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Plasma biomarkers for early detection of alzheimer's disease: a cross-sectional study in a Japanese cohort

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Abstract

Background Plasma biomarkers offer a promising alternative to amyloid beta (Aβ) positron emission tomography (PET) or cerebrospinal fluid (CSF) biomarkers for diagnosing Alzheimer's disease (AD). This cross-sectional study assessed the utility of multiple plasma biomarkers for diagnosing and staging AD in a Japanese cohort.

Methods The assessed plasma biomarkers included Aβ42/40, phosphorylated tau (p-tau181 and p-tau217), glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL), individually and in combination. Aβ42/40 was measured using the HISCL® platform, while all other biomarkers were measured using the Simoa® platform. Participants were classified based on Aβ PET imaging and neuropsychological testing into healthy controls (HC), AD continuum (preclinical AD, mild cognitive impairment [AD-MCI], and mild dementia [AD-D]), and non-AD cognitive impairment (CI) groups. Receiver operating characteristic analyses were performed to predict the Aβ PET status, correlation with Centiloid (CL) values and cognitive scores, and biomarker comparisons across AD stages.

Results Sixty-nine HC, 13 preclinical AD, 38 AD-MCI, 44 AD-D, and 79 non-AD CI participants were included. The area under the curves (AUCs) for predicting Aβ PET status were 0.937 (Aβ42/40), 0.926 (p-tau217), and 0.946 (p-tau217/Aβ42); results of pair-wise DeLong tests revealed no significant differences among these three metrics (all $p > 0.05$). In the cognitively normal group, the AUCs were 0.968 (Aβ42/40), 0.958 (p-tau217), and 0.979 (p-tau217/Aβ42), while in the cognitively impaired group, they were 0.919 (Aβ42/40), 0.893 (p-tau217), and 0.923 (p-tau217/Aβ42). Among HC and AD continuum participants, CL correlations were −0.74 (Aβ42/40), 0.81 (p-tau217), and 0.83 (p-tau217/Aβ42). In the HC and AD continuum, Aβ42/40 levels showed a bimodal distribution (cutoff = 0.096), with a shift from high to low occurring at 19.3 CL, compared to the PET positivity threshold of 32.9 CL. P-tau217 exhibited a linear increase with disease progression. All biomarkers correlated strongly with logical memory scores.

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Conclusions Plasma biomarkers, Aβ42/40 and p-tau217, and particularly their ratio (p-tau217/Aβ42), show strong potential as Aβ PET alternatives for AD diagnosis. HISCL-based plasma Aβ42/40 detects Aβ accumulation earlier than Aβ PET visual reading threshold, underscoring its utility as an early diagnostic marker.

Keywords Alzheimer's disease, Plasma biomarkers, Aβ42/40, p-tau217, p-tau217/Aβ42, HISCL, Simoa, Centiloid

Background

Plasma biomarkers have recently gained significant attention for diagnosing Alzheimer's disease (AD). Although amyloid beta (Aβ) positron emission tomography (PET) and cerebrospinal fluid (CSF) analyses remain the gold standard for confirming AD pathology [1], these methods are costly, invasive, and impractical outside specialized facilities. In contrast, plasma biomarker tests are more straightforward, minimally invasive, and feasible even in primary care settings, making them a promising tool for aiding AD diagnosis in broader clinical environments [2].

Key plasma biomarkers reflecting AD pathology include the Aβ42/40 ratio, phosphorylated tau (p-tau), glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL). The Aβ42/40 ratio indicates Aβ deposition in the brain and declines in AD because of a selective reduction in Aβ42, which has a higher propensity for aggregation. While plasma Aβ measurement was once challenging, technological advances, such as the HISCL® platform, an automated chemiluminescence immunoassay system with high stability and accuracy, have significantly improved its reliability [3–7]. P-tau181 and 217 also reflect Aβ toxicity, with different phosphorylation sites denoted by numeric markers. Among these, p-tau217 has demonstrated superior diagnostic accuracy and specificity for AD compared to p-tau181 [8]. Recent studies have revealed increased p-tau217 and p-tau231 levels in the early stages of Aβ accumulation, suggesting that these markers also function as sensitive indicators of Aβ pathology [9, 10]. GFAP is a marker of astrocyte reactivity and increases AD because of astrocytic activation in response to Aβ plaque formation [11]. NfL indicates axonal degeneration and is elevated in various neurodegenerative diseases, including AD [12]. Aβ42/40 and p-tau are considered AD-specific biomarkers, whereas GFAP and NfL are non-specific.

