

Cerebrospinal Biomarker Cut-off Methods Defined Only by Alzheimer's Disease Predict More Precisely Conversions of Mild Cognitive Impairment

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Background and Purpose The cerebrospinal fluid (CSF) biomarkers play an important supportive role as diagnostic and predictive indicators of Alzheimer's disease (AD). About 30% of controls in old age show abnormal values of CSF biomarkers and display a higher risk for AD compared with those showing normal values. The cut-off values are determined by their diagnostic accuracy. However, the current cut-off values may be less accurate, because controls include high-risk groups of AD. We sought to develop models of patients with AD, who are homogeneous for CSF biomarkers.

Methods We included participants who had CSF biomarker data in the Alzheimer's Disease Neuroimaging Initiative database. We investigated the factors related to CSF biomarkers in patients with AD using linear mixed models. Using the factors, we developed models corresponding to CSF biomarkers to classify patients with mild cognitive impairment (MCI) into high risk and low risk and analyzed the conversion from MCI to AD using the Cox proportional hazards model.

Results APOE $\epsilon 4$ status and age were significantly related to CSF A β_{1-42} . CSF t-tau, APOE $\epsilon 2$ status and sex were significant factors. The CSF p-tau₁₈₁ was associated with age and frequency of diagnosis. Accordingly, we modeled the three CSF biomarkers of AD. In MCI without APOE $\epsilon 4$, our models were better predictors of conversion.

Conclusions We can interpret CSF biomarkers based on the models derived from the data obtained from patients with AD.

Key Words Alzheimer's disease, cerebrospinal fluid biomarker, mild cognitive impairment, diagnosis, prediction, conversion.

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia. In the current clinical setting, AD is diagnosed by clinical criteria proposed by McKann et al.¹ Development of

disease modifying drugs requires accurate diagnostic tools. The cerebrospinal fluid (CSF) amyloid beta (A β_{1-42}), total tau (t-tau), and phosphorylated tau (p-tau₁₈₁) are promising diagnostic biomarkers.² They are correlated with the pathogenesis of AD. CSF biomarkers are not only an important diagnostic tool for AD, but also an important prognostic indicator of the disease decades before its onset.^{2,3}

Because the concentrations of CSF biomarkers are continuous values, arbitrary cut-off values should be determined for easy diagnosis. For example, AlzBio3 immunoassay kit (Inno-

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genetics, Ghent, Belgium) is one of the most popular kits for CSF protein assay.⁴ The cut-off points include: $A\beta_{1-42} < 192$ pg/mL, $t\text{-tau} > 93$ pg/mL, $p\text{-tau}_{181} > 23$ pg/mL, $t\text{-tau}/A\beta_{1-42} > 0.39$, or $p\text{-tau}_{181}/A\beta_{1-42} > 0.1$ for the diagnosis for AD.² Although the cut-off points were determined after considering the diagnostic accuracy, the values of patients and controls are centered around the cut-off points. In addition, the CSF biomarkers of controls who represent a high-risk group show an AD signature pattern. Therefore, we need to determine other ways to define the cut-off values for the CSF biomarkers.

The results of CSF biomarkers in AD are homogenous compared with those of the controls.⁵ In this study, we determined the factors associated with CSF biomarkers in AD. We then defined new cut-off values using appropriate models based on those factors.

