Frontal lobe functions. Curr Neurol Neurosci Rep 2001; 1(6): 547-52.
43. Leech R, Sharp DJ. The role of the posterior cingulate cortex in cognition and disease. Brain 2014; 137(Pt 1): 12-32.
44. Xiong YL, Meng T, Luo J, Zhang H. The potential of neurofilament light as a biomarker in Alzheimer's disease. Eur Neurol 2021; 84(1): 6-15.
45. Lin YS, Lee WJ, Wang SJ, Fuh JL. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. Sci Rep 2018; 8(1): 17368.
46. Zhao Y, Xin Y, Meng S, He Z, Hu W. Neurofilament light chain protein in neurodegenerative dementia: A systematic review and network meta-analysis. Neurosci Biobehav Rev 2019; 102: 123-38.
47. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018; 14(4): 535-62.

48. Skillback T, Farahmand B, Bartlett JW, Rosen C, Mattsson N, Nagga K, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology 2014; 83(21): 1945-53.
49. Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, Malaspina A, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS One 2013; 8(9): e75091.
50. Mayeli M, Mirshahvalad SM, Aghamollaii V, Tafakhori A, Abdolalizadeh A, Rahmani F. Plasma neurofilament light chain levels are associated with cortical hypometabolism in Alzheimer disease signature regions. J Neuropathol Exp Neurol 2019; 78(8): 709-16.
51. Chen Y, Therriault J, Luo J, Ba M, Zhang H, Initiative ADN. Neurofilament light as a biomarker of axonal degeneration in patients with mild cognitive impairment and Alzheimer's disease. J Integr Neurosci 2021; 20(4): 861-70.
52. Hu H, Chen KL, Ou YN, Cao XP, Chen SD, Cui M, et al. Neurofilament light chain plasma concentration predicts neurodegeneration and clinical progression in nondemented elderly adults. Aging (Albany NY) 2019; 11(17): 6904-14.
53. Benedet AL, Leuzy A, Pascoal TA, Ashton NJ, Mathotaarachchi S, Savard M, et al. Stage-specific links between plasma neurofilament light and imaging biomarkers of Alzheimer's disease. Brain 2020; 143(12): 3793-804.