Plasma neurofilament light, CSF Aβ, total tau, and p tau 181 is associated with altered cerebral blood flow across Alzheimer's disease spectrum

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Abstract

Neurofilament light (NFL) protein NFL is an axonal structural protein and a marker of neuroaxonal damage which is a consequence of neurologic diseases. Also, a decrease of the Regional cerebral blood flow (rCBF) is one of the earliest changes in AD patients. In line with the rising importance of the blood biomarkers and cerebral blood flow as a predictor of the onset of the AD, we investigated that whether there is a correlation between plasma NFL, CSF level of T tau, P tau, and Aβ, and the decrease of the blood flow in the several regions of the brain. We performed our analysis on three groups of subjects including AD (n=29), mild cognitive impairments (n=76), and cognitively healthy controls (n=39). Our results showed a significant correlation between plasma or CSF biomarkers with altered rCBF in different regions within groups. Our findings revealed that NFL is associated with rCBF, therefore, our results support the application of plasma NFL and other CSF biomarkers as a diagnosis tools for AD and neurodegenerations.

Introduction

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder that occurs in older patients and is characterized by symptoms such as cognitive dysfunction, personality changes and loss of memory (1). Due to the prospect of disease modification, the use of the biomarkers increased remarkedly in order to diagnose forms of dementia earlier in the preclinical stages of the disease (2). The core CSF biomarkers are total tau (T tau), phosphorylated tau (P tau), Amyloid beta (Ab 42), CSF neurofilament light (NFL) protein, and plasma T tau. These biomarkers were associated with mild cognitive impairment (MCI) (3). NFL is an axonal structural protein and a marker of neuro axonal damage which is a consequence of neurologic diseases (4). CSF levels of NFL can distinguish AD, frontotemporal dementia (FTD), and Amyotrophic lateral sclerosis (ALS) from cognitively unimpaired subjects (5). NFL can be also measured in the plasma, making it useful for repeated assessments, and is elevated considerably in the blood in many neurological disorders, including AD (6). According to recent study plasma levels of tau, NFL, and amyloid b can be used to define and stage AD (7). beside NFL , other CSF biomarkers that mentioned above are associated with the pathogenesis of the AD. CSF values of P tau, T tau, and Ab can improve the early diagnosis of the disease in the MCI patients (8).

Regulation of the cerebral blood flow is essential for normal function of the brain. Decrease of the Regional cerebral blood flow (rCBF) is one of the earliest changes in AD patients (9). Although it is unclear that the decrease of the rCBF is whether a cause or a consequence of AD, Some researchers hypothesize that there is a correlation between ischemic episodes and AD (10). Some studies revealed that there is a significance correlation between CSF tau protein and cerebral blood flow abnormalities (1).

The number of the patients with AD is rising each year and it is an economic burden on the health care system (11). At this situation the role of plasma biomarkers such as plasma NFL are crucial. Due to the noninvasiveness and lower costs of the blood tests compared to more invasive CSF puncture methods, plasma biomarkers can lessen the costs of the disease and predict the disease at the preclinical stages

(12). In line with the rising importance of the blood biomarkers and cerebral blood flow as a predictor of the onset of the AD we investigated that whether there is a correlation between plasma NFL, CSF level of T tau, P tau, and Aβ, and the decrease of the blood flow in the several regions of the brain. As our hypothesis plasma NFL, CSF level of T tau, P tau might correlate with altered rCBF across Alzheimer's disease spectrum.

Materials And Methods

Data acquisition

Data used in the provision of this paper were extracted from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was funded in 2003 as a public-private partnership, led by Michael W. Weiner, MD. The primary goal was to test that weather the combination of serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment could be used to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). We entered a population of subjects consists of 29 AD,76 MCI and 39 cognitively healthy. We enrolled all subjects which all required variables were available. According to the Stroke–Alzheimer's Disease and Related Disorders Association and National Institute of Neurological and Communicative Disorders Clinical Dementia Rating and MMSE scores are the factors for considering subjects as MCI, AD or CN (13).