METHODS

Participants⁶

We included subjects who had data involving CSF $A\beta_{1-42}$, $t\text{-tau}$, or $p\text{-tau}_{181}$ in the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The participants included 631 patients with amnesic mild cognitive impairment (aMCI) and 234 patients with AD whose diagnoses were determined at baseline. Among patients with AD, a patient who reverted to aMCI during a follow-up visit was excluded. The participants' ages ranged between 55 and 90 years (inclusive), good overall health to be included in the study. Other inclusion criteria were: treatment with medications stably for 4 weeks, appropriate visual and auditory acuity for neuropsychological testing, and scores on modified Hachinski scales ≤ 4 and geriatric depression scales < 6 .^{7,8} The patients with aMCI met the criteria proposed by Petersen et al.⁹ They had abnormal memory function which was tested by delayed paragraph recall of logical memory II. In addition, their Mini-Mental State Examination (MMSE) scores were 24–30 with a memory Clinical Dementia Rating (CDR) score of at least 0.5. Patients with AD met the 'probable' criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.¹ The ADNI includes patients with early stages of AD and MMSE scores of 20–26 and a sum-of-box CDR of 1–9 at their baseline visits. The exclusion criteria were: absence of other neurologic diseases such as Parkinson's disease, multi-infarct dementia, brain tumor, Huntington's disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma followed by persistent neurologic defaults or known structural brain abnormalities. Par-

Table 1. Characteristics of study participants

Factors	MCI (<i>n</i> =631)	AD (<i>n</i> =234)
Age, year	74.8±8.4	77.25±8.8
Women (%)	41.2	41.0
Education, year	16.1±2.8	15.1±3.0
<i>APOE</i> $\epsilon 2$ (%)		
Heterozygote	7.4	4.3
Homozygote	2.8	0.2
<i>APOE</i> $\epsilon 4$ (%)		
Heterozygote	38.8	47.0
Homozygote	10.9	20.9

The data are presented as mean±SD or percentile.

AD: Alzheimer's disease, MCI: mild cognitive impairment.

ticipants did not have major depression or bipolar disorder within the past 1 year, or alcohol/drug abuse within the past two years. They had no history of schizophrenia. Subjects who were contraindicated for MRI due to the presence of aneurysmal clips or pacemakers were excluded. We downloaded ADNI data on June 2014. The characteristics of people with normal cognition, aMCI and patients with AD including age, sex, years of education, *APOE* $\epsilon 2$ status, and *APOE* $\epsilon 4$ status are summarized in Table 1.

Baseline assessment and longitudinal assessment¹⁰

All subjects underwent neurological examination, physical examination, and neuropsychological assessments at baseline. Furthermore, CSF and blood samples were drawn, and MRI and FDG-PET scans were obtained. The protocols for cognitive testing, CSF, MRI, and PET are described in detail at <http://adni.loni.usc.edu/>. The follow-up interval was 6–12 months. In the present study we used results from the baseline and longitudinal results of CSF $A\beta_{1-42}$, $t\text{-tau}$, or $p\text{-tau}_{181}$.

CSF biomarkers

The patients underwent lumbar puncture (LP) in the morning after overnight fast.^{2,11} LP was carried out after MRI and before PET scan. If the test sequences of MRI and PET scan were reversed with LP, at least a three-day window was required between MRI and LPs, and at least a 12-hour window between PET scan and LPs. The patients repeated LPs annually. The "Run" means the test run which was assayed with the same kit and batch at the same time.

CSF $A\beta_{1-42}$, $t\text{-tau}$, or $p\text{-tau}_{181}$ were assessed using the multiplex xMAP Luminex platform (Luminex Corp., Austin, TX, USA) and Innogenetics/Fujirebio AlzBio3 immunoassay kits (Innogenetics).⁴

Statistical analysis

First, we developed mixed models with a random intercept and random slope, using age, sex, time from baseline, *APOE* $\epsilon 2$ alleles, and *APOE* $\epsilon 4$ alleles as fixed effects and run as a random effect. We used the compound symmetric as the covariate matrix. We also tested other covariate matrices, without any differences with the compound symmetric. The purpose of the first analysis was to identify the biological factors affecting the concentrations of CSF $A\beta_{1-42}$, t-tau, or p-tau₁₈₁ in AD. The allele number of *APOE* $\epsilon 2$ homozygote was regarded as 1, because *APOE* $\epsilon 2$ homozygotes were few. We arbi-

trarily selected the factors with $p \leq 0.10$ and used them in the final fitting models. In addition, we monitored the interactions among the significant factors. In the event of significant interactions among factors, we created fitting models by stratifications. We defined the upper 95% confidence interval curve for $A\beta_{1-42}$, the lower curve for t-tau, or p-tau₁₈₁ as new cut-off lines.

