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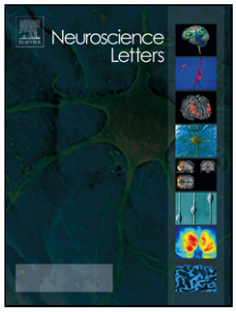

Association between CSF Aβ42 and amyloid negativity in patients with different stage mild cognitive impairment

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Title Page

Association between CSF Aβ42 and amyloid negativity in patients with different stage mild cognitive impairment

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Highlights

- CSF Aβ42 can discriminate between cognitively normal and either EMCI or LMCI patients only in the positive amyloid PET instead of the negative amyloid PET.
- CSF Aβ42 had strong correlations with other biomarkers and might help reduce risk of EMCI or LMCI in patients with amyloid negativity.

Abstract

Whether the cerebrospinal fluid (CSF) biomarkers of amyloid-positive and amyloid-negative patients with mild cognitive impairment (MCI) or Alzheimer's disease (AD) are significantly different is still unknown. The purpose of this study is to compare the differences in CSF total tau, P-tau and Aβ42 in patients with amyloid-positive positron emission tomography (PET) and amyloid-negative PET, and to explore related risk factors in cognitive normal (CN), early MCI (EMCI), late MCI (LMCI) and AD. 558 participants (140 CN; 233 EMCI; 125 LMCI; 60 AD) were recruited in this study from the AD Neuroimaging Initiative (ADNI) database. The associations between CSF biomarkers were assessed by partial correlation analysis. The relations between significant variables were determined by multinomial logistic regression. Compared with amyloid-positive PET patients, patients with amyloid-negative PET had higher CSF Aβ42 and lower P-tau in the whole samples. The concentration of Aβ42 in the positive amyloid PET was significantly different in different groups, but not the negative amyloid PET (CN vs. LMCI; CN vs. AD; EMCI vs. AD, all $P < 0.05$). When

amyloid PET was positive, a weak correlation was found between the levels of Aβ42 and P-tau only in CN group. However, a moderate degree of correlation between Aβ42 and P-tau was found in EMCI and LMCI when amyloid PET was negative. After covariates adjustment, CSF Aβ42 was significantly associated with EMCI [adjusted odds ratio (OR) = 0.99, 95% confidence interval (CI) = 0.99 – 1.00, *P* = 0.02) and LMCI (adjusted OR = 0.99, 95% CI = 0.99 – 1.00, *P* = 0.007)] in patients with negative amyloid PET, not in patients with positive amyloid PET. Our findings highlight that Aβ42 had strong correlations with other biomarkers and might help reduce risk of EMCI or LMCI in patients with amyloid negativity.

Keywords: Mild cognitive impairment; Aβ42; Cerebrospinal fluid; Amyloid PET negativity

1. Introduction

Mild Cognitive Impairment (MCI) represents the stage of cognitive impairment before the transition from healthy aging to Alzheimer's disease (AD). Due to the unsatisfactory results of clinical studies of drugs developed for AD [1], researchers begin to pay attention to how to delay the development of MCI to AD and MCI is further divided into early MCI (EMCI) and late MCI (LMCI) [2]. Compared with EMCI participants, LMCI participants have lower scores on various cognitive scales and follow-up results show a faster decline in cognitive level [3]. Also, in the brain function

test, amplitude of low-frequency fluctuations of MCI patients decreases in some brain areas, such as the posterior cingulate cortex and precuneus [4]. Moreover, LMCI had significantly higher levels of amyloid load and CSF total tau. Aβ42 and tau has been proven by many studies to predict the progression of MCI to AD [5, 6]. Similarly, Aβ42 also has higher accuracy in distinguishing MCI and AD, and Kallikrein-8 has even better diagnostic value than Aβ42 [7].

However, some recent studies have found that by grouping whether amyloid positron emission tomography (PET) is positive, experiments can get different results. The data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) indicate negative amyloid PET patients have higher CSF Aβ42 and lower P-tau, and is associated with better longitudinal cognitive performance than positive amyloid PET patients with LMCI or AD [8]. Another small sample study found negative amyloid PET patients have higher CSF Aβ42 levels than positive amyloid PET patients with suspected AD and the cut-off point of the identification of negative PET according to Aß42 level is 843 pg/ml [9].

