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Cerebrospinal Fluid Biomarkers for the Diagnosis of Alzheimer Disease in South Korea

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Abstract: Laboratory-specific reference values for cerebrospinal fluid (CSF) Alzheimer disease (AD) biomarkers are necessary. Our objective was to apply well-known CSF biomarkers and redetermine their diagnostic cutoff values for AD in South Korea. CSF samples from matched control subjects (n = 71), patients with AD dementia (ADD, n = 76), and other neurological disorders with cognitive decline (OND, n = 47) were obtained from 6 Korean dementia clinics according to a standardized protocol. CSF biomarker concentrations were measured using enzyme-linked immunosorbent assay. CSF biomarkers differed significantly between the ADD and control groups ($P < 0.001$ for all), and between the ADD and OND groups ($P < 0.001$ for all). The areas under the curve in differentiation of ADD from control subjects were 0.97 for Aβ42, 0.93 for total tau (tTau), 0.86 for pTau, and 0.99 for both tTau/Aβ42 and pTau/Aβ42 ratios. Our revised cutoff value for Aβ42 was higher than our previous one, whereas the values for the Tau proteins were similar. The tTau/Aβ42 ratio had the highest accuracy, 97%. Our findings highlight the usefulness of CSF AD biomarkers in South Korea, and the necessity of continually testing the reliability of cutoff values.

Key Words: Alzheimer disease, biomarker, cerebrospinal fluid, diagnosis

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A decrease in β-amyloid 1-42 (Aβ42) and an increase in total tau (tTau) and phosphorylated tau at threonine 181 (pTau181) cerebrospinal fluid (CSF) levels are useful for the early diagnosis of Alzheimer disease (AD).[1–3] These CSF profiles are incorporated as a supplementary tool to the recent AD diagnostic criteria recommended by the International Working Group-2[4] and the National Institute on Aging and the Alzheimer's Association workgroups.[5] However, high between-laboratory variability presents a serious obstacle to the sharing of biomarker data among research and clinical centers.[6,7] A global quality control program has been initiated to minimize interlaboratory variability[8] and develop a shared protocol for preanalytical procedures.[9,10] However, the establishment of laboratory-specific cutoff values is necessary to maintain internal consistency.[7,11] Furthermore, it is necessary to continually test the reliability of established CSF AD biomarker cutoffs in large populations using updated methods to ensure their clinical usefulness.[11]

Previously, we determined cutoff values for the diagnosis of AD in a preliminary study using a small number of CSF samples.[12] In the present study, we used a larger sample size, the updated version of the INNOTEST enzyme-linked immunosorbent assay (ELISA) kit, and the consensus protocol for preanalytical procedures[13] to determine new diagnostic cutoff values for the diagnosis of AD in the South Korea. Furthermore, we tested the validity of the cutoff values determined by laboratories using large sample sizes[14–18] by applying their values to our subjects.

METHODS

Subjects

CSF samples were obtained from 194 subjects [71 controls, 76 patients with AD dementia (ADD), and 47 patients with other neurological disorders with cognitive decline (OND)] from 6 Korean dementia clinics between April 2013 and 2016. The protocol was approved by the local Ethical Review Board and followed the principles of the Declaration of Helsinki. All subjects and their caregivers (in cases of dementia) provided written informed consent before participating in the study.

All participants with ADD (n = 76) and OND (n = 47) underwent comprehensive neurological, laboratory, and neuropsychological examinations, as well as magnetic resonance imaging before CSF collection (0.5 ± 0.2 mo interval)

following the protocol established by the Clinical Research Center for Dementia of South Korea (CREDOS).[19] Furthermore,[18] fluorodeoxyglucose-positron emission tomography and amyloid- positron emission tomography was performed, respectively, in 37 and 11 patients with dementia. The clinical diagnosis of AD was based on the revised clinical criteria for probable AD established by the National Institute on Aging and the Alzheimer's Association workgroups.[5] The OND group included autoimmune encephalitis (n = 3), corticobasal degeneration (n = 1), dementia with Lewy bodies (n = 5), epilepsy (n = 3), frontotemporal lobar degeneration (n = 12), metabolic encephalopathy (n = 2), major depression (n = 2), normal pressure hydrocephalus (n = 5), Parkinson disease dementia (n = 5), progressive supranuclear palsy (n = 1), spinocerebellar ataxia (n = 1), and vascular dementia (n = 7).