We compared MCI conversion to AD between old and new cut-off values using Cox proportional hazards model. The time variables included years from baseline visit to follow-up visit at the time of conversion.

Table 2. Factors affecting the concentration of cerebrospinal fluid $A\beta_{1-42}$, t-tau, and p-tau₁₈₁ of AD using linear mixed models

Factors	$A\beta_{1-42}$		t-tau		p-tau ₁₈₁	
	Estimated	<i>p</i>	Estimated	<i>p</i>	Estimated	<i>p</i>
Intercept	126.0	<0.001	223.7	<0.001	107.5	<0.001
Age	0.5	0.100	-0.8	0.128	-0.7	0.005
F/U time	-0.8	0.245	-1.7	0.170	2.21	0.004
Sex						
Female	4.4	0.342	34.1	<0.001	6.0	0.128
Male	Ref	Ref	Ref	Ref	Ref	Ref
<i>APOE</i> $\epsilon 2$						
-/-	-9.8	0.363	-48.0	0.009	-3.2	0.730
2/- or 2/2	Ref	Ref	Ref	Ref	Ref	Ref
<i>APOE</i> $\epsilon 4$						
-/-	42.6	<0.001	-13.6	0.227	-6.3	0.265
4/-	16.2	0.008	-10.0	0.337	-3.1	0.555
4/4	Ref	Ref	Ref	Ref		
Age × <i>APOE</i> $\epsilon 4$		0.020		-		-
Sex × <i>APOE</i> $\epsilon 2$		-		0.034		-
Age × F/U time		-		-		0.420

AD: Alzheimer's disease.

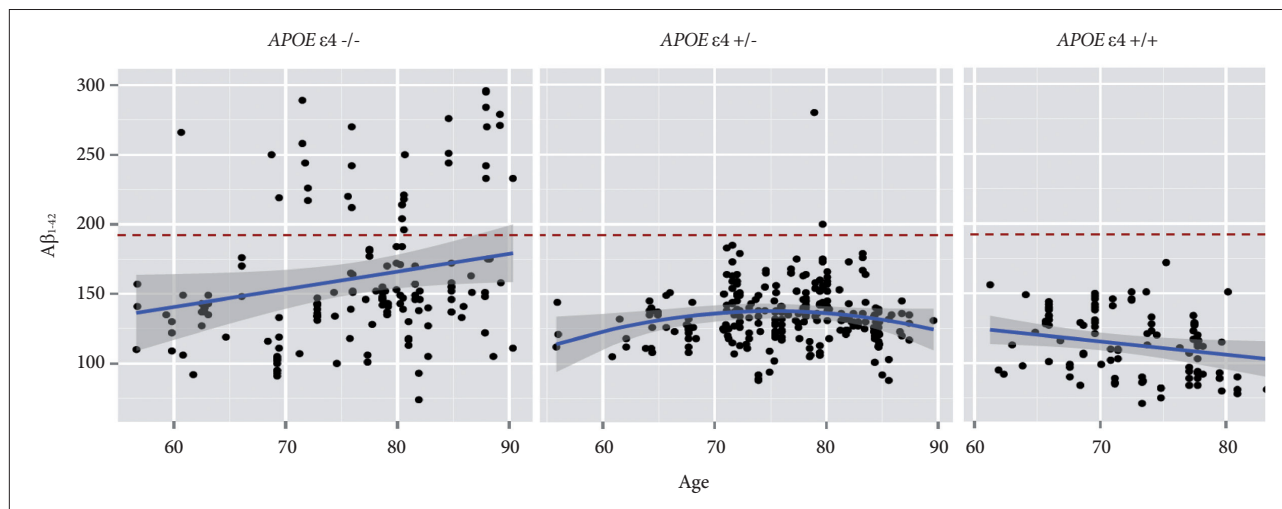


Fig. 1. Correlation of CSF $A\beta_{1-42}$ with age and *APOE* $\epsilon 4$ status in Alzheimer's disease. The blue lines represent the fitting models for concentration of CSF $A\beta_{1-42}$ and the surrounding gray zones denote 95% confidence intervals. The horizontal red dotted line is a cut-off values that distinguishes normality and abnormality as determined by a previous study. CSF: cerebrospinal fluid.

