Alzheimer's Cerebrospinal Biomarkers from Lumipulse Fully Automated Immunoassay: Concordance with Amyloid-Beta PET and Manual Immunoassay in Koreans

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Abstract

Background: Universal Alzheimer’s disease (AD) cerebrospinal fluid (CSF) biomarker cutoffs from immunoassays with low interlaboratory variability could improve diagnostic accuracy and help predict disease progression. To determine the diagnostic cutoffs of CSF AD biomarkers measured with three immunoassay platforms, including fully automated Lumipulse based on b-amyloid (Ab) positron emission tomography (PET) status, to determine diagnostic utility and clinical predictability.

Methods: Three hundred thirty-one Korean participants were enrolled from a prospective, 3-year longitudinal observational study of the validation cohort of Korean Brain Aging Study for the Early Diagnosis and Prediction of AD: 71 cognitively normal (CN), 99 with subjective cognitive decline (SCD), 89 with mild cognitive impairment (MCI), and 72 with AD. Among these 331 participants, 139 (29, 58, 29, and 23 from each group, respectively) provided CSF and 271 underwent baseline amyloid PET. Three annual cognitive and neuropsychiatric function tests were conducted. Ab42, Ab40, total-tau and phosphorylated-tau_181 were measured by Lumipulse fully automated immunoassay and two manual immunoassays (INNO-BIA AlzBio3, INNOTEST). Clinical utility of CSF biomarker cutoffs, based on 128 participants with Ab-PET, were evaluated.

Results: Cognitive and neuropsychological scores differed significantly among the groups, with descending performance among CN > SCD > MCI > AD. Biomarker levels among immunoassays were strongly intercorrelated, and using the cutoffs for Ab-PET status with maximal AD diagnostic accuracy (n = 215), the levels showed excellent agreement with Ab-PET. Use of Ab-PET-based cutoffs for CSF biomarkers showed excellent diagnostic discrimination between AD and CN (Ab42, Ab42/Ab40, t-tau/Ab42 and p-tau/Ab42) with overall AUC ranges to discriminate AD and CN (0.876–0.952). During follow-up, participants with AD-like CSF signature determined by Ab-PET-based cutoffs from Lumipulse showed rapid progression of clinical scores, after adjustment for potential confounders, compared with those with a normal CSF signature.

Conclusion: CSF AD biomarkers measured by different immunoassay platforms show strong intercorrelated agreement with Ab-PET. Ab-PET-based CSF biomarker cutoffs measured by immunoassays, including the Lumipulse, strongly predict progression of cognitive decline. The clinical utility of CSF biomarkers from fully automated immunoassay platforms should be evaluated in larger, more diverse cohorts.

Background

Given the pathologic characterization of Alzheimer’s disease (AD) by amyloid-β (Aβ) plaques and neurofibrillary tangles, measurement of AD biomarkers amyloid beta (1–42 and 1–40) (Aβ42, Aβ40), total tau (t-tau) and phosphorylated tau at Thr181 (p-tau) in cerebrospinal fluid (CSF) is recommended for accurate AD diagnosis and research.[1, 2] These biomarkers have been widely appreciated that the AD-like feature of “core” CSF AD biomarkers characterized by a lower Aβ42 and higher t-tau or p-tau levels in the CSF of patients with AD, compared with that of healthy older adults, reflects the abnormal Aβ plaque burden and tau pathology. Although the concentrations of each biomarker measured by single-plex or multiplex immunoassay platforms are not interchangeable, their concentrations are highly correlated and diagnostic performance is comparable.[3–6] In a qualified laboratory in which CSF AD biomarkers are measured routinely, the intra-laboratory precision for single-plex enzyme-linked immunosorbent assay (ELISA) method or multiplex xMAP-Luminex is excellent.[7, 8] Nevertheless, manual immunoassay-based concentration of Aβ42 and tau proteins across laboratories varies, even using equivalent CSF samples with standardization of preanalytical variables[8–10] or with unified test
procedure following comprehensive standard operating procedures (SOPs).[11] Implementation of unified SOPs in an experienced laboratory may decrease variability in determining their internal cutoffs for AD diagnosis; however, manual assays have inherent sources of analytical variability. Therefore, a fully automated immunoassay for routine clinical practice for CSF biomarker-based diagnosis of AD is desirable. Furthermore, using the reference method procedure such as liquid chromatography tandem mass spectrometry and certified reference material (CRM) for Aβ42 using neat CSF was recently introduced to harmonize immunoassays across platforms, to eliminate systemic bias in CSF Aβ42 levels, and across kit lots.[12] Currently, fully automated immunoassay systems have been developed,[13] including the Elecsys developed by Roche Diagnostics (Rotkreuz, Switzerland) and the Lumipulse developed by Fujirebio (Fujirebio Europe, Gent, Belgium), which show high concordance with amyloid positron emission tomography (PET) classification.[14–16]