Plasma NFL measurement

Analysis of plasma neurofilamentlight (NFL) on ADNI-1 samples performed by Blennow K in clinical neurochemistry laboratory of Gothenburg University, Sweden. He used single molecule array (Simoa) technique. The assay uses a combination of purified bovine NFL , monoclonal antibodies as a calibrator. All samples were measured in duplicate, except for one (due to technical reasons). Analytical sensitivity was <1.0 pg/mL, and plasma level of NFL wasn't below the limit of detection in any sample.

Apoe genotyping and CSF biomarkers assessment

Results for Apoe genotyping are available at ADNI. Careers are participants with at least one £4 allele. CSF collected samples were acquired by lumbar puncture and concentration of CSF biomarkers consist of T-tau, P-tau181 and Aβ1-42 have been measured by the micro-bead-based multiplex immunoassay, the INNO-BIA AlzBio3 RUO test (Fujirebio, Ghent, Belgium) (14), on the Luminex platform. All the details about CSF specimen collection and analytic measurement, are available at ADNI http://adni.loni.usc.edu/methods/documents/.)

rCBF measurement

The center for imaging of neurodegenerative disease (CIND) processing pipeline for Arterial Spine Label (ASL) imaging, provides perfusion-weighted images (PW1) and figures a quantitative map of cerebral blood flow (CBF) and a regional analysis. It is necessary to implant multiple instruments from the public domain to achieve the quantification of CBF and discrepancy to high-resolution anatomical MRI data such as various FSL tools, EPI nonlinear geometric distortion correction (15), SPM8, Insight Toolkit (ITK) (16), Free Surfer and in house MATLAB scripts.

The pipeline consummates 1) motion correction of the ASL frames, 2)computation of the PWI 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, 6) and CBF quantification in physical units by normalizing ASL to an estimated blood water density signal.

At the end ultimately there are two achievements consists of the PWI and a CBF image which both of them corrected for EPI distortion in two representations: 1) in native perfusion MRI space, 2) in the subject-specific space of the corresponding structural MRI data. Each achievement suggests users a manifold of options for more processing and analysis of the data.

Upon the interest to know the details you can visit the ADNI (http://adni.loni.usc.edu/methods)

Cogntive assessment

Cognitive condition of subjects estimated by Mini-Mental State Exam (MMSE), which is a common test to assess the cognitive function in the aged individuals . Language, memory, orientation, attention and visual-spatial are the skills that MMSE test measures. Each patient's MMSE score acquired from ADNI Mini-Mental examination.

Statistical analysis

Statistical analysis carried out using SPSS16. Non normal distributed variables log transformed for using parametric analysis. Demographical variables and rCBF difference between groups assessed by one way ANOVA. For comparison results we used Benjamini Hochberg correction for addressing type I error due to multiple comparisons. In the next step for investigation the linear relation between CSF or plasma biomarkers with rCBF, once among all subject and then within each group, we implemented the Pearson’s correlation models by entering CSF or plasma biomarkers, and rCBF in each region as variables, adjusted

for age, sex and APOE genotype as covariates. Bootstrapping method were used due to multiple comparisons in correlation models.

Results

Sample characteristics

Participants characteristics details, Cognitive scores, APOE genotyping shown in Table1. No group differences in age, education, and sex was detected. AD group had more APOE ε4 carriers than other groups. As expected, significant different in MMSE score was found between groups (AD<MCI<CN, P value<0.001).

rCBF different between groups

Results of one way ANOVA after correction showed a significant difference in cerebral blood flow in left entorhinal area, left and right hippocampus, left middle temporal gyrus, left inferior parietal lobule, and right parahippocampal gyrus (AD<MCI<CN). Details shown in Table2.

Biomarkers with rCBF among all participants

There is significant correlation between blood flow in different regions and plasma NFL levels among all participants. Results of partial correlation shown in Table3. Plasma NFL correlates with lower blood flow in inferior parietal lobule, right inferior temporal gyrus, right middle temporal gyrus, anterior and posterior part of left and right middle frontal gyrus, triangular and orbital part of right inferior frontal gyrus, right and left superior frontal gyrus (Tab3).

CSF Aβ was positively correlate with blood flow in right and left inferior temporal gyrus, and left middle temporal gyrus (Tab3). Also as described in Table3 CSF levels of total tau was associated with right lingual gyrus, and Central corpus callosum. As our analysis there is no correlation between CSF p tau 181 and cerebral blood flow among all participants (Tab3). However, pattern of changes due to total tau and p tau 181 were closely similar.