The statistical analyses were conducted using PASW (version 21.0; IBM Corp., Armonk, NY, USA). Graphical presentations were performed using R (version 3.0.1).

RESULTS

Table 2 displays the selection of the factors affecting CSF biomarker concentrations of patients with AD, using the mixed models. We selected factors with $p \leq 0.10$ and determined the interaction between the factors. Based on significant interactions, we separated factors to create a fitting model.

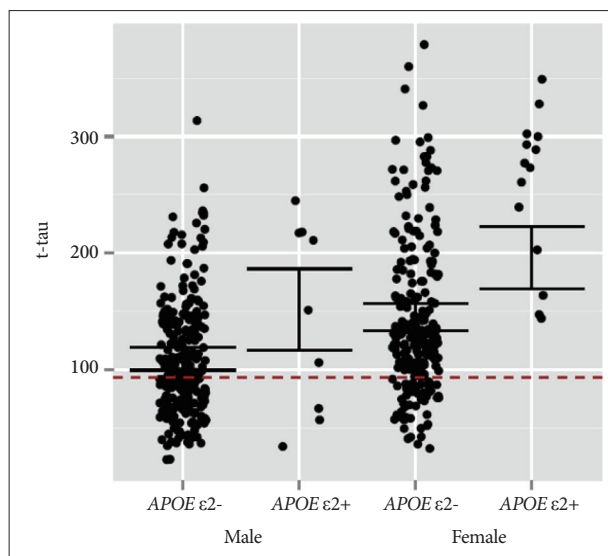


Fig. 2. The concentration of cerebrospinal fluid t-tau according to sex and APOE ε2 status in Alzheimer's disease. The horizontal bars are zones of mean ± standard deviation.

We selected APOE ε4 status ($p < 0.001$) and age ($p = 0.10$) as the factors significantly related with CSF Aβ₁₋₄₂. For CSF t-tau, APOE ε2 status ($p = 0.009$) and sex ($p < 0.001$) were significant factors. Age ($p = 0.005$) and time from diagnosis ($p = 0.004$) were the significant factors for CSF p-tau₁₈₁. We identified interactions between CSF Aβ₁₋₄₂ related factors (APOE ε4 status and age, $p = 0.020$) and between t-tau related factors (APOE ε2 status and sex, $p = 0.034$).

As shown in Fig. 1, the distribution of CSF Aβ₁₋₄₂ according to the number of APOE ε4 varies with respect to age. In the absence of APOE ε4, a linear increase was observed in age. In APOE ε4 heterozygotes, a parabolic shape with apex at about 67 was observed. In APOE ε4 homozygotes, the CSF Aβ₁₋₄₂ decreased linearly with respect to age. The CSF t-tau is

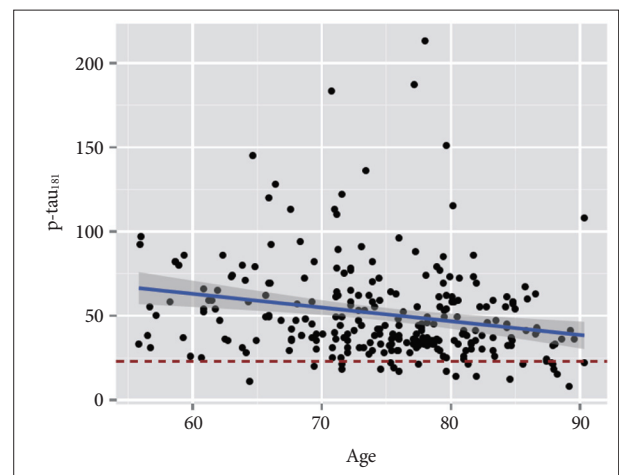


Fig. 3. Age and the concentration of cerebrospinal fluid p-tau₁₈₁ in Alzheimer's disease.

