

Original Article
Neuroscience



Cerebrospinal Fluid Biomarkers for the Diagnosis and Classification of Alzheimer's Disease Spectrum

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 OPEN ACCESS

Received: Jul 2, 2020

Accepted: Aug 27, 2020

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ABSTRACT

Background: Cerebrospinal fluid (CSF) biomarkers are increasingly used in clinical practice for the diagnosis of Alzheimer's disease (AD). We aimed to 1) determine cutoff values of CSF biomarkers for AD, 2) investigate their clinical utility by estimating a concordance with amyloid positron emission tomography (PET), and 3) apply ATN (amyloid/tau/neurodegeneration) classification based on CSF results.

Methods: We performed CSF analysis in 51 normal controls (NC), 23 mild cognitive impairment (MCI) and 65 AD dementia (ADD) patients at the Samsung Medical Center in Korea. We attempted to develop cutoff of CSF biomarkers for differentiating ADD from NC using receiver operating characteristic analysis. We also investigated a concordance between CSF and amyloid PET results and applied ATN classification scheme based on CSF biomarker abnormalities to characterize our participants.

Results: CSF A β 42, total tau (t-tau) and phosphorylated tau (p-tau) significantly differed across the three groups. The area under curve for the differentiation between NC and ADD was highest in t-tau/A β 42 (0.994) followed by p-tau/A β 42 (0.963), A β 42 (0.960), t-tau (0.918), and p-tau (0.684). The concordance rate between CSF A β 42 and amyloid PET results was 92%. Finally, ATN classification based on CSF biomarker abnormalities led to a majority of NC categorized into A-T-N-(73%), MCI as A+T-N-(30%)/A+T+N+(26%), and ADD as A+T+N+(57%).

Conclusion: CSF biomarkers had high sensitivity and specificity in differentiating ADD from NC and were as accurate as amyloid PET. The ATN subtypes based on CSF biomarkers may further serve to predict the prognosis.

Keywords: Alzheimer Disease; Cerebrospinal Fluid; Amyloid; tau; Positron Emission Tomography; Biomarkers; Classification

INTRODUCTION

Alzheimer's disease (AD), which is characterized by pathological hallmarks of β -amyloid (A β) and neurofibrillary tau tangles, is a continuum disorder largely with three different

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Funding

This research was funded by Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI14C2746 and HI19C1132).

Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Na DL. Data curation: Lee J, Jang H, Kang SH, Kim J, Kim JS, Kim JP, Kim HJ, Seo SW, Na DL. Formal analysis: Lee J, Jang H. Funding acquisition: Na DL. Investigation: Jang H, Seo SW, Kim HJ, Na DL. Project administration: Na DL. Resources: Lee J, Jang H, Kang SH, Kim JS, Kim HJ, Seo SW. Supervision: Na DL. Writing - original draft: Lee J, Jang H. Writing - review & editing: Na DL.

clinical stages: normal cognition (NC, or preclinical), mild cognitive impairment (MCI, or prodromal), and dementia.¹⁻³ Since previous diagnostic criteria for AD were based mainly on clinical manifestations rather than on biomarkers, early diagnosis for preclinical or prodromal AD was difficult. However, advances in AD biomarker development using positron emission tomography (PET) or cerebrospinal fluid (CSF) have enabled *in vivo* identification of pathologies, and therefore early diagnosis of patients even in the preclinical or prodromal stages has become possible. More recent research criteria for AD proposed by the National Institute on Aging and Alzheimer's Association (NIA-AA) task force incorporated both A β phenotyping and clinical status to characterize patients.³⁻⁵ Further, a new research framework using a multiple-biomarker-based definition of AD in living patients was recently proposed^{6,7}; it included biomarkers for A β (A), pathologic tau (T), and neurodegenerative/neuronal injury (N), called the ATN (amyloid/tau/neurodegeneration) system.⁴

Individuals with preclinical AD or prodromal AD show changes in CSF amyloid. Specifically, a decreased level of β -amyloid 1-42 (A β 42) and increased level of total tau (t-tau) and phosphorylated tau (p-tau) in CSF are useful for the early diagnosis of AD.⁸ CSF biomarkers may have several advantages over amyloid PET. First, CSF tests are less expensive and more accessible and have no radiation exposure issue compared to amyloid PET. Second, prior studies showed that changes in CSF A β 42 precede amyloid PET uptakes,^{9,10} suggesting that CSF biomarkers may be more sensitive in identifying individuals with preclinical AD. Third, the most noteworthy benefit of CSF biomarkers is that CSF gives more information about AD-related pathologies than does amyloid PET. That is, CSF biomarkers provide not only amyloid status (disease marker) but also information on the tau pathology biomarker (phosphorylated tau) and neurodegenerative marker (total tau) as well.¹¹ Finally, as novel biomarkers such as neurofilament or neurogranin are being developed,¹²⁻¹⁴ one lumbar puncture can provide a variety of information encompassing AD pathology or other neurodegeneration in the future.