Herein, we analyzed CSF samples from a validation cohort in the Korean Brain Aging Study for the Early Diagnosis and Prediction of Alzheimer’s Disease (KBASE-V study) using a fully automated immunoassay Lumipulse G and two manual immunoassay platforms: xMAP-Luminex INNOBIA-AlzBio3 multiplex assay (Luminex) and ELISA with INNOTEST kit (INNOTEST) for Aβ42, Aβ40 (only for INNOTEST and Lumipulse G), t-tau, or p-tau. We evaluated the overall agreement of these core CSF AD biomarkers with amyloid PET results, the correlations among the CSF biomarker levels measured with these three platforms, and the diagnostic performance of each biomarker using a cutoff based on Aβ-PET status. We also assessed the predictability of baseline CSF biomarkers for cognitive decline over 3 years.

**Methods**

**Methods**

**Participants**

From April 2015 to August 2016, we recruited 331 participants from nine memory clinics across South Korea (KBASE-V study participants); 71 were cognitively normal (CN), 99 showed subjective cognitive decline (SCD), 89 had been diagnosed with mild cognitive impairment (MCI), and 72 had been diagnosed with probable AD. Supplementary information (Supplementary Method 1) presents the criteria for clinical diagnosis of MCI and AD, and exclusion criteria.[17, 18] Among the 331 participants, 139 (29 CN, 58 SCD, 29 MCI, and 23 AD) agreed to provide CSF. Clinical assessments conducted at baseline and every year for 3 years included: the Korean version of the Mini-Mental State Examination (MMSE) in the CERAD assessment packet;[17] the Subjective Memory Complaints Questionnaire;[19] the Geriatric Depression Scale (GDS); the CDR and Global Deterioration Scale;[18] the Blessed Dementia Scale-Activities of Daily Living (BDS-ADL); and comprehensive neuropsychological testing. Demographic information including age, gender, and education years was collected for all participants. Peripheral blood was drawn for ApoE genotyping and laboratory tests. Ethical approval was given by the Institutional Review Board of each center (INHAUH 2015-03-021). All participants or their legal representatives voluntarily agreed to participate and provided written informed consent. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**CSF Collection and AD Biomarker Analysis Using Manual Immunoassay Systems**

139 participants underwent lumbar puncture in the morning. CSF was drawn in 15 mL polypropylene (PP) tubes (Falcon, Corning Science, NY, USA) and immediately centrifuged at 2000–g for 10 minutes at room temperature.
(RT). The supernatant (~ 10 mL) was frozen on dry ice and transferred to the laboratory at Inha University for AD biomarker analyses. Transported CSF was thawed at RT, gently mixed with a PP tip pipette, 0.4 mL aliquots divided into 0.5 mL PP tubes (Sarstedt AG & Co., Nümbrecht, Germany), and stored at ~ 80°C until analysis. We applied two manual immunoassay platforms (INNOTEST and Luminex-AlzBio3) and one fully automated immunoassay to measure AD biomarker levels, as previously described (Additional file: Supplementary Method 2).

Fully Automated Immunoassays For CSF AD Biomarkers Using Lumipulse

Using Lumipulse G1200 fully automated immunoassay system with Lumipulse® G p-tau$_{181}$, t-tau, Aβ42 or Aβ40 kit (Fujirebio Europe), additional CSF aliquots were analyzed. The Lumipulse G instruments use single analyte, ready-to-use, immunoreaction cartridges with a throughput of 60 and 120 tests/hour for the G600II and the G1200 instruments, respectively. The analyte is captured specifically by antibody-coated microparticles before the biotinylated detection antibodies (streptavidin labeled with alkaline-phosphatase, i.e. for Aβ42 and t-tau assays) or ALP labeled detection antibodies (i.e. for Aβ40 and p-tau assays) and substrate are added, each after a thorough washing step. Established monoclonal antibodies were used in the set-up for the Lumipulse G assays. Analysis of the CSF samples (from storage vials) was accomplished with a Lumipulse G 1200 series instrument using the Lumipulse G Aβ42 (CRM standardized), Aβ40, t-tau, and p-tau assays at the Fujirebio Gent facility. The concentrations were within the assay’s measurement range, except for 16 samples (all Aβ-PET negative) in which the t-tau measurements were all below the limit of quantitation (141 pg/mL). The excellent analytical performance for the assays has been described previously.[21–23]

Amyloid Positron Emission Tomography

271 subjects out of 331 participants underwent amyloid imaging via $^{11}$C-PiB PET or $^{18}$F-flutemetamol PET. We aligned PET images to the corresponding T$_1$-weighted MRI and the standard uptake value ratio (SUVR) of each region of interest (ROI), which was obtained by dividing the mean uptake value for all voxels within the ROI by the mean value of the reference region. Composite SUVR values were calculated by averaging the SUVR values for the prefrontal, orbitofrontal, parietal, lateral temporal, anterior cingulate, and posterior cingulate/precuneus regions.[24] We determined the amyloid PET positivity based on cutoff values from the composite SUVR of cortical PiB retention to differentiate CN from AD, as described previously.[25] Among the 139 participants who provided CSF, 128 underwent amyloid PET imaging analysis. With these samples, we used SUVR cutoff values for amyloid PET positivity from 271 participants to determine the cutoff values for CSF biomarkers following Youden’s index to differentiate participants with amyloid PET positivity. Finally, we evaluated the diagnostic and predictive performances of these predetermined cutoff values for CSF biomarkers among all 139 participants.