At present, whether the correlation between the CSF markers of the negative protein participants is different from that of the positive participants remains unknown in in cognitive normal (CN), EMCI, LMCI and AD. Thus, this work intended to quantify the extent with degree of correlation between CSF biomarkers, and further to detect the potential risk factors in individuals with negative or positive PET.

2. Methods

2.1 ADNI study

The data for this study were from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was propelled in 2003 as a public-private partnership under the leadership of Principal Investigator Michael W. Weiner, MD. So far, it consists primarily of CN individuals, EMCI, LMCI and AD. More details can be discovered at www.adni-info.org. At each ADNI site, an approval from the Institutional Review Board (IRB) was obtained. All participants or authorized representatives gave written informed consent.

2.2 Subjects

A total of 558 subjects (140 CN; 233 EMCI; 125 LMCI; 60 AD) participated in the study. Diagnosis criteria were depicted in the ADNI program manual [2]. The diagnosis of CN required older people with normal cognitive function that matches age, gender, and education level. The MCI group was divided into EMCI and LMCI. Briefly, compared with the EMCI, the LMCI adjusted the scores for measuring objective memory loss in combination with the level of education. A potential AD diagnosis needed a combination of dementia syndrome and National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria ((NINCDS/ADRDA)) for AD [10].

2.3 Neuropsychological assessment

The Mini-Mental State Examination (MMSE) scale was popular in assessing different domains of cognition in all the participants. The scale includes the following aspects: orientation, memory, attention and calculation, language, and visual space.

2.4 Measurement of CSF Aβ42, tau, and P-tau

The concentrations of CSF Aβ42, tau, and P-tau were detected using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) with the INNOBIA AlzBio3 kit (Fujirebio, Ghent, Belgium) at the ADNI biomarker core (University of Pennsylvania) in the previous publications [11-13].

2.5 Apolipoprotein E genotyping

Apolipoprotein E (APOE; gene map locus 19q13.2) genotypes of the study subjects were obtained from the ADNI database (https://adni.loni.usc.edu). All subjects were classified as APOE ε4 carriers with phenotypes ε2/ε4, ε3/ε4, and ε4/ε4, APOE ε4 non-carriers group with ε2/ε2, ε2/ε3 and ε3/ε3 genotypes.

2.6 [^{18}F] AV 45 (Florbetapir) PET scan

[^{18}F] AV 45 (Florbetapir) PET data had been obtained and analyzed according to previous reported methods [14]. The mean florbetapir standard uptake value ratios (SUVRs) were determined for every participant in four regions of interest (ROI),

including frontal, anterior cingulate, precuneus and parietal cortex, then normalized to the entire cerebellum reference region. All participants were divided into two subgroups, SUVRs >1.11 was identified as amyloid-positive, SUVRs ≤1.11 was identified as amyloid-negative based on a pre-determined threshold [15]. More information about the PET protocols and data can be discovered on the ADNI website.

2.7 Statistical analysis

Data that conforming to a normal distribution was presented by the mean ± standard deviation (SD), while a skewed distribution analysis result was described by median (M) and interquartile range (IQR). The ratio was expressed as a percentage. One-way analysis of variance (ANOVA) was adopted to estimate the divergence of age and CSF biomarkers across the CN, EMCI, LMCI, and AD groups. Gender, race, ethnicity, marital status, and Aβ positivity were assessed using the Chi-square test across the four diagnostic groups. Education and MMSE score were evaluated using Kruskal-Wallis test for group differences. Partial correlation was used to evaluate the association between different biomarkers after controlling for age, and r_s represented the correlation coefficient [16]. Multinomial logistic regression was utilized to analyze demographic and clinical variables in different groups [9]. Statistical analyses were executed with SPSS software (version 23.0; IBM SPSS). GraphPad Prism 7 was adopted to generate scatter plots. For all calculated tests, the significance was commonly set at $P < 0.05$.