One issue with CSF biomarkers, however, is that there exists variability in cutoff values between laboratories. It is therefore necessary to develop laboratory-specific cutoff values to enable application of these values in clinical judgement. In line with this need, a recent study using multicenter CSF data from Korea reported new cutoff levels of CSF AD biomarkers and compared them with those from Western studies.¹⁵ However, this study included participants based on clinical diagnosis only; investigating the concordance between CSF and PET positivity or accuracy of CSF biomarkers for detecting amyloid pathology was unavailable. Besides, the cutoff values developed in this study were intended to differentiate ADD from other neurodegenerative disorder. Considering that other neurodegenerative disorders may have concomitant amyloid pathology, developed cutoff values might not be pathology specific.

Therefore, in this study, we first investigated CSF biomarkers across three different cognitive groups (NC, MCI, and AD dementia [ADD]) in a single large center in Korea in order to establish cutoff values of CSF biomarkers that could differentiate ADD from NC. Second, we observed the concordance of CSF biomarkers and amyloid PET results to confirm the reliability of CSF biomarkers. Finally, we attempted to apply the ATN biomarker classification system to our patients based on CSF biomarker cutoffs derived from our study, which would have clinical implications, in that both amyloid and tau information rather than amyloid alone can provide more accurate prediction of patients' prognoses.

METHODS

Study participants

In this study, we included a total of 400 participants who had undergone CSF analysis at the Samsung Medical Center, Seoul, Korea, from February 2011 to July 2019. Participants received a detailed dementia evaluation, including clinical history, neurologic examination, neuropsychological tests, blood tests to exclude secondary causes of dementia, brain magnetic resonance imaging (MRI), and amyloid PET and APOE genotyping. Out of the initial 400 participants, we included only individuals with NC, amnesic MCI (aMCI), or ADD; we excluded 224 patients with other diagnoses, such as normal pressure hydrocephalus (NPH), subcortical vascular cognitive impairment (SVCI, cognitive impairment associated with severe deep white matter hyperintensities [WMH], which was defined as a deep WMH lesion ≥ 25 mm and periventricular WMH lesion ≥ 10 mm), neurodegenerative disorders with parkinsonism, traumatic brain injury, frontotemporal dementias, brain tumor, neurosyphilis, CO poisoning, epilepsy, autoimmune encephalitis, or Creutzfeldt-Jakob disease. When ADD, aMCI, or NC participants showed a gait disturbance and an Evan's ratio over 3.0 on MRI, they were referred to as ADD, aMCI, or NC with "combined hydrocephalus" and excluded from this study ($n = 37$).

ADD was clinically diagnosed when patients met the diagnostic criteria for probable ADD according to NIA-AA criteria, with cognitive impairment, such as memory loss, word-finding difficulties, or visual/spatial problems significant enough to impair a person's ability to function independently.¹⁶ AMCI was diagnosed according to Peterson's MCI criteria,¹⁷ which was characterized by memory complaints usually corroborated by an informant, objective memory impairment for age, essentially preserved general cognitive function, and largely intact functional activities. We recruited NC participants from our memory or headache clinic or orthopedic surgery department, where they received spinal anesthesia. All the NC individuals met the following criteria: no history of neurological or psychiatric disorders, normal cognitive function, normal activities of daily living with or without subjective memory complaints, and normal CSF cell count.

CSF collection and AD biomarker analysis

For most participants, CSF samples were collected from a lumbar puncture done in the L3-4 or L4-5 intervertebral spaces using a 20 or 22G needle. Fasting was not required. A subset of 28 AD and 12 aMCI patients had Ommaya reservoir insertion for a clinical trial of intraventricular stem-cell injection, 'Safety and Exploratory Efficacy Study of NEUROSTEM® Versus Placebo in Patients with Alzheimer's Disease (ClinicalTrials.gov Identifier: NCT02054208)'¹⁸ and the CSF obtained from the Ommaya reservoir before stem-cell injection was used for the current study. All CSF samples were collected into 15-mL polypropylene tubes at the time of the tap and were then sent to Samsung Medical Center laboratory within 30 minutes after collection. After samples were centrifuged at 2000 g for 10 minutes within 4 hours after collection, aliquots (1 mL) prepared from these samples at room temperature were immediately stored in bar-code-labeled polypropylene vials at -70°C .¹⁹ In our laboratory, we run assays for CSF biomarkers once CSF samples are collected from 30 to 40 patients, using INNOTEST enzyme-linked immunosorbent assay (ELISA) kits (Fujirebio Europe N.V.). The CSF biomarkers included levels of A β 42 (Amyloid- β (1-42)), t-tau, and p-tau (181 phosphorylated tau).