Statistical analysis

Groups were compared using chi-square tests for categorical variables or the Kruskal–Wallis test followed by post hoc Dunn's multiple comparisons for continuous variables. Receiver-operating characteristic curve (ROC) analyses were carried out to assess the diagnostic utility of various CSF biomarkers to distinguish AD from CN using Prism (v. 6.0; GraphPad Software, San Diego, CA, USA). Cutoffs with the highest agreement on Aβ-PET of CSF AD biomarkers using Youden's rule were used to determine diagnostic performance and to assess the predictability of clinical progression. For the latter analysis, we compared the clinical scores of 139 participants who provided CSF and one or more annual follow-up assessments over three years between the normal and AD-like signature groups.
(i.e., above or below the cutoffs for each biomarker) using mixed-effect modeling. We further assessed significant
time · group interaction effects for the ability of CSF biomarkers to predict change in cognitive decline of 139 total
subjects, MCI group, or cognitively normal subjects (CN plus SCD), adjusting for covariates (age, gender, education
years, and ApoE genotype) using analyses of covariance models (SPSS, ver. 19.0, Chicago, IL). \( P < .05 \) was
considered statistically significant.

**Results**

**Demographics And Clinical Characteristics**

Median values for age and education years, and frequency of ApoE e4 allele among the four groups (71 CN, 99
SCD, 89 MCI, and 72 AD) differed significantly, although there was no significant difference for these measures
between CN and SCD. Scores on cognitive function and neuropsychological tests among the entire sample
differed significantly between the four groups, with descending performance among CN > SCD > MCI > AD, as
reported previously.[25] Clinical characteristics and neuropsychological test results among the subgroup of 139
participants who provided CSF (29 CN, 58 SCD, 29 MCI, and 23 AD) were consistent with the total sample of 331
subjects. Ages within the CN and SCD groups were similar, and younger than MCI and AD. Education years among
the CN and MCI groups were comparable, and higher than SCD or AD. Gender distribution and ApoE e4 allele
frequency differed significantly among the groups (Tables 1 and Table S1).
### Table 1
Clinical Characteristics of 139 Participants who Provided CSF, According to the AD Clinical Spectrum.

| Characteristics | CN (n = 29) | SCD (n = 58) | MCI (n = 29) | AD (n = 23) | P value |
|-----------------|------------|-------------|-------------|-------------|---------|
| Age, median (95% CI), y | 63.0 (58–67) | 66.5 (63–69) | 72.0 (66–76) | 71 (67–79) | < 0.001 |
| Education, median (95% CI), y | 12 (9–16) | 6 (6–9) | 10 (6–12) | 6 (6–9) | < 0.001 |
| Gender (M : F) | 10 : 19 | 29 : 29 | 21 : 8 | 6 : 17 | < 0.004† |
| MMSE, median (95% CI) | 29 (28–29) | 26 (25–27) | 24 (21–26) | 16 (15–19) | < 0.001 |
| SMCQ, median (95% CI) | 1 (1–2) | 5 (4–6) | 5 (3–7) | 8 (5–12) | < 0.001 |
| SBT, median (95% CI) | 0 (0–2) | 4 (2–4) | 7 (4–11) | 18 (17–22) | < 0.001 |
| CERAD, mean Z, median (95% CI) | 0.48 (0.42–0.61) | 0.14 (0.01–0.23) | -0.81 (-1.13–0.33) | -1.65 (-2.02–0.94) | < 0.001 |
| BDS-ADL, median (95% CI) | 0 (0–0) | 0 (0–0.5) | 0 (0–1) | 2.5 (1–4.5) | < 0.001 |
| ESS, median (95% CI) | 5 (4–7) | 4 (4–5) | 3 (1–4) | 2 (1–5) | 0.0127 |
| CDR 0:0.5:1 | 29 : 0 : 0 | 58 : 0 : 0 | 0 : 29 : 0 | 0 : 8 : 15 | < 0.001† |
| CDR-SB, median (95% CI) | 0 (0–0) | 0 (0–0) | 0.5 (0.5–1.0) | 5 (3.5–6.0) | < 0.001 |
| GDS, median (95% CI) | 5 (3–8) | 8 (7–12) | 9 (5–15) | 12 (7–20) | 0.003 |
| Aβ PET(+), % (- : +) | 3.7 (27:1) | 19.2 (42:10) | 40.7 (16:11) | 81.0 (4:17) | < 0.001† |
| ApoE ε4 carrier, % (-:+) | 17.2 (24 : 5) | 20.7 (46 : 12) | 20.7 (23 : 6) | 56.5 (10 : 13) | 0.008† |