In our previous study [7], we found that plasma-based Aβ42/40 exhibited exceptional performance in detecting Aβ accumulation, surpassing p-tau181, GFAP, and NfL in diagnostic accuracy. In the present study, we expanded the sample size and included p-tau217, a biomarker with growing evidence of utility. Additionally, we explored the diagnostic performance of biomarker combinations and conducted a multifaceted analysis, including associations with neuropsychological performance and AD clinical staging, to better characterize disease progression.

Methods

Participants

Cognitively impaired patients who attended the Memory Center at Keio University Hospital and cognitively normal volunteers recruited through external organizations, as described in our previous papers, were recruited between July 2018 and May 2024 [7, 13, 14]. All participants were aged 40–85 years and had at least 12 years of education (YOE). The inclusion and exclusion criteria are shown in Table S1. All diagnoses were established based on standard clinical criteria [15–21]. After enrollment, all participants underwent a comprehensive neuropsychological assessment, Aβ PET imaging, plasma biomarker measurements, and apolipoprotein E (APOE) genotyping.

Ethical approval, registration, and informed consent

This study was approved by the Certified Review Board of Keio University (approval number N20170237) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants or their representatives when necessary. The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; ID: UMIN000032027, the Registration Date, 2018/3/31) and the Japan Registry of Clinical Trials (jRCT; ID: jRCTs031180225).

Aβ PET imaging

Details of the PET scan protocol have been described previously [13].

Aβ PET imaging was performed using 18 F-Florbetaben (FBB) [22, 23]. Images were reconstructed using ordered-subsets expectation maximization. Following standardized visual reading guidelines that have been approved by the U.S. Food and Drug Administration (FDA) and Japanese health insurance, trained neuroradiologists and a dementia specialist classified scans as Aβ-positive (Aβ+) or Aβ-negative (Aβ−).

Quantitative Aβ assessment was conducted using Amyquant, a semiautomated software tool [24] to calculate the Centiloid (CL) value [25]. The CL scale, a 100-point standardized system, facilitates data comparison across institutions and PET tracers. Regions of interest included the posterior cingulate cortex/precuneus, frontal lobe, temporal lobe, parietal lobe, and putamen, with the whole cerebellum serving as the reference region [26].

Plasma biomarker measurements

Plasma concentrations of p-tau181, p-tau217, GFAP, and NfL were measured using the Single Molecule Array (Simoa®) platform (Quanterix, Billerica, MA, USA) with the following kits: Simoa® ALZpath p-Tau 217 Advantage PLUS for p-tau217, Simoa® pTau-181 Advantage V2 Kit for p-tau181, Simoa® GFAP Discovery Kit for GFAP, and Simoa® NF-Light Advantage PLUS Reagent Kit for NfL. Plasma Aβ40 and Aβ42 concentrations were measured using the High Sensitivity Chemiluminescence Enzyme-immunoassay (HISCL®) platform (Sysmex, Kobe, Japan), a completely automated high-sensitivity chemiluminescence enzyme immunoassay system [27].

APOE genotyping

Genomic DNA was extracted using the Magnetic Nanoparticles DNA Extraction kit. APOE genotypes (ε2, ε3, and ε4) were determined by real-time PCR with TaqMan probes [28].

Cognitive assessment

We administered the following comprehensive neuropsychological tests to assess cognitive function: Clinical Dementia Rating (CDR), Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog), Wechsler Memory Scale (WMS), Logical Memory I Immediate Recall (LM-I) and LM-D, Word Fluency Test Category (WF-C) and Initial Letter (WF-I), and the Japanese version of the Trail Making Test Parts A (TMTJ-A) and B (TMTJ-B). Standard procedures were followed while administering these tests as described in a previous study [29].

Statistical analysis

At the time of study enrollment, clinical diagnoses of AD and MCI were re-confirmed using neuropsychological assessments. Participants who were Aβ + were classified as having mild AD dementia (AD-D) or MCI due to AD (AD-MCI), depending on cognitive impairment severity. Those with CDR = 0 or 0.5 who did not meet MCI criteria and showed no evidence of neuropsychiatric disorders were classified as cognitively normal. Among them, Aβ + participants were defined as preclinical AD, corresponding to stage 1 of the revised criteria for the diagnosis and staging of AD [2], while Aβ- participants were defined as healthy controls (HC). Aβ- individuals who did not qualify as HC were categorized as non-AD cognitive impairment (CI).