A β PET acquisition and definition of A β positivity

A total of 90 patients underwent A β PET (PiB, $n = 5$; florbetaben, $n = 80$; flutemetamol, $n = 5$). For 11C-PiB PET, a 30-minute emission static PET scan was done 60 minutes after bolus injection of a mean dose of 420 MBq 11C-PiB into an antecubital vein. For 18F-florbetaben PET, a 20-minute emission PET scan in dynamic mode (consisting of 4×5 min frames) was done 90 minutes after bolus injection of a mean dose of 381 MBq 18F-florbetaben into an antecubital vein. For flutemetamol PET, a 20-minute emission static PET scan in dynamic mode (consisting of 4×5 min frames) was done 90 minutes after bolus injection of a mean dose of 185 MBq flutemetamol into an antecubital vein.

We defined A β PET positivity (PET+) for the three different types of PET images as follows: 1) Global PiB SUVR (assessed from the volume-weighted average SUVR of 28 bilateral cerebral cortical VOIs) of greater than 1.5 as described in our previous study,²⁰ 2) visual rating score on florbetaben PET of 2 or 3 on the brain A β plaque load scoring system,²¹ 3) positive visual interpretation of ¹⁸F-flutemetamol PET in any one of the five brain regions (frontal, parietal, posterior cingulate and precuneus, striatum, and lateral temporal lobes) in either hemisphere.²²

Statistical analyses

We used ANOVA (Analysis of Variance) to compare clinical characteristics between NC, aMCI and ADD, and ANCOVA (Analysis of Covariates) to compare CSF biomarker levels with adjustment of age. We used paired t -tests to compare CSF biomarker levels between each of the two groups.

We did receiver operating characteristic (ROC) analyses to establish the cutoffs for each CSF biomarker (A β 42, t-tau, p-tau) and ratio (t-tau/ A β 42, p-tau/ A β 42) that best differentiated clinical ADD from NC. The cutoffs for biomarkers were defined as values that give the maximum Youden index (sensitivity + specificity - 1). We then calculated the sensitivity and specificity for each cutoff value. We additionally compared AUC of all models using Delong method.

We defined CSF biomarker levels as abnormal (CSF+) when CSF A β 42 was lower than cutoff values. Therefore, the concordance rate of amyloid PET and CSF biomarker results was calculated as the number of CSF+/PET+ plus CSF-/PET- cases over the total number of participants in the analysis. All statistical tests were conducted using the SPSS version 22 (SPSS, Inc., Chicago, IL) and STATA version 15 (StataCorp LLC).

Ethics statement

We obtained written informed consent for obtaining CSF data from each patient, and this study was approved by the Institutional Review Board at the Samsung Medical Center (2010-12-003 & 2015-06-093-001).

RESULTS

Demographics and clinical characteristics of study participants

Table 1 shows the clinical characteristics and CSF biomarker levels of the study participants. The mean age ($P = 0.205$) and education year ($P = 0.881$) were not significantly different across groups. The proportion of female was different across groups ($P = 0.025$), being highest at 82% in NC, followed by 61% in aMCI and 60% in ADD. The frequency of APOE4 carriers was higher in aMCI (15/22, 67%) and ADD (52/92, 55%) than in NC (1/6, 16%),

Table 1. Demographics and clinical characteristics of the study participants (n = 139)