The age-matched controls (n = 71) were recruited longitudinally from subjects who underwent neuroimaging for various reasons (headache, dizziness, or health screening) or who were scheduled to undergo spinal anesthesia for orthopedic surgery within a week. They underwent neuropsychological testing, neuroimaging, and CSF collection. Control subjects were excluded if they had a history of cognitive complaints or significant disorders that could potentially affect cognitive function or if abnormalities were revealed by the cognitive test or neuroimaging study (magnetic resonance imaging in 34 and computed tomography in 37).

All subjects were followed for at least 6 months, beginning either before or after the lumbar puncture, to ensure that the clinical diagnoses were accurate, and uncertain cases were excluded from the study. The results of the CSF AD biomarkers were not considered in the clinical diagnosis.

APOE Genotype

Genomic DNA was extracted from all participants using a commercially available kit (QIAGEN, Venlo, the Netherlands). APOE genotyping was performed by polymerase chain reaction using an APOE genotyping PrimerMix Kit (BioCore, Seoul, Korea) according to the manufacturer's recommendations.

CSF Sampling and Analysis

The CSF sampling and storage protocols have been described previously.[13] Briefly, CSF was obtained via lumbar puncture between 8:00 AM and noon. The first 1 to 2 mL of CSF was used for a routine evaluation and the next 10 mL were collected into 15-mL polypropylene tubes (#352096; BD Falcone, Bedford, MA). The CSF samples were centrifuged at 2000*g* for 10 minutes at room temperature within 4 hours of collection. Directly, 400 μL of supernatant was aliquoted into 500-μL polypropylene CryoTubes with screw caps (#72.730.006 or 72.730.005; Sarstedt, Nümbrecht, Germany) and stored at −80°C until assayed. Stored samples were packed in dry ice. CSF analyses were performed in a biomarker core laboratory. The samples were analyzed using the improved version of the INNOTEST ELISA kit (Fujirebio Diagnostics, Ghent, Belgium), which provides ready-to-use antibody calibrators and run validation controls in place of concentrated standards.[20–22] Eight CSF samples were analyzed, including 3 pooled samples, in the initial comparison between the runs. The 3 pooled specimens were then used to monitor additional runs.

Statistical Analysis

The normality of the continuous variables was tested using the Shapiro-Wilk test. CSF Aβ42, tTau, and pTau181 values were log-transformed because of skewed distributions, and the logarithmic values were used for between-group comparisons. Associations between the AD biomarkers and diagnostic groups were assessed using analysis of variance followed by Tukey post hoc test. χ^2 tests were used to compare categorical variables. Multiple linear regression analysis was used to investigate the influence of age and *APOEε4* carrier status on CSF AD biomarker validity. Receiver operating characteristic (ROC) curves were generated, and areas under the curve (AUCs) with 95% confidence intervals (CI) were used to identify CSF AD biomarkers that differentiated patients with ADD from control subjects. The cutoffs for individual biomarkers were the scores that yielded the maximum Youden index (sensitivity + specificity − 1). The sensitivity and specificity were calculated for each cutoff value. All statistical tests were conducted using the Statistical Package for the Social Sciences version 19.0 (SPSS Inc., Chicago, IL). $P < 0.05$ were deemed to indicate statistical significance. Bonferroni correction was used to adjust for multiple comparisons.

RESULTS

Demographic and clinical characteristics of the subjects were compared according to the clinical diagnosis (Table 1). The percentage of females ($P = 0.019$) was higher in the ADD and control groups than in the OND group. The Mini-Mental State Examination scores of the ADD (17.7 ± 6.7) was lowest followed by OND (20.3 ± 6.1) groups and then control subjects (28.0 ± 1.8; $P < 0.001$). The clinical dementia rating and sum of box scores were significantly higher in the ADD (1.1 ± 0.8 and 5.8 ± 4.8, respectively) and OND (1.0 ± 1.0 and 4.8 ± 5.3, respectively) groups compared with controls (0 ± 0; $P < 0.001$ for all). As expected, the percentage of *APOEε4* carriers was higher in the ADD than in the control and OND group ($P = 0.002$).