a p < 0.05 versus CN; b p < 0.05 versus SCD; c p < 0.05 versus MCI by Dunn's multiple comparison following the Kruskal-Wallis test. † Chi-square test. MMSE, Mini-Mental State Examination; SMCQ, Subjective Memory Complaint Questionnaire; SBT, Short Blessed Test; CERAD, Consortium to Establish a Registry for AD; BDS-ADL, Blessed Dementia Scale-Activities of Daily Living; ESS, Epworth Sleepiness Scale; CDR, Clinical Dementia Rating scale; CDR-SB, CDR-Sum of Boxes; GDS, Geriatric Depression Scale; ApoE, apolipoprotein E

Amyloid-PET positivity was determined by cutoff values for composite SUVR values. For 11C-PiB PET, the cutoff SUVR value was 1.20, with 100% sensitivity and 84.6% specificity to discriminate CN from AD participants following Youden's rule. For 18F-flumetamol PET, the cutoff SUVR value was 0.62, with 95.8% sensitivity and 90.0% specificity to discriminate CN from AD participants. Three (5%) of 56 CN participants, 19 (21.6%) of 88 SCD participants, 32 of 73 participants with MCI (43.8%), and 43 (79.6%) of 54 patients with AD showed amyloid deposition. When we analyzed the subgroup who provided CSF (n = 139), the results were similar to those from the
total sample: i.e., the percentages of amyloid PET-positivity for CN (n = 28), SCD (n = 52), MCI (n = 27) and AD (n = 21) were 3.6%, 19.2%, 40.7% and 81.0%, respectively.

Cerebrospinal Fluid Biomarker Levels

The Aβ42 levels measured by 3 immunoassays in patients with MCI and AD were significantly lower than those in the CN group, while the levels among those with SCD were comparable to the CN group. The Aβ40 levels measured by ELISA and Lumipulse G did not differ among the groups. The levels of t-tau, p-tau, t-tau/Aβ42, and p-tau/Aβ42 determined by the three platforms in the AD group were significantly higher than those measured in the CN group. For those with MCI, the p-tau/Aβ42 levels measured by Lumipulse and Luminex were significantly higher than in the CN group. The ratio of t-tau/Aβ42 in the MCI group measured by Lumipulse was higher than in the CN group, while the levels in the MCI group measured by other assays were comparable with the CN group. In all immunoassay platforms, the mean Aβ42 level in the AD group was approximately 50% of the CN group level (Table 2), consistent with a previous study.[26] When we compared the ratio of Aβ42/Aβ40 measured by INNOTEST or Lumipulse G, the ratio among those with AD was significantly lower compared with the CN and SCD groups. The ratio in the MCI group measured by Lumipulse, but not by INNOTEST, was significantly lower than in the CN or SCD groups. As shown in Figure, the biomarker levels from the various immunoassay platforms were strongly intercorrelated.
Table 2
CSF Biomarker Levels Measured by Three Immunoassay Platforms.

| Groups | CSF biomarker levels, median (95% CI) | Aβ<sub>42</sub> | Aβ<sub>40</sub> | T-tau | P-tau | T-tau/Aβ<sub>42</sub> | P-tau/Aβ<sub>42</sub> | Aβ<sub>42</sub>/Aβ<sub>40</sub> |
|--------|----------------------------------------|----------------|----------------|-------|-------|-------------------|-------------------|--------------------|
|        |                                        |                |                |       |       |                   |                   |                    |
| Luminex-AlzBio3 |                                      |                |                |       |       |                   |                   |                    |
| CN     |                                        | 573.2          | n.d.           | 46.1  | 16.1  | 0.09              | 0.03              | n.d.               |
|        |                                        | (511.3–600.6)  |                | (42.1–55.4) | (13.7–18.6) | (0.08–0.10) | (0.03–0.03) |                    |
| SCD    |                                        | 520.3          | n.d.           | 46.0  | 16.9  | 0.09              | 0.03              | n.d.               |
|        |                                        | (484.2–570.5)  |                | (41.4–55.6) | (14.0–20.1) | (0.08–0.11) | (0.03–0.04) |                    |
| MCI    |                                        | 411.2<sup>a,b</sup> | n.d.           | 48.4  | 21.2  | 0.12              | 0.05<sup>a</sup>  | n.d.               |
|        |                                        | (282.8–541.1)  |                | (42.0–63.0) | (15.1–25.5) | (0.08–0.18) | (0.03–0.08) |                    |
| AD     |                                        | 290.6<sup>a,b</sup> | n.d.           | 75.2<sup>a,b</sup> | 28.1<sup>a,b</sup> | 0.33<sup>a,b,c</sup> | 0.11<sup>a,b</sup> | n.d.               |
|        |                                        | (242.7–361.1)  |                | (49.5–107.0) | (22.7–49.4) | (0.17–0.44) | (0.06–0.22) |                    |
|        |                                        |                |                |       |       |                   |                   |                    |
| INNOTEST |                                      |                |                |       |       |                   |                   |                    |
| CN     |                                        | 756.7          | 6724           | 195.0 | 38.5  | 0.26              | 0.05              | 0.11               |
|        |                                        | (583.8–879.8)  | (5160–9070)    | (156.1–239.7) | (35.0–49.7) | (0.23–0.32) | (0.05–0.07) | (0.09–0.12) |
| SCD    |                                        | 794.9          | 7405           | 199.5 | 43.8  | 0.26              | 0.05              | 0.10               |
|        |                                        | (642.0–950.7)  | (6388–9565)    | (145.6–270.4) | (34.6–50.1) | (0.20–0.31) | (0.04–0.06) | (0.09–0.12) |
| MCI    |                                        | 565.1<sup>b</sup> | 7158           | 207.7 | 46.4  | 0.31              | 0.07              | 0.09               |
|        |                                        | (390.4–734.9)  | (5551–8164)    | (156.3–291.5) | (34.1–53.6) | (0.23–0.66) | (0.05–0.12) | (0.06–0.12) |