Using Python 3.10.16, analyses were conducted on 3 groups: the AD continuum (preclinical AD, AD-MCI, and AD-D), the HC group, and the non-AD CI group. For all analyses, a p-value of 0.05 was considered statistically significant.

- ROC Analyses for Aβ PET Prediction.
  We used ROC analyses to evaluate each biomarker's ability to distinguish Aβ + from Aβ- in the following groups:
  (1) Overall dataset: AD continuum vs. non-AD participants (HC and non-AD CI)
  (2) Cognitively impaired group: AD-MCI + AD-D vs. non-AD CI
  (3) Cognitively normal group: preclinical AD vs. HC
  Additionally, we examined all pairwise ratios (quotients) and products of the six biomarker values (Aβ42, Aβ40, p-tau181, p-tau217, GFAP, and NfL). For (1), (2), and (3), we selected the top five combinations based on the highest AUC values and plotted their ROC curves. The optimal cut-off values were determined using the Youden index (YI). Pairwise comparisons of AUCs were conducted using the DeLong test with False Discovery Rate (FDR) correction.
- Correlations with CL and Cognitive Scores
  In participants classified as HC or in the AD continuum, Spearman's correlation coefficients were used to analyze relationships between each biomarker and CL values and between each biomarker and cognitive scores. Cognitive scores were adjusted for sex, age, and YOE using a linear regression model.
- Changes Across AD Stages
  Biomarker values were compared among the HC, preclinical AD, AD-MCI, and AD-D groups. In HC and preclinical AD, comparisons were also performed based on CL level. Following criteria from a lecanemab trial in preclinical AD [30], CL values were divided into 3 groups: low Aβ (< 20), intermediate Aβ (20–40), and elevated Aβ (≥ 40). Between-group comparisons were conducted using the Kruskal–Wallis test. When significant differences were detected, Dunn's post-hoc test with Bonferroni correction was applied to determine which specific group differences were significant. Additionally, linear trends were evaluated using a polynomial contrast test, and equivalence was assessed using a two one-sided test (TOST).

Results

Descriptive statistics are presented in Table 1. After excluding participants with coexisting neurological disorders (one each with CBS, LBD, and history of dura mater transplant due to a skull tumor) from the AD continuum group because of difficulties in interpreting the results, the final analysis included 69 HC and 13 preclinical AD, 38 AD-MCI, 44 AD-D, and 79 non-AD CI participants. The non-AD CI group clinically comprised 15