Characteristics	NC (n = 51)	aMCI (n = 23)	ADD (n = 65)	P value
Clinical characteristics				
Age, yr	64.1 ± 11.0	67.5 ± 8.1	63.3 ± 9.3	0.205
Female sex	42 (82)	14 (61)	39 (60)	0.025
Education, yr	11.8 ± 4.4	12.4 ± 4.3	11.9 ± 4.1	0.881
APOE4 carriers, % (No.)	16 (1/6)	67 (14/21)	55 (33/60)	0.096
Amyloid PET positivity, % (No.)	0 (0/2)	78 (18/23) ^a	94 (61/65) ^a	< 0.001
CSF biomarkers				
Aβ42, pg/mL	1,033.5 ± 330.5	468.5 ± 299.7 ^a	372.2 ± 169.1 ^a	< 0.001
T-tau, pg/mL	185.3 ± 96.1	400.5 ± 175.4 ^a	560.4 ± 309.0 ^{a,b}	< 0.001
P-tau, pg/mL	50.3 ± 17.4	57.5 ± 23.3	70.4 ± 34.7 ^a	0.001
T-tau/Aβ42	0.195 ± 0.141	1.230 ± 0.840 ^a	1.668 ± 0.896 ^{a,b}	< 0.001
P-tau/Aβ42	0.051 ± 0.026	0.155 ± 0.078 ^a	0.201 ± 0.090 ^{a,b}	< 0.001

Values are represented as mean ± standard deviation or number (%) unless otherwise indicated.

NC = normal control, aMCI = amnesic mild cognitive impairment, ADD = Alzheimer's disease dementia, APOE4 = apolipoprotein E4 gene, PET = positron emission tomography, CSF = cerebrospinal fluid, Aβ42 = beta amyloid 1-42, T-tau = total tau, P-tau = phosphorylated tau.

^aP < 0.05 compared to NC; ^bP < 0.05 compared to aMCI.

although the difference was not statistically significant ($P = 0.096$). The frequency of amyloid PET positivity was different across groups ($P < 0.001$) with 78% in aMCI and 94% in ADD, and two NC participants who underwent amyloid PET tested negative.

Levels of CSF biomarkers in different cognitive groups

CSF biomarkers levels (Aβ42 [pg/mL], t-tau [pg/mL], p-tau [pg/mL], t-tau/Aβ42, p-tau/Aβ42) in the three diagnostic groups are shown in **Table 1** and **Fig. 1**. CSF Aβ42 levels were 1033.5 ± 330.5, 400.5 ± 175.4, and 372.2 ± 169.1 pg/mL in NC, aMCI, and ADD, respectively (ANCOVA, $P < 0.001$). CSF t-tau levels were 185.3 ± 96.1, 391.0 ± 177.9, and 507.0 ± 348.5 (ANCOVA, $P < 0.001$), and CSF p-tau levels were 50.3 ± 17.4, 57.1 ± 22.8, and 65.0 ± 39.9 in NC, aMCI and ADD (ANCOVA, $P = 0.001$), respectively. CSF t-tau/Aβ42 ratios were 1.668 ± 0.896, 1.230 ± 0.840, and 0.195 ± 0.141 (ANCOVA, $P < 0.001$), and CSF p-tau/Aβ42 ratios were 0.201 ± 0.090, 0.155 ± 0.078, and 0.051 ± 0.026 in NC, aMCI, and ADD (ANCOVA, $P < 0.001$). All biomarkers were significantly different between the three groups, even after controlling for age. *Post hoc* analysis of paired *t*-tests showed that Aβ42 was different between NC vs. aMCI ($P < 0.001$) and NC vs. ADD ($P < 0.001$) groups, but it was not significantly different between ADD and aMCI ($P = 0.391$). However, t-tau, t-tau/Aβ42, and p-tau/Aβ42 showed differences between NC vs. aMCI, NC vs. ADD, and aMCI vs. ADD, whereas p-tau differed only between NC and ADD ($P = 0.001$).

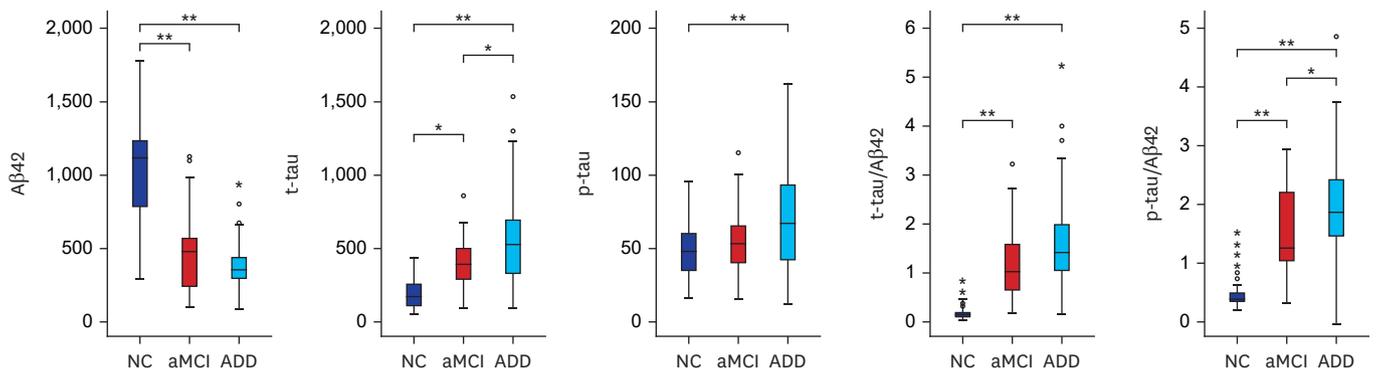


Fig. 1. Comparison of CSF biomarkers among NC, aMCI and ADD groups.