3. Results

3.1 Demographic features

558 participants (including 140 CN, 233 EMCI, 125 LMCI and 60 AD) were involved in this study. The four cohorts did not differ in gender, race and ethnicity. The cohorts differed in age (F = 5.8, $P = 0.001$), education (F = 2.7, $P = 0.04$), marital status ($\chi^2 = 10.1$, $P = 0.01$) and APOE ε4 ($\chi^2 = 10.1$, $P < 0.001$). Besides, significant differences were found in CSF Aβ42, tau, P-tau, MMSE score and Aβ positivity across the four cohorts (all $P < 0.001$).

3.2 Associations between CSF tau, P-tau and Aβ42

Table 2 showed the concentrations of CSF biomarkers in patients with negative or positive amyloid PET. Compared with the positive amyloid PET, the negative amyloid PET had significantly higher levels of Aβ42 and lower levels of P-tau (all $P < 0.001$). We found that there were significant trends toward the concentrations of Aβ42 and tau in the positive amyloid PET (CN vs. LMCI; CN vs. AD; EMCI vs. AD, all $P < 0.05$). Disparately, only the levels of tau and P-tau, not Aβ42, had significant difference in the negative amyloid PET (tau: LMCI vs. AD; P-tau: EMCI vs. AD, LMCI vs. AD, all $P < 0.05$). Interestingly, there was significant difference toward CSF Aβ42 only in the positive amyloid PET, and only 2% (5/263) subjects with negative amyloid PET was diagnosed AD.

To further explore the associations between these biomarkers, we adopted partial correlation to evaluate them in the whole sample and found P-tau and tau were weakly negatively related to Aβ42 (r_s = -0.223, $P < 0.0001$; r_s = -0.140, $P < 0.0001$, respectively). As expected, Subgroup analysis also showed that P-tau and Aβ42 had a weak negative correlation in EMCI and LMCI (Fig. 1D and 1F), while tau and P-tau were weakly positively correlated to Aβ42 in CN (Fig. 1A and 1B). However, no relationships were discovered between tau, P-tau and Aβ42 in AD (Fig. 1G and 1H). Interestingly, these points in the scatter plot are almost parallel to the y-axis, indicating that Aβ42 can hardly affect tau, see Fig. 1G and 1H.

We next estimated the difference of CSF biomarkers in both negative and positive amyloid PET. Aβ42 was not linked with tau and P-tau in all positive amyloid PET subjects (all $P > 0.05$). Subgroup analysis showed P-tau was only positively correlated to Aβ42 in CN group (r_s = 0.312, P = 0.045, Fig. 2B). However, the analysis results for negative amyloid PET subjects showed that these biomarkers were more closely related. Both tau and P-tau were positively related to Aβ42 (r_s = 0.559, P < 0.001; r_s = 0.493, P < 0.001, respectively). Although tau and P-tau had a strong positive correlation with Aβ42 in CN group (r_s = 0.645, P < 0.001; r_s = 0.596, P < 0.001, respectively), correlation coefficient gradually decreased from EMCI to LMCI (Fig. 3C, 3E, 3D and 3F), and there is even no correlation in AD (all $P > 0.05$).

3.3 Multiple logistic regressions for the risk factors in different diagnostic groups

As shown in Table 3, both EMCI and LMCI subjects were associated with age, MMSE score, and CSF Aβ42 (all $P < 0.05$). Moreover, factors that individually related to AD include age, MMSE score, and Aβ42/P-tau (all $P < 0.05$). Subgroup analysis found age and MMSE scores were related with EMCI, LMCI, and AD in the positive amyloid PET, but Aβ42 was independently associated with EMCI and LMCI in the negative amyloid PET patients compared with CN (all OR = 0.99, Table 4) (all $P < 0.05$).

4. Discussion

This study found the concentrations of CSF Aβ42 in whole individuals with negative amyloid PET were higher than that of patients with positive amyloid PET. CSF Aβ42 can discriminate between cognitively normal and either EMCI or LMCI patients only in the positive amyloid PET instead of the negative amyloid PET. Amyloid-positive PET patients had a weak correlation between Aβ42 and P-tau in CN, while a moderately positive correlation existed between Aβ42 and P-tau in amyloid-negative CN, EMCI and LMCI. After covariates adjustment, CSF Aβ42 was associated with EMCI and LMCI in negative amyloid PET but not in the positive one.