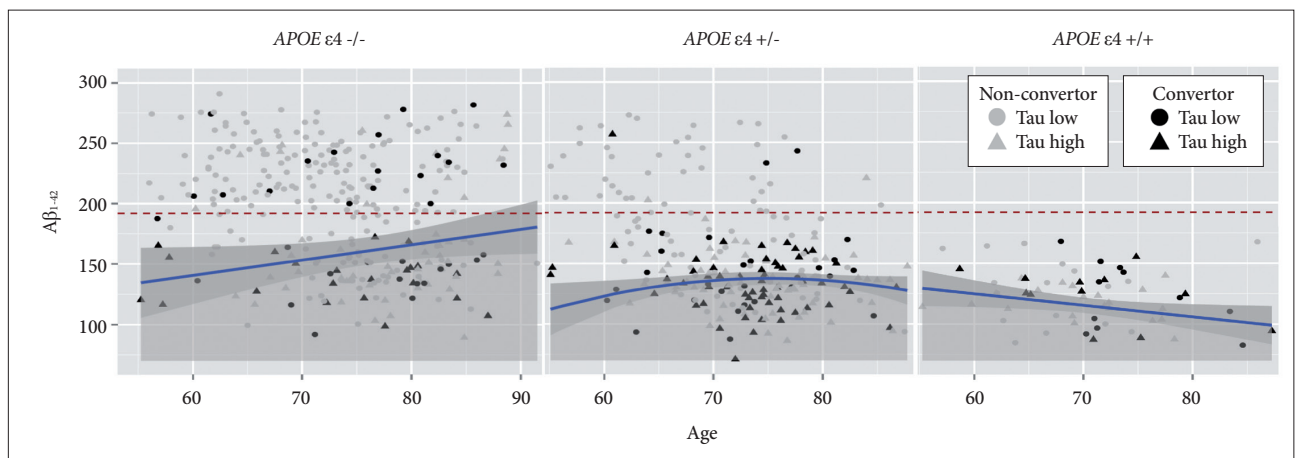


Fig. 4. Classification of patients with MCI based on the new cut-off lines of CSF biomarkers. The converters with high CSF tau (dark triangle) above upper 95% CI line (MCI with abnormalities in Aβ₁₋₄₂, t-tau, and p-tau₁₈₁) represent false negatives. The cut-off lines of Aβ₁₋₄₂ represent the upper 95% CI lines in Fig. 1. The cut-off lines of t-tau and p-tau₁₈₁ are the lower 95% CI lines of Figs. 2 and 3, respectively. Patients who showed the low tau (black circles) may have non-Alzheimer's disease dementia. CI: confidence interval, CSF: cerebrospinal fluid, MCI: mild cognitive impairment.

related to gender by *APOE* $\epsilon 2$ (Fig. 2). The distribution of CSF p-tau₁₈₁ decreased linearly with age at baseline visit (Fig. 3).

We assumed that the conversion to AD may be predicted accurately in patients with MCI using the new models. To test this hypothesis, the CSF results of MCI, based on age, sex, *APOE* $\epsilon 2$, and *APOE* $\epsilon 4$ were used to distinguish the normal/abnormal conditions as shown in Fig. 4. As shown in Fig. 5, in MCI without *APOE* $\epsilon 4$, our cut-off methods showed lower false positives in the predicted normal group and lower false

negatives in the predicted AD group (Fig. 5). However, in MCI with *APOE* $\epsilon 4$ heterozygotes, our cut-off methods produced higher false negative rate in the predicted normal group. In *APOE* $\epsilon 4$ homozygotes, it played no role in predicting the conversion, because *APOE* $\epsilon 4$ homozygote itself was a significant predictor of disease.