Table 1 Demographic, cognitive, and biomarker profiles

		The AD continuum					
	HC (*n*=69)	**preclinical AD (*n*=13)**	**AD-MCI (*n*=38)**	**AD-D (*n*=44)**	**Difference Test**	**Normality Test**	**non-AD CI (*n*=79)**
Male	35 (50.7%)	9 (69.2%)	21 (55.3%)	20 (45.5%)	0.474		41 (51.9%)
Age	69 [63-76]	73 [67-76]	76 [70-79]	75 [62-80]	<0.05	<0.05	73 [64-78]
YOE	16.0 [14.0-16.0]	16.0 [15.0-16.0]	16.0 [12.0-16.0]	14.0 [12.0-16.0]	0.09	<0.05	16.0 [12.0-16.0]
MMSE	29.0 [29.0-30.0]	29.0 [27.0-29.0]	27.0 [26.0-29.0]	21.0 [17.0-22.0]	<0.05	<0.05	26.0 [23.0-28.0]
ADAS-Cog	4.0 [2.7-5.0]	5.7 [4.1-8.7]	8.2 [6.0-9.7]	16.5 [12.0-21.7]	<0.05	<0.05	9.2 [5.4-12.6]
LM-I	13.0 [11.0-16.0]	11.0 [9.0-13.0]	5.0 [4.0-6.0]	2.0 [1.0-3.0]	<0.05	<0.05	6.0 [3.0-8.0]
LM-D	12.0 [10.0-14.0]	10.0 [9.0-11.0]	2.0 [0.0-4.0]	0.0 [0.0-1.0]	<0.05	<0.05	3.0 [0.0-5.0]
WF-C	42.0 [38.0-47.0]	38.0 [27.0-43.0]	33.0 [27.2-38.2]	22.5 [16.0-30.0]	<0.05	0.274	26.0 [19.0-35.0]
WF-I	27.0 [22.0-32.0]	28.0 [19.0-33.0]	24.5 [21.0-30.8]	17.0 [12.0-22.2]	<0.05	0.151	18.0 [13.8-24.0]
TMTJ-A	48.0 [39.0-56.0]	50.0 [37.0-68.0]	59.5 [47.5-72.5]	91.5 [69.2-124.8]	<0.05	<0.05	66.0 [48.5-93.5]
TMTJ-B	73.0 [57.0-95.0]	99.0 [81.0-109.0]	108.5 [86.0-154.5]	155.5 [120.0-251.5]	<0.05	<0.05	117.5 [77.8-169.5]
Aβ42 (pg/mL)	18.7 [17.3-21.2]	13.3 [12.9-14.1]	16.1 [14.5-18.4]	15.0 [13.2-16.9]	<0.05	<0.05	19.0 [17.5-20.2]
Aβ40 (pg/mL)	171.4 [161.9-190.2]	159.8 [154.4-184.9]	184.5 [169.5-205.1]	173.9 [160.7-195.4]	<0.05	<0.05	179.0 [165.0-196.6]
Aβ42/40	0.109 [0.104-0.114]	0.083 [0.081-0.086]	0.085 [0.083-0.089]	0.085 [0.082-0.089]	<0.05	<0.05	0.105 [0.097-0.112]
p-tau181 (pg/mL)	17.8 [15.9-23.7]	34.2 [25.3-38.8]	34.3 [27.9-44.2]	37.7 [34.5-46.4]	<0.05	<0.05	23.6 [17.6-30.4]
p-tau217 (pg/mL)	0.24 [0.19-0.34]	0.74 [0.54-0.92]	0.97 [0.78-1.21]	1.08 [0.77-1.28]	<0.05	<0.05	0.32 [0.24-0.50]
NfL (pg/mL)	16.5 [10.6-20.4]	25.4 [19.4-27.1]	23.7 [17.1-33.7]	25.3 [18.6-30.1]	<0.05	<0.05	25.4 [18.4-40.8]
GFAP (pg/mL)	213.0 [167.1-278.8]	320.2 [268.7-542.4]	503.2 [300.4-780.6]	458.5 [340.9-650.9]	<0.05	<0.05	287.8 [204.8-408.0]
CL	-2.3 [-5.7-5.1]	62.3 [58.0-84.6]	83.6 [58.3-105.0]	94.3 [64.3-118.2]	<0.05	<0.05	-1.1 [-6.8-10.0]
APOE4 carrier	15 (21.7%)	7 (53.8%)	20 (52.6%)	27 (61.4%)	<0.05		14 (17.7%)

Abbreviations: AD, Alzheimer's disease; AD-MCI, mild cognitive impairment due to AD; AD-D, AD dementia; CI, cognitive impairment; HC, healthy control; CI, cognitive impairment; YOE, year of education; MMSE, Mini-Mental State Examination; ADAS-Cog, Alzheimer's Disease Assessment Scale–Cognitive Subscale; Aβ, amyloid beta; p-tau, phosphorylated tau; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; CL, Centiloid; ApoE4, Apolipoprotein E ε4

Continuous variables are shown as median [IQR]. Differences among HC, preclinical AD, AD-MCI, and AD-D were assessed using the Kruskal-Wallis test; normality was tested using the Shapiro-Wilk test on the combined sample of these groups

FTLD, 7 PSP, 6 CBS, 3 LBD, 3 TBI, 1 NPH, 1 encephalitis, 1 myotonic dystrophy, and 42 other participants without a specific clinical diagnosis. Missing data were identified for MMSE ($n = 4$), ADAS-Cog ($n = 4$), LM-D ($n = 5$), LM-I ($n = 5$), WF-C ($n = 4$), WF-I ($n = 4$), TMTJ-A ($n = 10$), TMTJ-B ($n = 25$), p-tau181 ($n = 4$), NfL ($n = 4$), and CL ($n = 4$). Missing values in neuropsychological assessments were mainly attributed to impaired verbal output, communicative difficulties due to hearing loss, or time-limit violations during task performance. For each analysis, participants with missing data for the variables involved were excluded. No imputation was performed. No significant differences in sex or YOE were observed between the HC and the AD continuum groups.