<sup>a</sup>p<0.05 versus CN; <sup>b</sup>p<0.05 versus SCD; <sup>c</sup>p<0.05 versus MCI by Dunn's multiple comparison following Kruskal-Wallis test.
Agreement on Amyloid PET and CSF Biomarker Levels Determined by Three Platforms

CSF amyloid positivity in the subgroup of 139 participants who provided CSF was determined using the cutoff of mean SUVR of amyloid PET from 215 participants (i.e., 1.20 for PiB and 0.62 for \(^{18}\)F-flutemetamol retention), which showed the highest discriminability between AD and CN. Based on the amyloid PET results, we determined CSF biomarker cutoffs at the highest agreement rate for amyloid deposition in the 128 participants who provided CSF and underwent the amyloid PET test. In all immunoassay platforms, A\(_\beta42\) (AUC = 0.884–0.920), t-tau/A\(_\beta42\) (AUC = 0.880–0.947), p-tau/A\(_\beta42\) (AUC = 0.894–0.921) and A\(_\beta42\)/A\(_\beta40\) (AUC = 0.880–0.904) showed higher agreement than did t-tau (AUC = 0.636–0.852) or p-tau (AUC = 0.751–0.861), as expected (Table 3).
Table 3
ROC Parameters for CSF Biomarkers Measured by Different Immunoassay Platforms to Discriminate Participants with Amyloid-PET Positivity from Those with Amyloid-PET Negativity.

| Assay Platforms | Parameters | $\text{A}^{\beta}_{42}$ | $\text{A}^{\beta}_{40}$ | T-tau | P-tau | T-tau/ $\text{A}^{\beta}_{42}$ | P-tau/ $\text{A}^{\beta}_{42}$ | $\text{A}^{\beta}_{42}$/ $\text{A}^{\beta}_{40}$ |
|-----------------|------------|----------------|----------------|-------|-----|----------------|----------------|----------------|
| Luminex-AlzBio3 | n*         | 128           | -              | 127   | 128 | 127            | 127            | 128            |
|                 | ROC AUC    | 0.920         | -              | 0.636 | 0.838 | 0.880          | 0.921          | -              |
|                 | Cut-off value | 466.9 pg/mL | -              | 63.75 pg/mL | 21.02 pg/mL | 0.133 | 0.045          | -              |
|                 | PPA (%)    | 89.7          | -              | 56.0  | 76.9 | 82.1           | 89.7           | -              |
|                 | NPA (%)    | 84.3          | -              | 87.5  | 82.0 | 93.2           | 88.8           | -              |
| INNOTEST        | n          | 126           | 126            | 118   | 112 | 118            | 112            | 126            |
|                 | ROC AUC    | 0.891         | n.s.           | 0.852 | 0.751 | 0.947          | 0.902          | 0.904          |
|                 | Cut-off value | 499.7 pg/mL | -              | 247.3 pg/mL | 41.29 pg/mL | 0.484 | 0.079          | 0.091          |
|                 | PPA (%)    | 82.1          | -              | 83.3  | 94.3 | 88.9           | 82.9           | 83.9           |
|                 | NPA (%)    | 94.3          | -              | 74.4  | 45.5 | 96.3           | 88.3           | 94.9           |
| Lumipulse       | n          | 126           | 126            | 126   | 126 | 126            | 126            | 126            |
|                 | ROC AUC    | 0.884         | n.s.           | 0.825 | 0.861 | 0.904          | 0.894          | 0.880          |
|                 | Cut-off value | 653.4 pg/mL | -              | 337 pg/mL | 36.0 pg/mL | 0.315 | 0.051          | 0.060          |
|                 | PPA (%)    | 84.6          | -              | 61.5  | 82.1 | 87.2           | 87.2           | 87.2           |
|                 | NPA (%)    | 88.5          | -              | 89.7  | 78.2 | 88.5           | 93.1           | 92.0           |