The interrun variability between the ELISA measurements was 5.8 ± 4.7% for Aβ42, 16.3 ± 4.2% for tTau, and 11.5 ± 8.0% for pTau181 for the coefficients of variance. The CSF Aβ42 protein levels were lowest in the ADD group (316.1 ± 105.7 pg/mL) compared with the control (676.0 ± 175.1 pg/mL) and OND (565.8 ± 187.9 pg/mL) groups ($F_{2,\ 191} = 85.6$; $P < 0.001$), whereas the ADD group had the highest tTau (583.0 ± 286.4 pg/mL) and pTau (73.8 ± 28.8 pg/mL) protein levels compared with the control (212.5 ± 67.3 pg/mL for tTau and 41.9 ± 12.8 pg/mL for pTau) and OND (227.9 ± 120.0 pg/mL for tTau and 37.0 ± 15.4 pg/mL for pTau) groups ($F_{2,\ 191} = 90.2$; $P < 0.001$ for tTau; $F_{2,\ 191} = 51.1$; $P < 0.001$ for pTau) (Fig. 1). Post hoc analysis revealed that the Aβ42 levels were lower in the OND group than in the control group ($P = 0.006$). However, the CSF levels of tTau and pTau were similar between the OND and control groups ($P = 0.974$, 0.205, respectively). As CSF AD biomarkers can be affected by age and the *APOEε4* allele,[23–25] and the *APOEε4* allele and sex differed between the groups in our study, we performed multiple linear regression analysis adjusting for age, sex, and *APOEε4* status (Table 2). The analysis revealed that the CSF biomarkers differed significantly between the ADD and control groups ($\beta = -0.758$, 0.743, 0.549 for Aβ42,

TABLE 1. Demographic Data According to Clinical Diagnosis

	ADD	CON	OND
Number	76	71	47
Sex, female [n (%)]	47 (62)§	50 (70)§	21 (45)*,†
Age (y)	61.8 ± 8.2	60.1 ± 7.1	64.2 ± 12.8
Education (y)	10.2 ± 4.6	10.1 ± 3.8	9.1 ± 4.4
MMSE	17.7 ± 6.7*,§	28.0 ± 1.8†,§	20.3 ± 6.1*,†
CDR	1.1 ± 0.8*	0 ± 0†,§	1.0 ± 1.0*
CDR-SOB	5.8 ± 4.8*	0 ± 0†,§	4.8 ± 5.3*
APOEε4 carrier [n (%)]	33 (43)*,§	14 (20)†	9 (19)†

Values are shown as the means ± SD. Analysis of variance followed by Tukey post hoc test, and χ^2 tests were used to assess continuous and categorical variables, respectively.

ADD indicates Alzheimer disease dementia; CDR-SOB, clinical dementia rating-sum of box; CON, control; MMSE, Mini-Mental State Examination; OND, other neurological disorder with cognitive decline.

*$P < 0.05$ versus CON.

†$P < 0.05$ versus ADD.

§$P < 0.05$ versus OND.

tTau, and pTau, respectively; $P < 0.001$ for all), and between the ADD and OND groups ($\beta = 0.617, -0.666, -0.642$ for Aβ42, tTau, and pTau, respectively; $P < 0.001$ for all).