As shown in Fig. 1, Aβ42/40 and p-tau217 demonstrated high AUCs as single biomarkers, whereas p-tau217/Aβ42 was the best-performing among all biomarker combinations evaluated. The AUCs for Aβ42/40 and p-tau217 were 0.937 and 0.926 (Fig. 1A; AD continuum vs. non-AD participants), 0.919 and 0.893 (Fig. 1B; AD-MCI + AD-D vs. non-AD CI), and 0.968 and 0.958 (Fig. 1C; preclinical AD vs. HC), respectively. Among all evaluated combinations, p-tau217/Aβ42 and p-tau217/Aβ40 ranked within the top five AUCs across all three classification tasks. The AUCs for p-tau217/Aβ42 were 0.946 (Fig. 1D), 0.923 (Fig. 1E), and 0.979 (Fig. 1F), numerically the largest among all single biomarkers; however, none of the pair-wise comparisons reached statistical significance (all DeLong's $p > 0.05$). Table S2 provides the cutoff, AUC, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for single biomarkers and biomarker combinations and the pair-wise DeLong test results with FDR correction. NfL vs. Aβ42/40, p-tau217 or p-tau217/Aβ42 showed significant AUC differences. As shown in Fig. 2, p-tau217/Aβ42 exhibited the strongest correlation with CL, followed by p-tau217 and Aβ42/40, with correlation coefficients of 0.83, 0.81, and − 0.74, respectively.

Figure 3 compares the changes in biomarker levels across AD stages, and detailed statistical test results are summarized in Table S3. Although Aβ42/40 decreased markedly from HC to preclinical AD, it remained relatively stable in later disease stages; specifically, TOST demonstrated significant equivalence only between AD-MCI and AD-D. In contrast, p-tau181, p-tau217, and p-tau217/Aβ42 showed an increasing trend across AD stages, beginning from preclinical AD. Notably, significant linear contrasts were observed for p-tau217 and p-tau217/Aβ42 in the polynomial contrast test. When examining CL and biomarkers limited to cognitively normal participants (HC + preclinical AD) (Fig. 4) to further focus on preclinical Aβ pathology, Aβ42/40 significantly