*T-tau measured by Luminex of one sample and t-tau and p-tau measured using INNOTEST kits of 10 and 16 samples respectively were excluded following acceptance criteria of SOP, and two samples could not be measured due to loss of samples. ROC AUC, area under the receiver operating characteristic curve; PPA, positive percent agreement; NPA, negative percent agreement.

When we applied the predetermined cutoffs of each biomarker to 139 participants, including 11 without amyloid-PET results, the ability to discriminate AD from CN is summarized in Table S2 (Additional file). The AUC of t-tau/Aβ42 (AUC = 0.913, 0.927, and 0.952 in Luminex, INNOTEST, and Lumipulse, respectively), p-tau/Aβ42 (AUC = 0.897, 0.912, and 0.946 in Luminex, INNOTEST, and Lumipulse, respectively) and Aβ42/Aβ40 (AUC = 0.922 and 0.952 in INNOTEST and Lumipulse, respectively) were higher than Aβ42 alone (AUC = 0.907, 0.876, and 0.889 in Luminex, INNOTEST, and Lumipulse, respectively), except for p-tau/Aβ42 versus Aβ42 in Luminex. Aβ42 (AUC = 0.808, P < .0001), t-tau/Aβ42 (AUC = 0.765, P = .0008), p-tau/Aβ42 (AUC = 0.780, P = .0004) and Aβ42/Aβ40 (AUC = 0.769, P = .0006) from the Lumipulse assay showed significant discriminability between the MCI and CN groups. These biomarkers showed low sensitivity (48.1–51.9%) but higher specificity (85.7–96.4%). In the other immunoassay platforms, the diagnostic performance for discrimination between MCI and CN groups was like that...
for Lumipulse. As expected, when we compared the CSF biomarker levels between amyloid PET-positive and -negative groups, all biomarkers determined by the three assay platforms except Aβ40 differed significantly (Additional file: Table S3). In all assay platforms, the Aβ42 level in amyloid PET-positive participants was 50.6–53.8% of the amyloid PET-negative group.

Performance Of CSF Biomarkers For Predicting Clinical Progression

To test clinical predictability of the baseline PET-based cutoffs for cognitive decline, we followed participants up to 3 years. When we compared the progressive decline of cognitive function between above or below the CSF biomarker cutoffs, groups with AD-like CSF biomarker signature (i.e., lower Aβ42 or Aβ42/Aβ40, and higher p-tau/Aβ42 or t-tau/Aβ42 than cutoffs) showed more rapid decline in cognitive function (e.g., CDR, CDR-SB, MMSE, BDS-ADL, construction praxis, clock drawing and Short Blessed Test scores) compared with the groups with normal CSF biomarker signatures (P < .05). This significant difference remained after adjusting for either age and ApoE genotype or age, ApoE genotype, gender, and education years (Table 4). In the MCI group, although we observed a trend toward more rapid MMSE score decline in the group with an AD-like CSF signature (n = 14) compared with the group with a normal CSF signature (n = 13), the difference in the progressive cognitive decline did not reach statistical significance (Additional file: Supplementary Figure). A more rapid increase in CDR-SB scores among those in the MCI group with a higher t-tau/Aβ42, higher p-tau/Aβ42 or lower Aβ42/Aβ40 than among those in the MCI group with a normal CSF signature was observed, though the difference was not statistically significant. During follow-up, we observed that in the groups with normal cognition or without significant cognitive dysfunction (i.e., CN plus SCD, n = 80), there was more rapid progression of CDR scores (F = 3.107, P = .032) and delayed recognition (F = 4.870, P = .004) in the group with low Aβ42, more rapid progression of MMSE scores (F = 3.405, P = .023) in the group with high t-tau/Aβ42, and more rapid progression of construction recall (F = 3.432, P = .022) in the group with higher t-tau.
Table 4
Predictive Performance of CSF Biomarkers for Clinical Progression Among Overall Sample