Several studies from the ADNI or other center have demonstrated that patients with negative amyloid PET have higher CSF Aβ42 and lower P-tau than patients with positive amyloid PET in LMCI and AD [8, 9]. Our results also support the above reports and are extended to CN and EMCI. In fact, Table 1 showed that there were significant

differences in the levels of CSF biomarkers in different groups. However, when subgroup analysis was performed, we found CSF Aβ42 can discriminate between cognitively normal and either EMCI or LMCI patients only in the positive amyloid PET, but CSF P-tau can help distinguish EMCI or LMCI from AD only in the negative amyloid PET. Although the above article did not compare the Aβ42 or P-tau of LMCI and AD patients with negative or positive amyloid PET, it is not difficult to draw the same conclusion from the data in the literature table [8]. The potential explanation for this phenomenon is as follows. On one hand, lower CSF Aβ42 predicts Aβ deposition and has a higher agreement with amyloid PET [17]. On the other hand, the concentration of Aβ42 deposited in the brain tissue is gradually increasing from CN to AD [18]. This indicates that when the patient reaches the standard of amyloid positive, the Aβ42 that has been deposited in the brain may begin to impair cognition, and as more concentration of Aβ42 is deposited, the cognitive impairment will aggravate. Therefore, the difference in Aβ42 deposited in the brain tissue can be distinguished by CSF Aβ42.

The relationship between Aβ42 and tau will change from a positive linear relationship to a negative linear relationship with age in preclinical AD [16]. A survey of 3565 patients with cognitive impairment found that Aβ42 and tau have negative correlation. Among them, 36% of patients with amyloidosis and neurodegeneration have a weaker relationship between Aβ42 and tau [19]. Despite controlling age factors, our results found the correlation coefficients of Aβ42 and P-tau or tau were small in

each group, and there is no correlation even in AD which is not consistent with the above studies [19]. The explanation is that the sample size in this study is not large enough, but the correlation coefficient of the above study is less than 0.1, which is not convincing. However, compared with the above studies without subgroup analysis, we had newly discovered that Aβ42 were stronger positive correlated to P-tau whether in CN, EMCI, or LMCI carrying amyloid negativity (Fig. 3B, 3D and 3F), not amyloid positivity. The authors explained the interesting phenomenon as follows. The correlation between CSF Aβ42 and tau is positive in CN [16]. Amyloid positivity indicates that the clearance rate of Aβ42 in the brain decreases, and the corresponding CSF Aβ42 will also undergo abnormal changes. This change will destroy the correlation between CSF Aβ42 and P-tau. Therefore, the relationship between biomarkers can only be observed on negative amyloid patients.

Tau hypothesis provides a way of thinking that when Aβ42 reaches a certain threshold in the brain, it may play a role of trigger point and lead to the rapid development from MCI to AD [18]. Our results showed that CSF Aβ42 was negatively linked with tau or P-tau in EMCI and LMCI, but those points represented Aβ42 were almost parallel to the y-axis, representing tau or P-tau in AD (see Fig. 1G and 1H). Basic research proves that Aβ increases the expression of tau, and the extracellular tau further increases the level of Aβ in AD [20]. When the content of CSF Aβ42 gradually decreases from high to below a certain threshold [9], it indicates that Aβ42 in CSF gradually deposits into the brain tissue to form senile plaques, then inducing

hyperphosphorylation of tau in AD. Previous study has observed an inverse relationship between abnormal amyloid PET intake and CSF Aβ42 levels [21]. This may explain why 92% (55/60) of AD patients in our study had a positive amyloid PET. Whether there is a linear or non-linear relationship between Aβ and tau is still unknown, although there is mutual influence between the two to some extent.