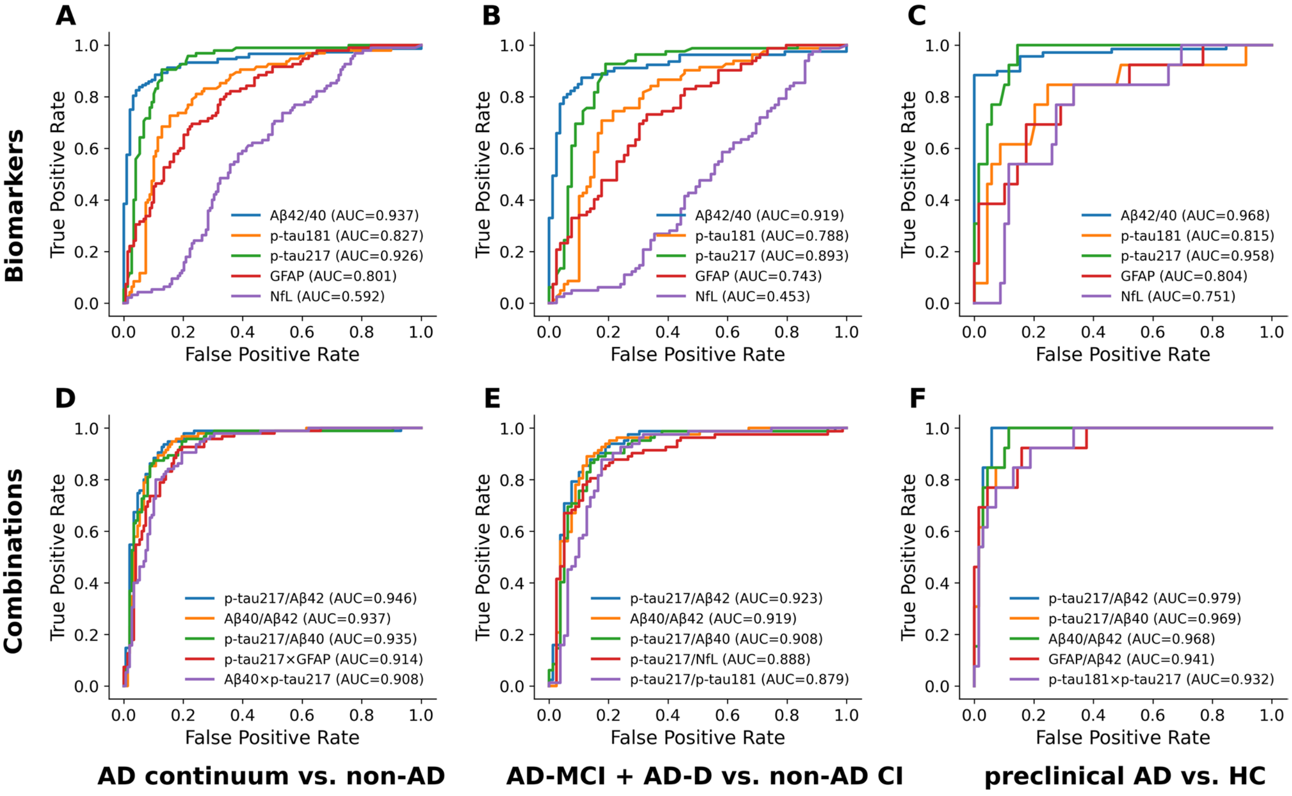

Fig. 1 ROC analysis of single biomarkers (**A**–**C**) and their combinations (**D**–**F**). The combinations represent the top five biomarker pairs with the highest AUC. The comparison groups are as follows: **A** & **D**: AD continuum ($n = 95$) vs. non-AD ($n = 148$); B & E: AD-MCI + AD-D ($n = 82$) vs. non-AD CI ($n = 79$); C & F: preclinical AD ($n = 13$) vs. HC ($n = 69$)

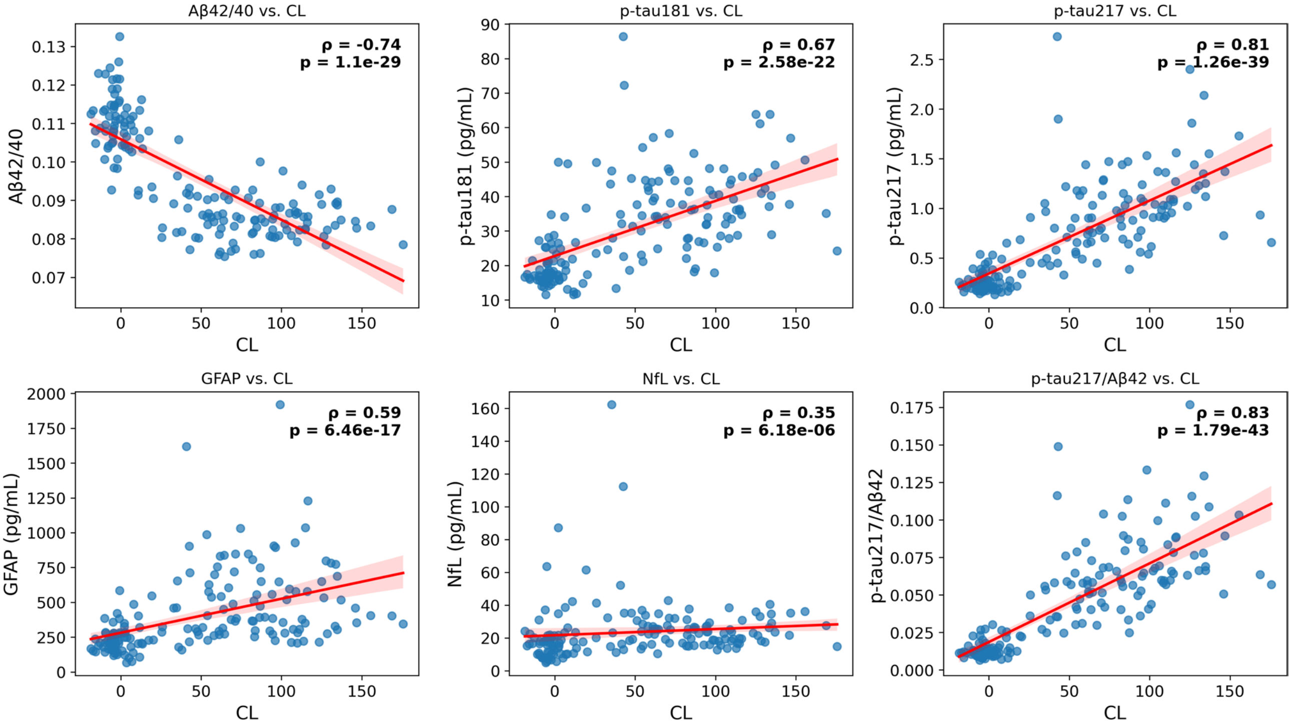

Fig. 2 Scatter plots between CL and each biomarker (including p-tau217/Aβ42), with Spearman's correlation coefficients (ρ) and the best-fit linear regressions