Biomarkers with rCBF within each group

We observed that higher CSF Aβ was significantly correlate with higher blood flow in right frontal pole and CSF p tau 181 negatively correlate with blood flow in Optic chiasm within AD group (Tab4). The correlation models failed to reach significance for CSF total tau and plasma NFL in AD group.

Analysis among MCI group revealed a strong relation between CSF total tau and p tau 181 with blood flow in several regions (Tab4). CSF total tau and p tau 181 was positively correlate with blood flow in left cuneus, left pericalcarine, and Central corpus callosum (Tab4). Moreover there is strong correlation between CSF p tau 181 and blood flow in left postcentral gyrus, right cuneus, right lingual gyrus, and caudal part of left anterior cingulate gyrus in addition to previuos regions (Tab4). Plasma NFL and CSF Aβ did not show any significant correlation with rCBF in MCI patients.

Partial correlation model adjusted for age, sex, and APOE genotyping revealed a significant correlation between plasma NFL and altered blood flow in widespread regions (Tab4). Higher Plasma NFL was associated with lower blood flow in right middle and superior temporal gyrus, triangular and orbital part of right inferior frontal gyrus, right and left posterior cingulate gyrus, posterior part of left middle and anterior part of right middle frontal gyrus, left superior frontal gyrus, and Right vessel CN group (Tab4). There is no meaningful correlation between regional cerebral blood flow and CSF biomarkers including Aβ, total tau, and p tau 181 among healthy participants (Tab4).

Discussion

In a cross sectional study based on ADNI cohort, we investigate the association between plasma levels of neurofilament light protein, some other CSF biomarkers, and regional cerebral blood flow in three groups of objects consists of (CN, MCI, AD). Exclusive of grouping analysis showed a significant correlation between plasma and CSF biomarkers with rCBF changes in broad regions of the brain.

Regional cerebral blood flow decreases by up to 50% in patients with AD (17, 18). This reduction in the rCBF affects the normal function of the Na/K pump, maintenance of the resting potential level and, glutamate uptake (19). subsequently, we see various changes in cell biology including imbalance protein synthesis and degradation (20).previous studies revealed that reduced rCBF is a consequence of declining the number of blood vessels as well as decreasing the diameter of the vessels (18). according to the findings of N.Mattsson et al, the functional and synaptic loss is one of the earliest events in the Alzheimer’s pathology and cane lead to reduced rCBF even before considerable grey matter loss (21). it is suggested that changes in the rCBF are present earlier than Ab accumulation (22), meanwhile it is unclear that whether the reduction in the rCBF initiates the amyloid cascade or caused by amyloid production (18). rCBF change in the MCI group can predict the chance of conversion to AD during the time (1). Our one-way ANOVA analysis found a reduction of the CBF in the AD group compared to the normal subjects in the left entorhinal, left and right hippocampus, left middle temporal gyrus, left inferior parietal lobule, and right parahippocampal gyrus. Our findings are in line with Zheng et al. which showed that the CBF is different in the left posterior cingulated cortex, the left and right dorsolateral prefrontal cortex, the left inferior parietal lobule, the right middle temporal gyrus, the left middle occipital gyrus, and the left precuneus between AD patients and normal subjects (23). We found that CBF in MCI patients is more than AD group. This finding approves the fact that cerebral blood flow decreases during the improvement of Alzheimer's disease (24).