The current study on whether Aβ led to the progression of MCI to AD remained contradictory [22, 23]. The Aβ was capable of protecting against innate immunity, but it might become a destructive factor due to inflammatory stimuli [22]. Multinomial logistic regression in our study supported the hypothesis that higher levels of CSF Aβ42 were protective factor in negative amyloid PET patients with MCI. Previous studies have confirmed that Aβ42 was the main component involved in the formation of amyloid plaques and CSF Aβ42 levels were inversely proportional to Aβ load [18]. Standards for Aβ42 concentrations in very high ranges were often associated with negative amyloid PET [9]. Moreover, we observed only 2% (5/263) of patients with negative amyloid PET suffered from AD in this study. Based on the above theory, we speculate that when the CSF Aβ42 level was high, it indicates that the Aβ42 deposited in the brain will reduce. Once CSF Aβ42 begins to decrease and form amyloid plaques, amyloid plaques activate tau and then deposit it in the brain tissue together with stronger synergy [17], which ultimately impaired cognitive function. Furthermore, CSF Aβ42/40 ratio is better than Aβ42 as a potential marker for evaluating amyloid PET

[24]. Thus, Aβ42/40 ratio may also be a better choice for predicting the progression of amyloid-negative MCI [25].

However, certain limitations were existed in this work. First, the simple cross-sectional study is not sufficient to analyze the dynamic changes in the relationships between CSF biomarkers as progress from MCI to AD. If the results of follow-up also confirm that CSF Aβ42 is an independent factor, it will be more convincing. Second, a larger sample of AD patients with amyloid negativity is necessary for multivariate regression analysis.

5. Conclusion

In summary, the present results highlight that the level of CSF Aβ42 is significantly associated with EMCI and LMCI in patients with negative amyloid PET, not in patients with positive amyloid PET. Longitudinal studies with larger samples sizes considering Aβ40 level are needed to further investigate the role of Aβ42/40 ratio in the pathogenesis of AD.

6. Ethics approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or

comparable ethical standards. Written informed consents were obtained from all participants or authorized representatives included in the study.

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Credit Author Statement
Bingjie He: Conceptualization, Methodology, Writing-Original Draft; Lijun Wang: Conceptualization, Resources, Data Curation. Bingdong Xu: Conceptualization, Software, Writing-Original Draft. Yusheng Zhang: Conceptualization, Writing-Review & Editing, Supervision.

8. Conflicts of interests

The authors declared that they had no conflicts of interest.

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Figure. 1 Correlations between CSF tau, P-tau and Aβ42 in the CN, EMCI, LMCI, and AD groups

A-B. Correlations between CSF tau, P-tau and Aβ42 in the CN group. C-D. Correlations between CSF tau, P-tau and Aβ42 in the EMCI group. E-F. Correlations between CSF tau, P-tau and Aβ42 in the LMCI group. G-H. Correlations between CSF tau, P-tau and Aβ42 in the AD group. *P* values were tested by partial correlation after controlling for age factor.

AD, Alzheimer's disease; CN, cognitive normal; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment.

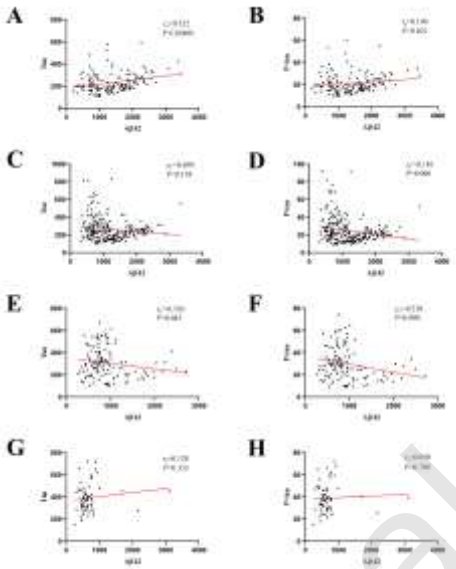

Figure. 2 Correlations between CSF tau, P-tau and Aβ42 in Aβ positive patients

A-B. Correlations between CSF tau, P-tau and Aβ42 in the Aβ positive CN group. C-D. Correlations between CSF tau, P-tau and Aβ42 in the Aβ positive EMCI group. E-F Correlations between CSF tau, P-tau and Aβ42 in the Aβ positive LMCI group. G-H. Correlations CSF tau, P-tau and Aβ42 in the Aβ positive AD group. *P* values were tested by partial correlation after controlling for age factor.