We generated ROC curves to identify CSF biomarkers that differentiated patients with ADD from control subjects. The AUCs were 0.97 (95% CI, 0.95-0.99) for Aβ42, 0.93 (95% CI, 0.89-0.96) for tTau, and 0.86 (95% CI, 0.80-0.93) for pTau. The AUCs for the tTau/Aβ42 and pTau/Aβ42 ratios were 0.99 (95% CI, 0.98-1.0) for both, which was more accurate than the individual protein levels. The cutoff values that yielded the best Youden index for ADD diagnosis were 481 pg/mL for Aβ42, 326 pg/mL for tTau, 57 pg/mL for pTau, 0.55 for tTau/Aβ42, and 0.10 for pTau/Aβ42. The reliability of the CSF biomarkers increased when the tTau/Aβ42 and pTau/Aβ42 ratios were considered (≥ 95% for sensitivity and specificity) instead of individual concentrations (Table 3).

We applied the cutoffs from ADD versus controls to differentiate ADD from OND. There were 7 subjects who had tTau/Aβ ratios above the cutoffs for ADD (> 0.55) and 8 with pTau/Aβ ratios > 0.10 in OND. Instead, the ability of the CSF biomarkers to differentiate between ADD and OND patients was newly validated using a separate ROC analysis. The AUCs were 0.88 (95% CI, 0.80-0.95) for Aβ42, 0.90 (95% CI, 0.85-0.95) for tTau, 0.89 (95% CI, 0.83-0.95) for pTau, 0.94 (95% CI, 0.89-1.0) for tTau/Aβ42, and 0.94 (95% CI, 0.88-0.99) for pTau/Aβ42. The cutoff values were 478 pg/mL for Aβ42, 327 pg/mL for tTau, 48 pg/mL for pTau, 0.76 for tTau/Aβ42, and 0.12 for pTau/Aβ42. The sensitivity and specificity of these values were lower than those found in the ADD versus controls comparison: 93% and 70% for Aβ42, 83% and 85% for tTau, 86% and 85% for pTau, 93% and 92% for tTau/Aβ42, and 95% and 89% for pTau/Aβ42, respectively. However, they were higher than when we applied the cutoffs from ADD versus controls to the OND subjects. Of those, the tTau/Aβ42 ratio had the greatest accuracy.

We then compared our CSF AD biomarker cutoffs with those used in other laboratories to investigate the location-specificity of these values. We restricted our investigation to studies including large sample sizes[14–18,26] and those differentiating patients with AD and normal controls (Table 3). They commonly used the INNOTEST ELISA kit (Innogenetics, Zwijndrecht or Ghent, Belgium). We found that the cutoff values for Aβ42 and Tau proteins determined by other laboratories were accurate when applied to our subjects. In particular, the cutoff for the tTau/Aβ42 ratio > 0.52 from Duits et al[14] had the highest accuracy, with 99% sensitivity and 93% specificity

DISCUSSION

We determined new cutoff values for CSF AD biomarkers that differentiate patients with ADD from control subjects in South Korea. The individual cutoff values for Aβ42, tTau, and pTau showed good specificity and sensitivity; however, the tTau/Aβ42 and pTau/Aβ42 ratios were more accurate, with ≥ 95% for all statistical measures. This finding suggests that combined Aβ42 and Tau protein levels are a more accurate indicator of AD than individual levels, which is consistent with previous findings.[7,13,14,17,25] The reliability of our CSF biomarker cutoff values was higher than that of previous studies reporting 75% to 90% accuracy in distinguishing patients with AD from control subjects using ELISA.[14–16,18,26,27] Several factors may have contributed to the improved accuracy in our study, including following the consensus protocol to reduce