CSF = cerebrospinal fluid, NC = normal control, aMCI = amnesic mild cognitive impairment, ADD = Alzheimer's disease dementia, Aβ42 = beta amyloid 1-42, t-tau = total tau, p-tau = phosphorylated tau.

* $P < 0.05$; ** $P < 0.01$.

Cutoff values of CSF AD biomarkers

Table 2 shows the ROC curve-derived cutoff values of the CSF biomarkers that best distinguished ADD from NC. T-tau/Aβ42 showed the highest AUC (0.994) followed by p-tau/Aβ42 (0.963), Aβ42 (0.960), and t-tau (0.918). In contrast, p-tau alone showed a low AUC of 0.684 (**Fig. 2**). The cutoff values were 667.91 pg/mL (Sensitivity [Sn] 88%, Specificity [Sp] 97%) for Aβ42, 355.27 pg/mL (Sn 73%, Sp 96%) for t-tau, 61.82 pg/mL (Sn 59%, Sp 82%) for p-tau, 0.641 (Sn 97%, Sp 98%) for t-tau/ Aβ42, and 0.103 (Sn 92%, Sp 94%) for p-tau/Aβ42. When we compared the AUC between models, the AUC for p-tau was lower than others ($P < 0.001$), and the AUC for Aβ42 model did not differ from other models except for p-tau model. Among the three models, the AUC for t-tau/Aβ42 was significantly higher than those of p-tau/Aβ42 ($P = 0.032$) and t-tau ($P < 0.001$), and the AUC for p-tau/Aβ42 was higher than that of t-tau ($P = 0.048$).

Concordance of CSF Aβ biomarker and amyloid PET

We further investigated the concordance of amyloid PET results and CSF Aβ biomarker cutoff-based results. Among 90 participants who underwent amyloid PET, the number of CSF+PET+ and CSF-PET- cases were 77 and 6, which resulted in a 92% (83/90) concordance rate. Among the seven discordant cases for PET and CSF Aβ42, two were PET+/CSF- and five were PET-/CSF+. The clinical characteristics and CSF biomarker levels of the discordant cases are described in **Table 3**. Two PET+/CSF- cases were aMCI and ADD who showed CSF

Table 2. CSF biomarker cutoff values that best discriminate ADD from NC

CSF biomarker	Cutoff	AUC	Sensitivity, %	Specificity, %
Aβ42, pg/mL	667.9	0.960	97	88
T-tau, pg/mL	355.3	0.918	73	96
P-tau, pg/mL	61.8	0.684 ^{a,b}	59	82
T-tau/Aβ42	0.641	0.994 ^{b,c}	97	98
P-tau/Aβ42	0.103	0.963 ^{b,c,d}	92	94

CSF = cerebrospinal fluid, ADD = Alzheimer's disease dementia, NC = normal control, AUC = area under curve, Aβ42 = beta amyloid 1-42, T-tau = total tau, P-tau = phosphorylated tau.

^a $P < 0.05$ compared to AUC of the model for Aβ42; ^b $P < 0.05$ compared to AUC of the model for T-tau; ^c $P < 0.05$ compared to AUC of the model for P-tau; ^d $P < 0.05$ compared to AUC of the model for T-tau/Aβ42.