Plasma NFL is a reliable biomarker to diagnose patients with AD, and is released into CSF and subsequently into the blood as a result of neural damage (25). Plasma NFL level is a marker for measurement of neurodegeneration in AD patients so an increased level of Plasma NFL is correlated with future atrophy, hypometabolism, and cognitive decline (26). Meanwhile plasma level of biomarkers is less expensive and easily accessible (27). heretofore most studies emphasized the correlation between CSF level of biomarkers and regional cerebral blood flow changes in neurodegenerative diseases. For example, Stomrud et al. investigated the correlation between CSF biomarkers and cerebral blood flow in healthy elderly (1). They found out that the CSF level of p-tau correlated positively with rCBF in the left frontotemporal border zone area. They also realized a negative significant correlation between rCBF in the right superior posterior medial frontal lobe in healthy elderly (1). Similarly, our analysis shows a significant correlation between the plasma level of NFL and CBF changes in widespread areas of the brain between healthy subjects. We found that Higher Plasma NFL was associated with lower blood flow in the right middle and superior temporal gyrus, triangular and orbital part of right inferior frontal gyrus, right and left posterior cingulate gyrus, posterior part of the left middle, and anterior part of right middle frontal gyrus, left superior frontal gyrus, and Right vessel in CN group. In the present study, we investigate the correlation between plasma NFL and rCBF with not only healthy objects but also with AD and MCI patients. The pathological role of some of the regions mentioned above was approved previously. hypoperfusion in the cingulate cortex as a predictor factor in the MCI patients (28), and reduced blood supply in the temporal region in AD patients (29) were the topics that have been discussed in the earlier studies .We also found that Plasma NFL correlates with lower blood flow in inferior parietal lobule, right inferior temporal gyrus, right middle temporal gyrus, anterior and posterior part of the left and right middle frontal gyrus, triangular and orbital part of right inferior frontal gyrus, right and left superior frontal gyrus Exclusive of grouping among all participants. Surprisingly, we couldn't find a reasonable correlation between plasma NFL and rCBF changes in neither AD nor MCI patients. Recent research found that there is substantial hypoperfusion in the cognitively healthy older participants (1). the fact that there is no significant correlation between NFL and rCBF in the MCI and AD patients makes it an important issue for further research.

CSF biomarkers such as Aβ, total tau, and phosphorylated tau are applied predictors for the chance of converting to AD in MCI patients. Although the changes in the CSF level of biomarkers and rCBF changes reflected different mechanisms in AD progress, the use of them as a combined biomarker has shown higher reliability in the prediction of converting to AD in MCI patients comparing with use each one separately. The studies researching about the correlation between the CSF level of biomarkers and r CBF would approach a possibility of a relation between biomarkers pathology and CBF abnormality in Alzheimer's disease (1, 30). So far, few studies have been conducted in this field and in one of them, Habert et al. investigated this correlation in two groups of participants consisting of AD and MCI patients (31). They found out that there is no significant correlation between Aβ42 and brain perfusion, meanwhile, the correlation between T-tau and P-tau concentration with the perfusion in left angular, inferior parietal, and precuneus cortices was so significant and clear (31). We investigated the correlation between CSF levels of Aβ and r CBF changes in every 3 groups of our subjects and we only found that Aβ

was significantly correlated with higher blood flow in the right frontal pole in AD patients. Our analysis shows a correlation between CSF levels of T-tau and P-tau181 with r CBF changes in MCI patients too. We found that CSF total tau and p tau 181 were positively correlated with blood flow in the left cuneus, left pericalcarine, and Central corpus callosum. Moreover, there is a strong correlation between CSF p tau 181 and blood flow in the left postcentral gyrus, right cuneus, right lingual gyrus, and caudal part of the left anterior cingulate gyrus in addition to the previous regions. meanwhile, CSF p-tau 181 negatively correlates with blood flow in optic chiasm in the AD group. analysis failed to reach a correlation for CSF t-tau in this group of subjects. Based on the result of our analysis none of the CSF t-tau and p-tau 181 are correlated with r CBF in healthy participants.

Conclusion

Our findings revealed that NFL is associated with rCBF, therefor, our results support the application of plasma NFL for tracking neurodegeneration and decline in cerebral blood flow. However, this is first study that investigated the association between plasma NFL and rCBF in AD. Further research is needed for rule out the underlying mechanism of rCBF changes and the association with Aβ and tau accumulations in AD progress.

Declarations

Data used in this article's preparation were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI investigators contributed to the design and implementation of ADNI and/or provided data but did not participate in the 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

Competing interests

The authors declare no competing interests.