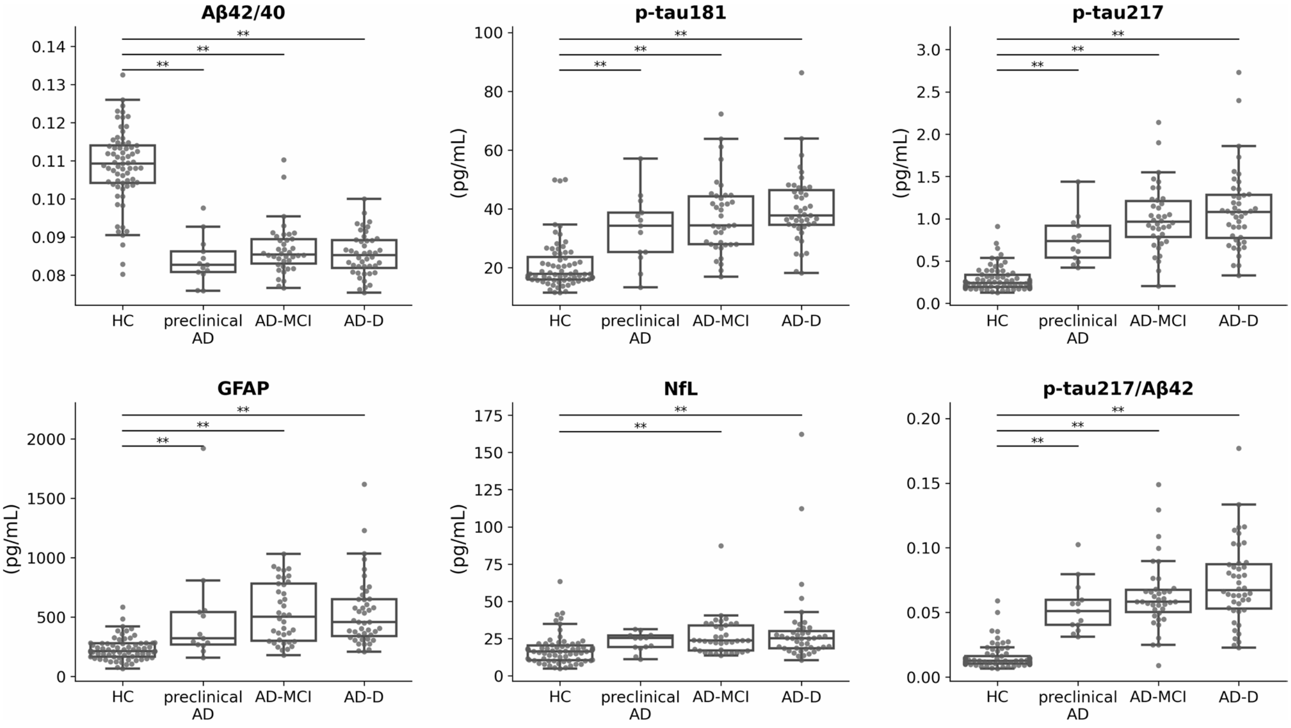

Fig. 3 Comparison of biomarker values at each AD stage in HC ($n=69$) + the AD continuum (preclinical AD [$n=13$], AD-MCI [$n=38$], and AD-D [$n=44$]). *$p<0.05$, **$p<0.01$