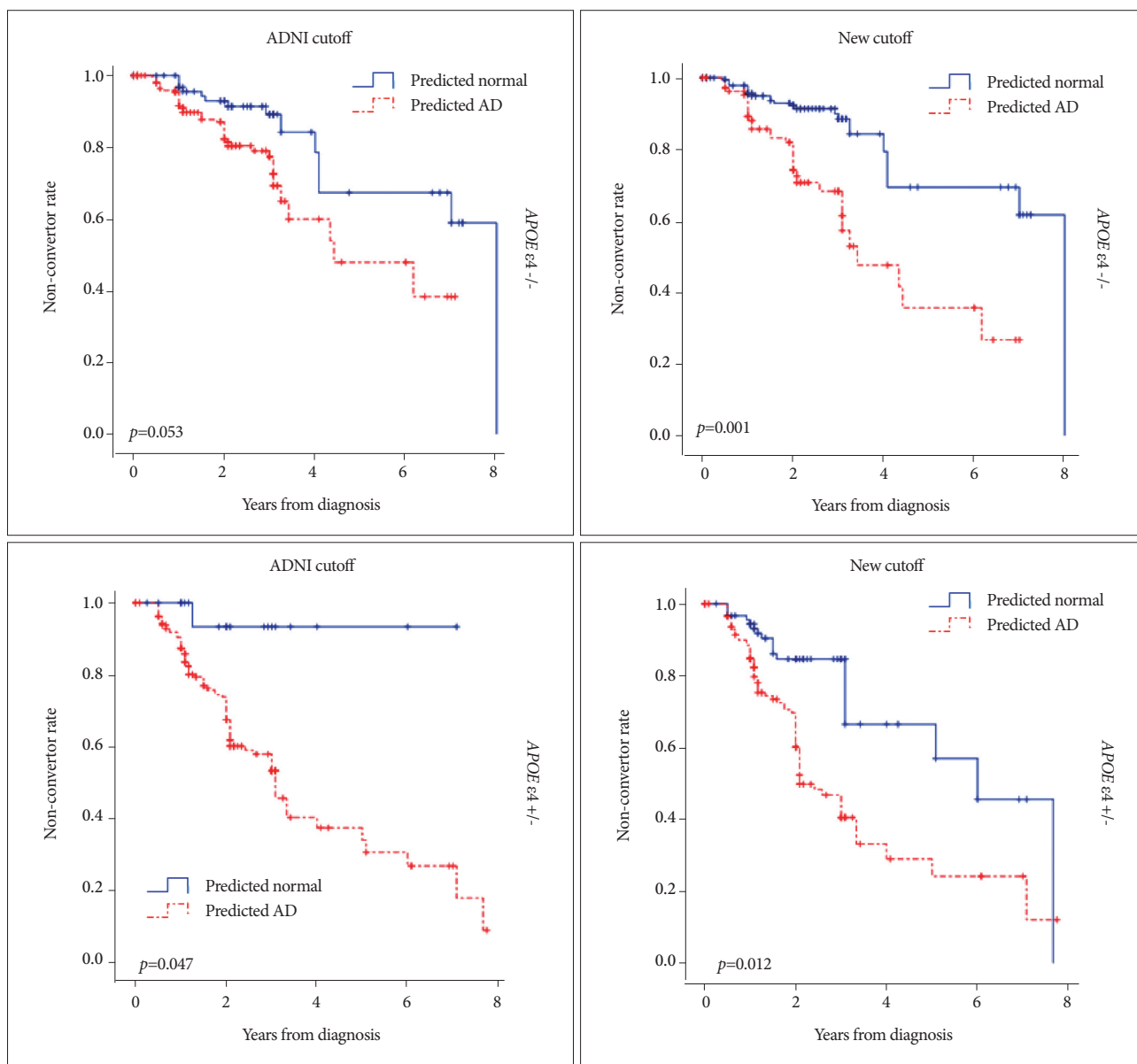


Fig. 5. Kaplan-Meier curves comparing conversion from MCI to dementia using existing/new diagnostic methods. *p* was obtained by the Cox proportional hazards model adjusting for age, gender, education years, and clinical dementia rating sum of box. The left column represents the results of current diagnostic methods, and the right column displays results of new diagnostic methods. The upper panels show results for MCI without *APOE* $\epsilon 4$, and the lower panels displays results of MCI with *APOE* $\epsilon 4$ heterozygotes. AD: Alzheimer's disease, ADNI: Alzheimer's Disease Neuroimaging Initiative, MCI: mild cognitive impairment.