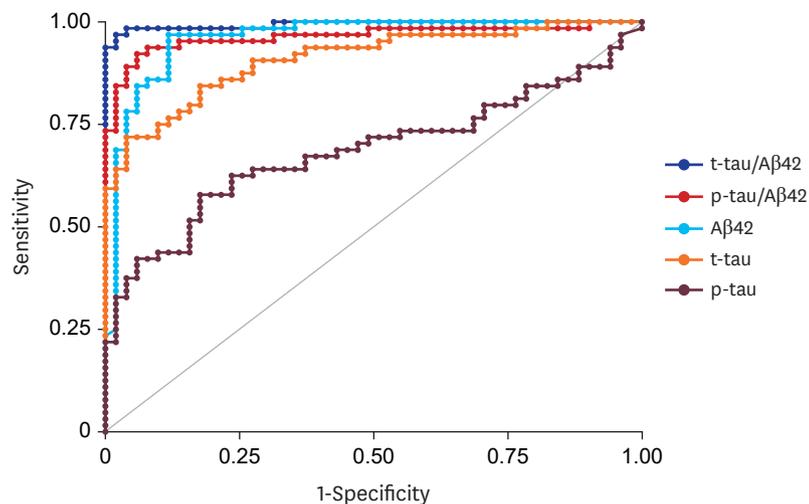


Fig. 2. ROC curves of CSF biomarkers for discrimination between NC and ADD.

ROC = receiver operating characteristic, CSF = cerebrospinal fluid, NC = normal control, ADD = Alzheimer's disease dementia, Aβ42 = beta amyloid 1-42, t-tau = total tau, p-tau = phosphorylated tau.

Table 3. PET-CSF discordant cases

Case	Patient characteristics		CSF biomarkers (cutoff values)				
			Aβ42 (667.9)	t-tau (355.3)	p-tau (61.8)	t-tau/Aβ42 (0.641)	p-tau/Aβ42 (0.103)
PET+CSF-							
M/79	aMCI		777.9	305.7	63.9	0.393	0.082
F/84	ADD		794.6	713.7	89.9	0.898	0.113
PET-CSF+							
M/77	aMCI		446.4	612.8	100.6	1.373	0.225
F/63	aMCI		548.4	391.3	57.9	0.714	0.106
F/69	Clinically diagnosed as AD as memory impairment was the main symptom, but the diagnosis was changed to bvFTD with detailed clinical interview along with negative amyloid PET		274.1	628.3	97.8	2.293	0.335
F/55	Clinically diagnosed as AD. However, the diagnosis was changed to bvFTD after the review of clinical course along with negative amyloid PET, and positive flortaucipir (tau) PET that demonstrated increased uptakes in both frontotemporal areas.		337.0	408.4	61.1	1.212	0.181
M/82	Clinically diagnosed as AD, but found to have minimal amount of chronic SDH.		486.7	1,529.2	24.1	3.142	0.050

aMCI = amnesic mild cognitive impairment, ADD = Alzheimer’s disease dementia, APOE4 = apolipoprotein E4 gene, PET = positron emission tomography, CSF = cerebrospinal fluid, Aβ42 = beta amyloid 1-42, T-tau = total tau, P-tau = phosphorylated tau, bvFTD = behavioral variant frontotemporal dementia, SDH = subdural hemorrhage.

Aβ42 levels of 777.9 and 794.6. Among the five PET-/CSF+ cases, two were diagnosed as ADD when CSF tests were done but we changed the diagnosis to behavior variant frontotemporal dementia (bvFTD) when we followed them up with detailed history, MRI review, and the support from the negative amyloid PET scan. Another PET-/CSF+ patient was diagnosed with ADD at first but was found to have chronic subdural hemorrhage (SDH). Therefore, we conducted ROC analysis again after excluding these two bvFTD patients, and the cutoff of Aβ42 and the AUC remained the same (cutoff 667.9, AUC 0.959).

ATN biomarker classification using CSF AD biomarker cutoff

We used cutoffs of CSF Aβ42 (= 667.9), p-tau (= 61.8), and t-tau levels (= 355.3) to define abnormal Aβ (A+), abnormal tau (T+), and abnormal neurodegeneration (N+) to apply ATN biomarker classification in our study subjects. As shown in Fig. 3, a majority of NC was categorized into A-T-N- (73%), followed by A-T+N- (14%). A+T-N- and A+T+N+ occupied 30%