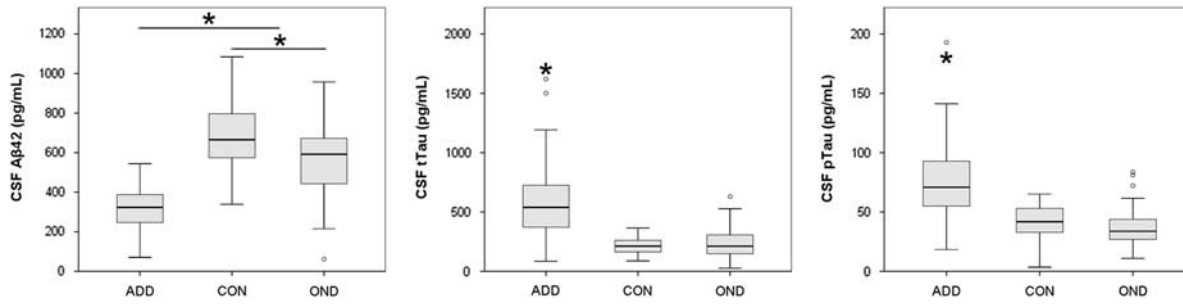

FIGURE 1. The CSF levels of Aβ42 (left), tTau (middle), and pTau181 (right) proteins depending on the diagnostic group. The CSF Aβ42 concentrations are lowest in ADD followed by OND and then control group, whereas CSF tTau and pTau181 proteins are most abundant in ADD than both OND and control subjects ($P < 0.001$ in all). ANOVA with post hoc analysis using log-transformed CSF values of the individual AD biomarkers were used for group comparisons. The box plots show median and interquartile range with the whiskers representing 95% confidence interval (*$P < 0.05$). ADD indicates Alzheimer disease dementia; ANOVA, analysis of variance; CON, controls; CSF, cerebrospinal fluid; OND, other neurological disorders with cognitive decline.

TABLE 2. Results of Multiple Linear Regression Analyses

	CSF Aβ42		CSF tTau		CSF pTau	
	β	*P*	β	*P*	β	*P*
Age (y)	0.059	0.287	−0.037	0.521	−0.032	0.647
APOEε4 +	−0.004	0.941	−0.042	0.480	0.020	0.778
Sex	0.024	0.667	0.011	0.856	−0.033	0.639
Diagnosis (ADD vs. CON)	−0.758	< 0.001*	0.743	< 0.001*	0.549	< 0.001*
Age (y)	0.018	0.810	0.003	0.962	−0.038	0.600
APOEε4 +	0.006	0.937	0.005	0.941	0.038	0.601
Sex	−0.025	0.736	−0.098	0.173	−0.118	0.105
Diagnosis (ADD vs. OND)	0.617	< 0.001*	−0.666	< 0.001*	−0.642	< 0.001*

β, regression coefficient.
ADD indicates Alzheimer disease dementia; CON, control; CSF, cerebrospinal fluid; OND, other neurological disorder with cognitive decline.
*$P < 0.025$ was used to correct for multiple comparisons.

preanalytical inconsistencies, use of the updated version of the ELISA, and enrolling well-defined control subjects.

Worldwide efforts to reduce interlaboratory variability by standardizing the analytical protocol, improving the ELISA kits, and using large sample sizes is expected to improve the reliability of CSF AD biomarkers.[7,28] However, individual laboratories are reluctant to adopt established cutoff values from other institutions because laboratory-specific cutoff values are necessary to maintain internal consistency.[7] This concern was highlighted recently when the variability in CSF biomarker cutoffs reported by 2 well-qualified laboratories resulted in frequent changes to the diagnosis of AD.[29] We found that established cutoff values used in other laboratories demonstrated good reliability when used in our subjects. Our cutoff values for the individual proteins Aβ42, tTau, and pTau were similar to those reported by Mattsson et al,[17] and our tTau/Aβ42 ratio was similar to that of Duits et al[14] In fact, the tTau/Aβ42 ratio cutoff value determined by Duits et al[14] had the highest reliability in our subjects (96% overall accuracy) and exceeded the original value of 88%. Furthermore, although the accuracy of the individual biomarker levels was not good, the reliability of the Aβ42 and Tau protein ratios was consistently better than that of the individual values. However, these established cutoff values may not demonstrate the same level of accuracy in subjects from other institutions. Further studies using larger sample sizes from multiple institutions are necessary to determine universal cutoff variables. However, our findings suggest that the Aβ42 and Tau protein ratio cutoff values are more reliable than those of the individual biomarker levels for the extrapolation of laboratory-specific cutoffs to other populations.