| Clinical variables | Biomarkers | \( A\beta 42 \) | T-tau | P-tau | \( A\beta 42/A\beta 40 \) | t-tau/\( A\beta 42 \) | p-tau/\( A\beta 42 \) |
|-------------------|------------|----------------|-------|-------|----------------|----------------|----------------|
|                   | \( F, P \) value | \( F, P \) value | \( F, P \) value | \( F, P \) value | \( F, P \) value | \( F, P \) value | \( F, P \) value |
| CDR               | 4.013, 0.009, 1.512, 0.341 | 1.127, 0.015, 3.627, 0.041 | 2.831, 0.015, 3.639, 0.015 |
|                   | 7.283, 0.0001, 1.584, 0.197 | 1.011, 0.001, 5.982, 0.005 | 4.472, 0.005, 6.416, 0.0001 |
| CDR-SB            | 2.529, 0.061, 1.634, 0.525 | 0.749, 0.034, 2.975, 0.092 | 2.199, 0.092, 3.095, 0.030 |
| BDS-ADL           | 1.389, 0.250, 1.500, 0.661 | 0.661, 0.578, 2.002, 0.074 | 2.365, 0.074, 1.720, 0.167 |
|                  | 2.694, 0.050, 2.362, 0.260 | 1.358, 0.007, 4.227, 0.007 | 4.277, 0.007, 5.030, 0.003 |
|                  | 3.670, 0.016, 1.596, 0.800 | 0.335, 0.109, 2.071, 0.088 | 2.244, 0.088, 2.088, 0.107 |
|                  | 3.224, 0.026, 3.482, 0.261 | 1.356, 0.004, 4.751, 0.003 | 5.091, 0.003, 5.034, 0.003 |

Ten subjects with AD-like CSF signature (2, 1 and 7 at first, second and third follow-up, respectively) and 9 subjects with normal CSF signature (1, 4 and 4 at third, second and first follow-up, respectively) were lost to follow-up. CDR, Clinical Dementia Rating scale; CDR-SB, CDR-Sum of Boxes; BDS-ADL, Blessed Dementia Scale-Activities of Daily Living; SBT, Short Blessed Test; MMSE, Mini-Mental State Examination.

**Discussion**

While AD diagnosis is largely based on clinical and neuropsychological test performance and cognitive function, postmortem diagnosis based on autopsy has shown that ~30% of AD cases are misdiagnosed.[27] Given that CSF immunoassays and amyloid PET analyses have shown promise as biomarkers reflecting the trajectory of AD pathology,[28] amyloid PET analysis has been widely accepted for its strong agreement with pathological amyloid
aggregates,[29, 30] and increasing diagnostic confidence.[31, 32] In addition, the use of amyloid-PET as the standard for establishing CSF AD biomarker cutoffs may reduce inter-center variability compared with clinical-based cutoffs[33] and may be useful for identifying AD pathology antemortem.[14] Therefore, we determined CSF biomarker cutoffs for evaluating diagnostic and predictive performance based on the best levels of agreement with amyloid PET status. When we compared agreement among CSF biomarkers with PET results using three immunoassay platforms, Aβ42, t-tau/Aβ42, p-tau/Aβ42, and Aβ42/Aβ40 showed a higher overall agreement than did t-tau or p-tau alone. For the diagnostic performance of the ratios, compared with Aβ42, t-tau or p-tau alone, using the predetermined cutoffs was clearly better. In addition, we observed strong correlations among the biomarker levels determined by the three immunoassay platforms (Figure). These results indicate that CSF biomarkers measured by a novel Lumipulse automated immunoassay with CRM-based method validation provide a more accessible, antemortem alternative to evaluating patients underlying AD pathophysiology, and an opportunity to discriminate symptomatic AD patients in the clinic or in AD trials.

Compared with previous studies,[20, 34–36] cutoffs of Aβ42 and t-tau by INNOTEST or Luminex-AlzBio3 for AD diagnosis were higher and lower, respectively, leading to a lower t-tau/Aβ42 ratio cutoff herein. Compared with the t-tau or p-tau cutoffs from INNOTEST-based diagnostics reported in another Korean population, ours were lower.[37] These cutoff discrepancies for Aβ42, t-tau, or p-tau may have been caused by either inter-laboratory variability in determining the CSF AD biomarker levels using a manual immunoassay, assay concepts, or preanalytical variables. Significant intra-laboratory analytical variability in the levels of Aβ42, t-tau and p-tau from Luminex-AlzBio3 is unlikely, as we observed similar biomarkers levels between the KBASE-V cohort and another small, independent cohort (Additional files: Supplementary Method 3 and Table S4) and a low between-run %CV.