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Tables

Table1. Participants characteristic

Demographic and health characteristic	CN(39)	MCI(76)	AD(29)	P value
Age, years	71.6(±6.9)	70.2(±7.6)	72.6 (±6.8)	0.275
Sex(M/F)	15/24	37/39	14/15	0.562
Education, years	16.3(±2.4)	16.4(2.7)	16.4(±2.2)	0.987
MMSE	29(±1.4)	28.4(±1.6)	23.7(±1.9)	0.000
APOE genotype				0.001
With out ε4	27	49	9	
One ε4	11	20	13	
Two ε4	1	7	7	

Values are showed as mean(±SD), Mini Mental State Examination(MMSE), results of ANOVA analysis between groups noted as p value

Table2. Significant Results of comparison regional cerebral blood flow between groups

Regions	*df*	F	P value
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
Gray matter of right parahippocampal gyrus	2, 141	3.782	0.025

Results of one way ANOVA analysis showed as *df*, F, and P value, Benjamini Hochberg correction performed

Table3. Significant Results of partial Correlation Analyses of regional cerebral blood flow and Plasma NFL, CSF Aβ, tau, and p tau 181 Levels among all participants

Regions	Aβ	Tau	P tau 181	NFL
	Correlation Coefficient	*Correlation Coefficient*	*Correlation Coefficient*	*Correlation Coefficient*
inferior parietal lobule	0.059	0.020	0.005	-0.197*
anterior part of left middle frontal gyrus	0.115	-0.036	-0.039	-0.179*
right inferior temporal gyrus	0.105	-0.088	-0.106	-0.244**
right middle temporal gyrus	0.103	-0.049	-0.070	-0.194*
orbital part of right inferior frontal gyrus	0.117	-0.028	-0.028	-0.204*
triangular part of right inferior frontal gyrus	0.115	0.025	0.035	-0.200*
posterior part of left middle frontal gyrus	0.074	0.016	0.019	-0.224**
left superior frontal gyrus	0.122	0.028	0.042	-0.172*
posterior part of right middle frontal gyrus	0.141	-0.011	-0.010	-0.217*
anterior part of right middle frontal gyrus	0.130	-0.072	-0.060	-0.202*
right superior frontal gyrus	0.136	-0.001	0.014	-0.190*
left inferior temporal gyrus	0.283**	0.036	0.022	-0.003
left middle temporal	0.235*	0.063	0.042	-0.124
right inferior temporal	0.260*	-0.018	-0.043	-0.119
right lingual gyrus	-0.076	0.206*	0.199	-0.099
Central corpus callosum	0.029	0.256**	0.234	-0.004

*p < 0.05

***p < 0.01

partial correlation coefficient of DTI metrics value of the brain regions and plasma p tau 181 levels controlled for age and sex.

Table4. Significant Results of partial Correlation Analyses of regional cerebral blood flow and Plasma NFL, CSF Aβ, tau, and p tau 181 Levels among all participants

Regions	Aβ	Tau	P Tau 181	NFL
	Correlation Coefficient	*Correlation Coefficient*	*Correlation Coefficient*	*Correlation Coefficient*
AD				
Optic chiasm	0.054	-0.323	-0.461*	-0.257
right frontal pole	0.470*	0.158	0.080	-0.061
MCI				
left cuneus	-0.066	0.330**	0.290*	0.062
left pericalcarine	-0.052	0.273*	0.308*	0.037
left postcentral gyrus	0.058	0.219	0.256*	-0.058
right cuneus	0.020	0.233	0.268*	0.017
right lingual gyrus	-0.063	0.252	0.263*	0.039
caudal part of left anterior cingulate gyrus	0.062	0.255	0.251*	0.003
Central corpus callosum	-0.039	0.347**	0.333**	0.132
CN				
right middle temporal gyrus	0.090	0.291	0.117	-0.360*
orbital part of right inferior frontal gyrus	0.043	0.212	0.204	-0391*
triangular part of right inferior frontal gyrus	-0.053	0.136	0.107	-0.381*
right posterior cingulate gyrus	0.024	0.174	0.173	-0.483**
right superior temporal gyrus	-0.139	0.264	0.241	-0.392*
Right vessel	-0.097	0.184	0.211	-0.362*
posterior part of left middle frontal gyrus	0.024	0.037	0.027	-0.369*
left superior frontal gyrus	0.243	0.066	0.045	-0.339*
anterior part of right middle frontal gyrus	0.014	0.074	0.047	-0.380*
left posterior cingulate gyrus	0.091	0.148	0.147	-0.497**

*$p < 0.05$

**p<0.01

partial correlation coefficient of regional cerebral blood flow of the brain regions and plasma NFL, CSF Aβ, tau, and p tau 181 levels controlled for age, sex, and APOE genotype.