AD, Alzheimer's disease; CN, cognitive normal; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; PET, positron emission tomography.

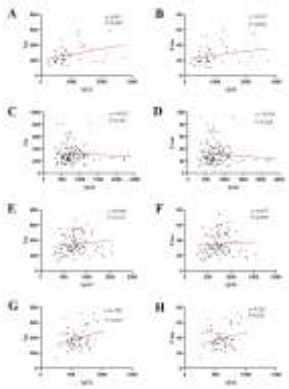

Figure. 3 Correlations between CSF tau, P-tau and Aβ42 in Aβ negative subjects

A-B. Correlations between CSF tau, P-tau and Aβ42 in the Aβ negative CN group. C-D. Correlations between CSF tau, P-tau and Aβ42 in the Aβ negative EMCI group. E-F. Correlations between CSF tau, P-tau and Aβ42 in the Aβ negative LMCI group. G-H. Correlations between CSF tau, P-tau and Aβ42 in the Aβ negative AD group. *P* values were tested by partial correlation after controlling for age.

AD, Alzheimer's disease; CN, cognitive normal; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; PET, positron emission tomography.

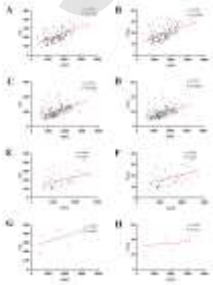

Table 1 Demographics and clinical characteristics of subjects included in the study

Characteristics	CN (n=140)	EMCI (n=233)	LMCI (n=125)	AD (n=60)	*P* value
Age, years	73.2 ± 6.2	70.8 ± 7.1	71.2 ± 7.6	74.2 ± 8.3	0.001
Gender, n (% Female)	69 (49.3)	100 (42.9)	58 (46.4%)	29 (47.5)	0.66
Education, years	16 (14-18)	16 (14-18)	17 (15-19)	16 (14-18)	0.04
Race, n (% White)	125 (89.3)	215 (92.3)	118 (94.4)	57 (93.4)	0.45
Ethnicity, n (% Not Hisp/Latin)	137 (97.9)	223 (95.7)	123 (98.4)	61 (100)	0.25
Marital status, n (% Married)	96 (68.6)	183 (78.5)	94 (75.2)	53 (88.5)	0.01
MMSE score	29 (29-30)	29 (28-30)	28 (26-29)	23 (22-25)	<0.001
Aβ positivity, n (%)	43 (30.7)	109 (46.8)	89 (71.2)	57 (95.0)	<0.001
APOE ε4, n (%)	37 (26.4%)	100 (42.9%)	72 (57.6%)	47 (78.3%)	<0.001
CSF Aβ42 (pg/ml)	1409.1 ± 661.9	1193.8 ± 585.3	933.8 ± 477.6	689.3 ± 430.7	<0.001
CSF tau (pg/ml)	233.2 ± 87.8	261.7 ± 125.5	306.5 ± 133.2	390.3 ± 128.0	<0.001
CSF P-tau (pg/ml)	21.2 ± 8.7	24.8 ± 14.1	29.8 ± 14.4	38.9 ± 13.0	<0.001

AD, Alzheimer's disease; CN, control; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; MMSE, Mini-Mental state examination.

Data are represented as mean ± SD, median and the interquartile range. *P* values were tested by ANOVA test, Kruskal-Wallis test and Chi-square test.

Table 2 CSF biomarkers in all subjects and according to patients with negative or positive amyloid PET *

	CN		EMCI		LMCI		AD	
	PET − (n=98)	PET + (n=42)	PET − (n=124)	PET + (n=109)	PET − (n=36)	PET + (n=89)	PET − (n=5)	PET + (n=55)
CSF Aβ42	1610.5 (1142.5-1943.0)	242.2 (192.5-334.0)	1459.5 (1144.2-1897.0)	285.2 (233.7-371.9)	1217.0 (925.6-1834.7)	321.5 (254.9-458.3)	1694.0 (1351.2-2638.4)	369.2 (304.1-461.0)
CSF tau	203.4 (168.1-259.3)	22.1 (18.2-33.2)	201.4 (156.1-247.7)	27.5 (21.3-38.6)	186.7 (125.4-235.1)	31.0 (24.7-46.2)	382.3 (228.6-450.1)	37.1 (29.5-46.5)
CSF P-tau	17.7 (14.8-22.6)	74.1 (70.6-80.4)	17.8 (12.9-21.1)	72.7 (67.9-77.5)	16.1 (11.3-21.7)	72.6 (66.7-76.6)	39.1 (21.6-44.1)	75.3 (68.6-79.9)

AD, Alzheimer's disease; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment.