TABLE 3. Comparisons of Various Diagnostic Cutoff Values in Differentiating Patients With ADD From Control Subjects

Study	Cutoff Value (pg/mL)	SE (%)	SP (%)
Current	Aβ42 < 481	**94**	87
	tTau > 326	84	**96**
	pTau > 57	72	**90**
	tTau/Aβ42 > 0.55	**99**	**95**
	pTau/Aβ42 > 0.10	**96**	**96**
Duits et al[14]	tTau/Aβ42 > 0.52	**99**	**93**
	pTau/Aβ42 > 0.08	**99**	87
Schoonenboom et al[15]	(152 + 8.25 × pTau)/Aβ42 > 1	**100**	89
Mulder et al[16]	Aβ42 < 550	**100**	76
	tTau > 375	74	**100**
	pTau > 52	80	73
	(373 + 0.82 × tTau)/Aβ42 > 1	**100**	79
Mattsson et al[17]	Aβ42 ≤ 482	**95**	86
	tTau ≥ 320	84	**93**
	pTau ≥ 52	80	73
	(3.694 + 0.0105 × Tau)/(Aβ42/pTau) > 1	87	**99**
Shoji et al[26]	tTau > 323	84	**94**
Hulstaert et al[18]	Aβ42 < 643	**100**	56
	tTau > 252	**92**	70
	(240 + 1.18 × tTau)/Aβ42 > 1	**100**	83

Values in bold indicate ≥ 90% accuracy.
AD indicates Alzheimer disease; CON, controls; SE, sensitivity; SP, specificity.

Compared with studies conducted in western countries, few investigations of CSF Aβ42, tTau, and pTau levels have been conducted in Asian populations.[26,30,31] Furthermore, previous studies were hampered by small sizes,[30,31] the use of poorly defined control groups,[30] and restricting the analysis to Tau protein data.[26] We used the established tTau protein cutoff of 323 pg/mL from a Japanese group[26] for comparisons with our cutoff value. This value, which was similar to our tTau protein cutoff value, demonstrated high accuracy in discriminating patients with AD from control subjects.

The revised Aβ42 cutoff values were significantly higher than those we reported previously, whereas those of tTau and pTau were comparable with the previous values.[12] Given that our new cutoff value for Aβ42 was similar to that of other laboratories that measured CSF Aβ42 levels using the ELISA kits, the disparity between the 2 studies may be due to errors in the preanalytical or analytical procedures during our prior measurements. Furthermore, the previous study, with its small sample size, may have included a sampling error resulting in much lower Aβ42 levels in ADD and enrolled preclinical AD patients as controls.

This study has shortcomings. First, AD subjects in this study were younger than the typical age distribution seen in Korean memory clinics.[19] We are concerned that the difference in age distribution could affect the results

regarding the CSF AD biomarkers. However, this does not seem likely because age was not significantly related to the CSF AD biomarker levels in the multivariate analysis. However, an additional validation study including more elderly subjects would be valuable for answering this question clearly. Second, the validity of AD biomarkers for differentiating between the ADD and OND groups was tested including an OND group with a smaller sample size and various diseases entities. However, our data revealed that the levels of CSF AD biomarkers in the ADD group were distinctly different from those of the OND group, and the AUCs in the ROC analysis demonstrated good reliability, 0.88 to 0.94, which again demonstrates the utility of the AD biomarkers. For clinical practice, it is very important to establish a cutoff for AD biomarkers in the differential diagnosis of dementia. However, this is challenging due to the frequency of mixed pathologies and the low sensitivity of the clinical diagnosis of non-ADD.[15] This would require a study with a large sample size of autopsy-confirmed cases.

In conclusion, we determined new CSF AD biomarker cutoff values that differentiate patients with ADD from control subjects. Our cutoffs were in a similar range to those previously reported by other laboratories, particularly the combined values of Aβ42 and Tau protein levels. We revised our previous cutoff values using a larger sample size, the updated ELISA kit, which provides standardized solutions, and standardized protocols for the preanalytical procedures. In contrast to the Tau protein cutoff values, the new Aβ42 cutoffs differed significantly from those obtained in our previous pilot study, which had a relatively small sample size and used an earlier version of the ELISA kit.[12] Our findings highlight the necessity of continually testing the reliability of CSF AD biomarker cutoffs to ensure their clinical usefulness.

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