Considering the fully automated Lumipulse immunoassay, a previous study reported higher cutoffs for Aβ42, t-tau, and t-tau/Aβ42 (approximately 0.54) for discriminating amyloid PET positivity compared with our study.[14] In another study, cutoffs for Aβ42, t-tau, and p-tau and their ratios were higher than herein.[38] However, another study reported lower diagnostic cutoffs for Aβ42, higher cutoffs for p-tau, p-tau/Aβ42 (0.086), and t-tau/Aβ42 (0.578), and a similar cutoff for t-tau compared with our amyloid-positive results herein.[23] Those previous studies showed an approximately 0.53–0.62 t-tau/Aβ42 for discriminating amyloid PET positivity or AD diagnosis, which is approximately twofold higher than our study cutoff of t-tau/Aβ42 (0.315). However, those investigators reported a similar Aβ42/Aβ40 cutoff (~ 0.06), indicating that the Aβ42/Aβ40 ratio may be more reliable for AD diagnosis than a single biomarker or other ratios. Given that fully automated immunoassay has low analytical variability in the CRM-adjusted CSF AD biomarker levels compared with manual immunoassay,[14] comparing the discrepancy in the Aβ42 level or tau proteins measured by Lumipulse in our study with other studies may be due to clinical variables or pre-analytics. Recently, racial disparity has been identified in CSF tau-based biomarker levels in both patients with MCI and community-living older adults, which remained after covariate-adjusted analysis.[39, 40] Although CSF biomarkers in these studies were measured via manual assays rather than fully automated, their reported tau protein levels reflected interethnic differences (i.e., lower levels among African-American compared with white participants). To our knowledge, ours are the first CSF AD biomarker data from a Korean population which were measured by both manual and fully automated immunoassay systems. In the future, direct comparison of CSF AD biomarkers across different AD disease continuums for race-specific AD diagnostic cutoffs, or evaluation of amyloid PET agreement among different races, including Korean, will clarify possible interethnic differences in CSF AD biomarker levels. Other possible influences on CSF AD biomarker levels may be different covariates, including different levels of mixed pathologies, comorbidities, or ages.[41, 42]
Regardless of differing CSF AD biomarkers cutoffs between immunoassay platforms, amyloid PET-based cutoffs of biomarkers showed significant diagnostic performance for discriminating between AD and CN groups in all immunoassay platforms. Furthermore, using PET-based cutoffs, we observed that Aβ42 and ratios (i.e., Aβ42/Aβ40, t-tau/Aβ42, and p-tau/Aβ42) measured by Lumipulse predicted progression of cognitive decline and deterioration of daily living over 3 years in our subgroup of 139 participants, after adjusting for covariates. Despite our relatively small sample, the significance of these results are due in part to this being the first report of the clinical predictability of amyloid PET-based cutoffs for CSF AD biomarkers, determined by a fully automated immunoassay in a multicenter Korean cohort. Considering the possible confounding effects of clinical variables on diagnostic and/or predictive performance of CSF AD biomarker cutoffs, further studies with larger, more diverse samples are warranted. The predictability of these CSF biomarkers for clinical progression was not significant among those with MCI. We observed a trend toward different clinical trajectories between the MCI group with an AD-like CSF signature and those with normal levels; thus, this may have been due to our relatively small number of participants with MCI. Although the number of participants without significant cognitive dysfunction was small, some biomarkers, Aβ42, Aβ42/Aβ40, t-tau/Aβ42, and particularly p-tau/Aβ42, may predict cognitive decline; hence, this result finding should be replicated in other cohorts with larger sample sizes.

This study's limitations include its relatively small number of participants on whom CSF analyses were performed, which may have resulted in failure to detect statistical significance in subgroup analyses. Despite this, it is important to note the clinical utility of these findings. To our knowledge, ours is the first report of the significant clinical utility of CSF AD biomarkers simultaneously measured by three immunoassay platforms using Aβ PET status-based cutoffs in a Korean sample. Interethnic differences in CSF AD biomarker levels are possible, hence our results may be specific to Koreans, which warrants future comparisons using a fully automated immunoassay among different races.

**Conclusion**

Despite limitations, our study demonstrates the clinical utility of Ab PET-based cutoffs of CSF AD biomarkers in Koreans using a fully automated immunoassay, which agrees with manual immunoassays. Although it remains to be determined whether CSF AD biomarker levels differ among various ethnic groups,[39, 40] our study indicates that a fully automated immunoassay with minimal inter-laboratory variability can replace manual immunoassays to differentiate AD from CN populations, incorporate the framework's amyloid/tau/neurodegeneration classification scheme for AD and predict clinical progression. Regarding accelerating AD trials for developing disease-modifying drugs through multinational studies, the use of automated CSF AD biomarker measurements will be vital.

**Declarations**

**Conflicts of Interest**

The authors have no disclosure for any conflicts of interest, including financial interests, relationships, activities and affiliations.

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Figures
Figure 1

Correlations Among CSF Biomarker Levels Determined by Three Immunoassay Platforms. The Spearman correlation coefficients between Aβ42, t-tau and p-tau levels measured by Luminex and INNOTEST were 0.87, 0.74, and 0.71, respectively, and the coefficients between the Luminex and Lumipulse G were 0.89, 0.69, and 0.82, respectively. Aβ42, t-tau, p-tau and Aβ40 levels measured by INNOTEST and Lumipulse G showed strong correlation coefficients: i.e., 0.91, 0.83, 0.91, and 0.86, respectively. Solid and dotted lines indicate the fitted lines of linear regression and 95% CI, respectively.

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