DISCUSSION

In our study, we considered only patients with AD for the determination of the cut-off values for the CSF biomarkers. In addition, conversion from MCI to AD can be more accurately predicted by considering age, sex, *APOE* $\epsilon 2$, and *APOE* $\epsilon 4$ status. The new cut-off methods were particularly accurate for subjects without *APOE* $\epsilon 4$.

Our study provided insight into the role of CSF biomarkers by plotting their concentrations according to the related factors. The interaction between age and *APOE* $\epsilon 4$ affected the concentrations of CSF $A\beta_{1-42}$, and therefore, age and the *APOE* $\epsilon 4$ status were analyzed separately (Fig. 1). In the *APOE* $\epsilon 4$ non-carriers, the concentration of CSF $A\beta_{1-42}$ was correlated linearly with age, although it showed a negative quadratic curve according to age in the *APOE* $\epsilon 4$ heterozygotes. In the *APOE* $\epsilon 4$ homozygotes, it was reduced to a negative correlation depending on age. Interestingly, the t-tau levels varied between patients with and without *APOE* $\epsilon 2$ (Fig. 2). The levels of t-tau also differed according to sex. The CSF p-tau₁₈₁ concentration was decreased according to age.

Our cut-off methods were accurate predictors of conversions particularly in MCI without *APOE* $\epsilon 4$ (Fig. 5). Introduction of preventive medicines may decrease the cost of our methods by decreasing falsely classified MCI with AD signatures. In addition, it increases the effectiveness of preventive medicines by decreasing the falsely classified MCI without AD signatures. However, our methods may decrease the specificity for MCI with *APOE* $\epsilon 4$.

Age was an important factor associated with CSF $A\beta_{1-42}$ in AD without *APOE* $\epsilon 4$ and with p-tau₁₈₁. The older patients with AD show mixed pathologies of subcortical vascular dementia, hippocampal sclerosis, argyrophilic grain disease, and suspected non-amyloid pathology.¹²⁻¹⁴ The additional pathologies can cause dementia in patients with less severe amyloid or tau burden in their brain. However, further studies are required to elucidate the role of gender in CSF t-tau. In addition, the further studies are needed to account for the age-related changes in CSF $A\beta_{1-42}$ found in the *APOE* $\epsilon 4$ heterozygotes, because of the lower number of patients in young (<70) and old (>80) groups compared with the 70–80 year age group.

We found two noble points. First, we found factors that affect CSF biomarkers in patients with AD. Recently, Li et al.¹⁵ and Lautner et al.¹⁶ reported that age, sex, and *APOE* $\epsilon 4$ are factors that affect CSF $A\beta_{1-42}$ in subjects with normal cognition. These two studies overlooked that the CSF biomarkers in subjects with normal cognition showed average results for two completely different groups—high risk and low risk groups for AD. The proportion of high risk patients for AD increased with

age in subjects with normal cognition. Second, we have redefined the cut-off methods of CSF biomarkers using factors that affect CSF biomarkers. In this study, we indicated that the current cut-off values for *APOE* $\epsilon 4$ noncarrier was less specific and resulted in several false negatives.

In conclusion, we established better cut-off values of CSF biomarkers, which were made only from patients with AD, and not from controls. In addition, age, sex, and *APOE* genotype play a key role in the interpretation of the cut-off values of CSF biomarkers.

Conflicts of Interest

The authors have no financial conflicts of interest.

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