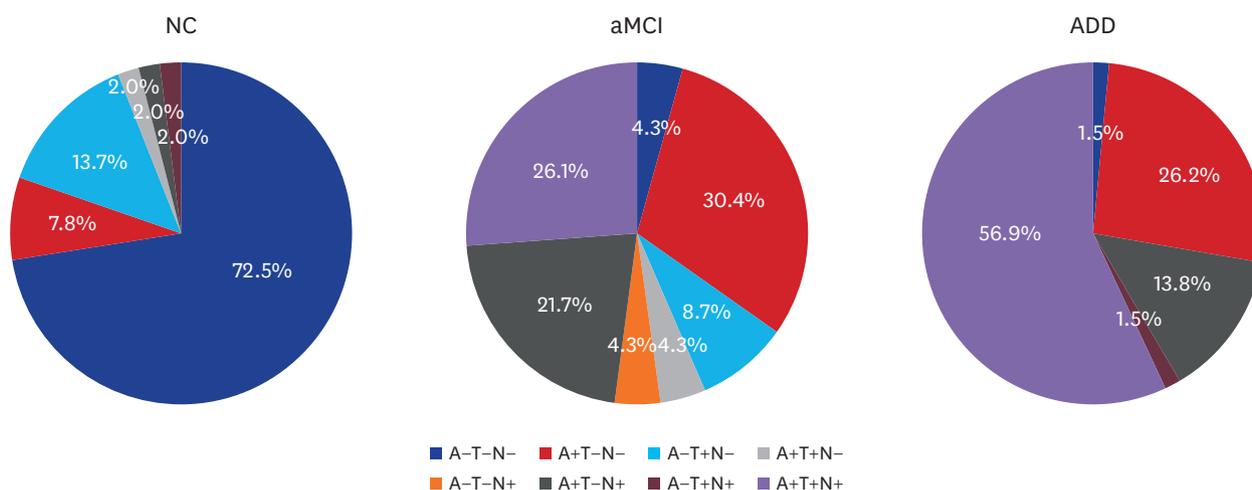


Fig. 3. Application of ATN system using CSF biomarker cutoff values. ATN = Amyloid-Tau-Neurodegeneration, CSF = cerebrospinal fluid, NC = normal control, aMCI = amnesic mild cognitive impairment, ADD = Alzheimer’s disease dementia.

and 26% of aMCI, followed by A+T-N+ (22%), and most ADD patients were categorized into A+T+N+ (57%), and subsequently into A+T-N- (26%) and A+T-N+ (14%).

DISCUSSION

In this study, we recruited 139 participants and compared CSF biomarker levels across three groups with different cognition levels (NC, aMCI, and ADD) and developed CSF biomarker cutoffs to differentiate ADD from NC. We also tried to investigate the reliability and clinical utility of CSF biomarkers by investigating concordance rates between amyloid PET and CSF results. Our major findings were as follows. First, CSF biomarkers were significantly different between NC, aMCI and ADD groups. Second, the cutoff values we obtained were accurate in differentiating ADD from NC and were highly concordant with amyloid PET results. Third, whereas amyloid PET can only categorize patients into amyloid negative versus positive groups, CSF biomarkers can further categorize patients according to their amyloid and tau status. Taken together, we consider that CSF biomarkers are useful in clinical and research field.

The first major finding of our study was that CSF biomarkers were significantly different between the three diagnostic groups. CSF A β 42 level was different between NC and aMCI or ADD. However, it was not different between aMCI and ADD, which may be in line with the fact that CSF A β 42 levels change before neurodegeneration and cognitive impairment occurs, as has been explained by the sigmoid-shaped trajectory of CSF A β 42 levels.⁹ Unlike CSF A β 42, CSF t-tau, t-tau/A β 42, and p-tau/A β 42 ratios were significantly different between aMCI and ADD. These findings are also consistent with previous notions that CSF t-tau is a well-known marker for neurodegeneration.⁶ Therefore, we considered that a combination of tau and amyloid markers (such as the ratio of CSF t-tau/A β 42 or CSF p-tau/A β 42) could represent disease progression better than could an amyloid marker alone.

The second major finding of our study was that we were able to obtain cutoff values that showed high accuracy (represented as AUC) in differentiating clinical ADD from NC. Specifically, the AUC from t-tau/A β 42 was highest (0.994), followed by those from p-tau/A β 42 (0.963) and A β 42 (0.960). Such high accuracy may be explained by the fact that we took extra caution when selecting patients by excluding patients with possible NPH and patients with severe WMH (patients with SVCI). In fact, recent studies showed that CSF A β 42 levels can be decreased in pure NPH.²³⁻²⁵ We compared our cutoffs with previously established cutoffs obtained from other memory clinics in Korea. As a result, our A β 42 cutoff was comparable with Jung et al.'s (A β 42 < 631.8, p-tau > 56.4, and t-tau > 271.6) and our t-tau cutoff was comparable with Park et al.'s (A β 42 < 481.8, p-tau > 57.0, and t-tau > 326.0). However, all three groups showed different cutoff levels for each biomarker, which suggests that we need to establish ELISA cutoffs for Koreans through a multicenter study involving a large number of patients with amyloid PET information.