* There was a significant difference ($p < 0.001$), compared to the amyloid PET negative subjects by Mann–Whitney test in all subgroups.

Table 3 Multinomial logistic regression of severity of cognitive impairment

Characteristics	EMCI (n=233)		LMCI (n=125)		AD (n=60)	
	Adjusted OR (95 % CI)	*P* value	Adjusted OR (95 % CI)	*P* value	Adjusted OR (95 % CI)	*P* value

Not cognitive impairment (reference)						
Age	0.92 (0.89-0.96)	<0.0001	0.91 (0.87-0.95)	<0.0001	0.90 (0.83-0.98)	0.01
Gender						
Male	1		1		1	
Female	0.65 (0.40-1.04)	0.07	1.00 (0.55-1.80)	0.99	1.79 (0.54-5.94)	0.33
Education	0.96 (0.87-1.05)	0.38	1.14 (1.01-1.28)	0.02	1.16 (0.94-1.44)	0.15
MMSE score	0.71 (0.59-0.86)	0.001	0.51 (0.42-0.64)	<0.0001	0.15 (0.09-0.24)	<0.0001
Aβ positivity						
Positivity	1		1		1	
Negativity	0.70 (0.36-1.36)	0.29	0.46 (0.19-1.07)	0.07	1.27 (0.10-16.22)	0.85
APOE ε4	0.90 (0.52-1.56)	0.71	0.96 (0.49-1.88)	0.92	0.71 (0.19-2.65)	0.62
CSF Aβ42	0.99 (0.98-1.00)	0.007	0.99 (0.98-0.99)	0.001	1.00 (0.99-1.03)	0.09
CSF tau	1.00 (0.99-1.01)	0.40	1.01 (0.99-1.02)	0.06	1.01 (0.99-1.03)	0.24
CSF P-tau	1.00 (0.89-1.14)	0.88	0.94 (0.82-1.08)	0.39	0.84 (0.66-1.06)	0.15
CSF Aβ42/P-tau	1.01 (1.00-1.03)	0.05	1.01 (0.99-1.04)	0.13	0.84 (0.74-0.96)	0.01

AD, Alzheimer's disease; CI, confidence interval; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; MMSE, Mini-Mental state examination; OR, odds ratio.

Table 4 Multinomial logistic regression of severity of cognitive impairment in the subjects with amyloid negativity

Characteristics	EMCI (n=122)		LMCI (n=36)	
	Adjusted OR (95 % CI)	P value	Adjusted OR (95 % CI)	P value
Age	0.91 (0.87-0.96)	<0.0001	0.93 (0.87-0.99)	0.03
Gender				
Male	1		1	
Female	0.93 (0.51-1.70)	0.13	1.07 (0.45-2.52)	0.87
Education	0.91 (0.80-1.03)	0.13	1.08 (0.90-1.29)	0.38
MMSE score	0.73 (0.58-0.93)	0.01	0.61 (0.45-0.82)	0.001
APOE ε4	1.21 (0.57-2.57)	0.61	2.64 (0.77-9.05)	0.12
CSF Aβ42	0.99 (0.99-1.00)	0.02	0.99 (0.99-1.00)	0.007
CSF tau	1.01 (0.99-1.04)	0.06	1.00 (0.97-1.03)	0.93
CSF P-tau	0.94 (0.74-1.20)	0.66	1.19 (0.85-1.67)	0.29
CSF Aβ42/P-tau	1.03 (0.99-1.07)	0.07	1.04 (1.00-1.09)	0.04

CI, confidence interval; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; MMSE, Mini-Mental state examination; OR, odds ratio.