Unexpectedly, there was a discrepancy between the AUC from t-tau (0.918) and p-tau (0.684), a finding inconsistent with previous studies which state that CSF p-tau has been considered to be more closely related to neurofibrillary tangles pathologies than is to CSF t-tau.²⁶⁻²⁸ However, CSF p-tau levels might change even in ADD according to the speed of progression.²⁹ That is, unlike tau PET imaging showing accumulating tau pathology, CSF p-tau may not reflect the total amount of neurodegeneration that have occurred up to that point, but rather may be the marker of ongoing progression. Further studies to investigate

longitudinal CSF p-tau change in AD are required. Alternatively, the NC group in our study might have included preclinical participants who had neurofibrillary tangles (restricted to Braak stage I/II or even involving III/IV stage regions), although they did not complain of any cognitive symptoms.

The third major finding was that, when we compared the results of CSF A β 42 with those of amyloid PET, the concordance rate was as high as 92%. When we looked at discordant cases, CSF A β 42 levels of two PET+CSF- patients were 777.9 and 794.6, which were higher than the cutoff values. One of these two patients had higher t-tau, p-tau, and their ratio with A β 42 than cutoffs, therefore, a combination with the tau biomarker could be more accurate than is CSF A β 42 alone for diagnosis of ADD. There were also five PET-CSF+ cases, among which two were aMCI cases as expected, because CSF biomarker changes occur prior to amyloid uptake on PET. One interesting case was an 82-year-old man who was diagnosed with ADD but had a markedly high t-tau level of 1,529.2, but a low p-tau of 24.1. When we reviewed his case later because of the exceptionally high t-tau levels, we found a minimal amount of chronic SDH on his CT image conducted before CSF tapping. Therefore, we need to reconsider a diagnosis in cases where t-tau levels are higher than the p-tau levels. It is also noteworthy that there are other conditions causing false positive CSF A β 42 and t-tau results: CSF A β 42 is reported to be decreased, albeit inconsistently, in frontotemporal dementia, vascular dementia, dementia of Lewy body,³⁰ or in normal pressure hydrocephalus.³¹ CSF t-tau, which is a neuronal injury marker, can increase in various brain disorders such as stroke, trauma, and non-AD dementias, especially in rapidly progressing dementias such as Creutzfeldt-Jakob Disease.

Nevertheless, a high concordance between amyloid PET and CSF results in our study has a clinical implication, in that CSF biomarkers can be an alternative to amyloid PET in clinical practice, since CSF biomarkers are less expensive and more accessible than an amyloid PET scan in most clinical settings. Although lumbar puncture is invasive than amyloid PET and requires a bed rest, CSF study still has its strengths in that it can detect various neurodegenerative biomarkers simultaneously once CSF is sampled. As in our study, information on amyloid and tau can be obtained from a single CSF sample, unlike a PET scan that should be done separately to detect two different biomarkers. In addition, new CSF biomarkers, such as neurofilament or neurogranin, are increasingly considered as promising biomarkers, which suggests that the potential of new CSF biomarkers for a variety of neurodegenerative diseases may increase.

Finally, when we used our CSF cutoff values to apply ATN biomarker classification to study participants, most of the NC were A-T-N-, whereas most of the AD were A+T+N+, as expected. Interestingly, in the MCI group, the majority of patients was classified into either A+T-N- (30%) or A+T+N+ (26%), followed by A+T-N+ (22%). The percentages of these groups are largely comparable to those from a previous study.^{32,33} Another feature that our study and a previous study had in common was that there was an A-T+(N-/N+) and A-T-(N-/N+) population in the MCI group, recapitulating the heterogeneity of MCI patients. In the era of molecular targeting treatment, delineating biomarker profiles in MCI patients may help clinicians to select the potential candidates by predicting different prognoses.

The strength of our study is that it was based on comprehensive and detailed clinical and imaging data from a single center, which probably minimized biases with homogeneous study protocols. On the other hand, there are limitations in our study. First, most of the NC did not undergo amyloid PET scans. Second, we used three different PET ligands requiring

the use of different standards for amyloid positivity. However, previous studies confirmed that PiB PET uptake highly correlates with the uptakes of three ^{18}F ligands PET (florbetapir, FMM and FBB),³⁴ and FMM and FBB uptakes were highly correlated with each other as well³⁵ Third, we had 40 participants who had CSF taken from the Omayya reservoir, but others had it taken by lumbar puncture, which disturbs the homogeneity of a single-center study. Finally, one ADD patient was proven to have SDH before CSF drainage, which could disturb CSF results that were shown as a PET-CSF discordant case. Despite these limitations, considering that every center and country needs their own cutoff values, we hope that the cutoff values derived from a single center of a large memory clinic may be useful in research and clinical fields.

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