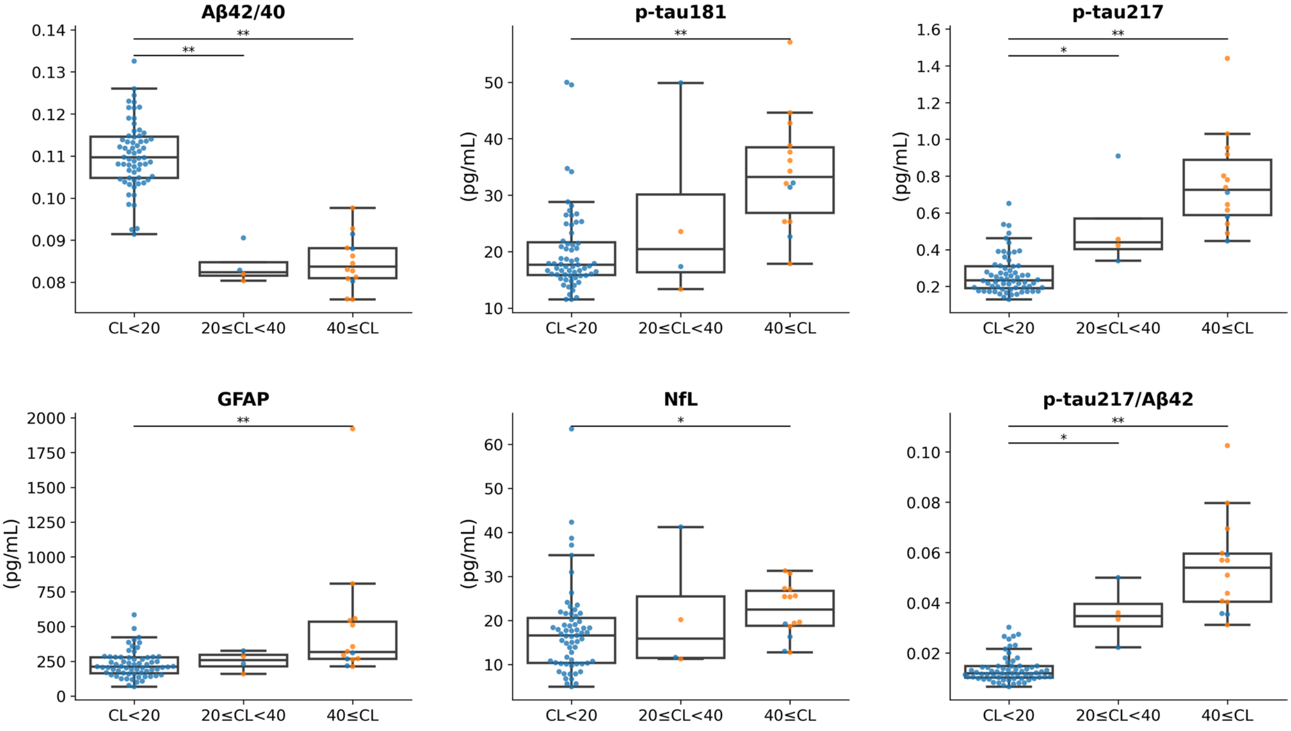

Fig. 4 Comparison of biomarker values at each CL level in HC ($n=69$; blue dots) + preclinical AD ($n=13$; orange dots). *$p<0.05$, **$p<0.01$

differed between the low and intermediate Aβ groups but not between the intermediate and high Aβ groups. For p-tau217 and p-tau217/Aβ42, a step-wise upward trend was observed; however, statistical significance was present only between the low-Aβ ($CL < 20$) and intermediate/high-Aβ groups ($CL \geq 20$).

Based on these findings, we concluded that Aβ42/40 effectively differentiates Aβ accumulation qualitatively. Therefore, additional analyses were conducted to compare Aβ42/40 with Aβ PET in the HC and AD continuum cohort. The optimal CL threshold for discriminating Aβ PET positivity from negativity based on visual reading, as determined by YI, was 32.90 (sensitivity 0.968, specificity 0.957; Fig. 5A). As shown in Fig. 5B, the Aβ42/40 distribution was bimodal (Ashman's $D > 2$). Using the intersection of the two components in the fitted Gaussian mixture model (GMM) at 0.096 as the cutoff, participants were clearly separated into low and highAβ42/40 groups. Bootstrap resampling ($n = 1000$) consistently confirmed Ashman's $D > 2$ (Fig. S1) and a narrowly distributed cutoff around 0.096 (Fig. S2). The optimal CL threshold distinguishing these groups was 19.35 (sensitivity 0.969, specificity 0.954; Fig. 5C), which was substantially lower than the visual reading threshold for Aβ PET.

In the correlation analyses with cognitive scores (Fig. 6), Logical Memory (LM-I and LM-D) had the highest correlations with biomarkers, followed by ADAS-Cog. Among the biomarkers, p-tau217, p-tau217/Aβ42, and CL demonstrated particularly strong correlations, with absolute correlation coefficients of approximately 0.7 for

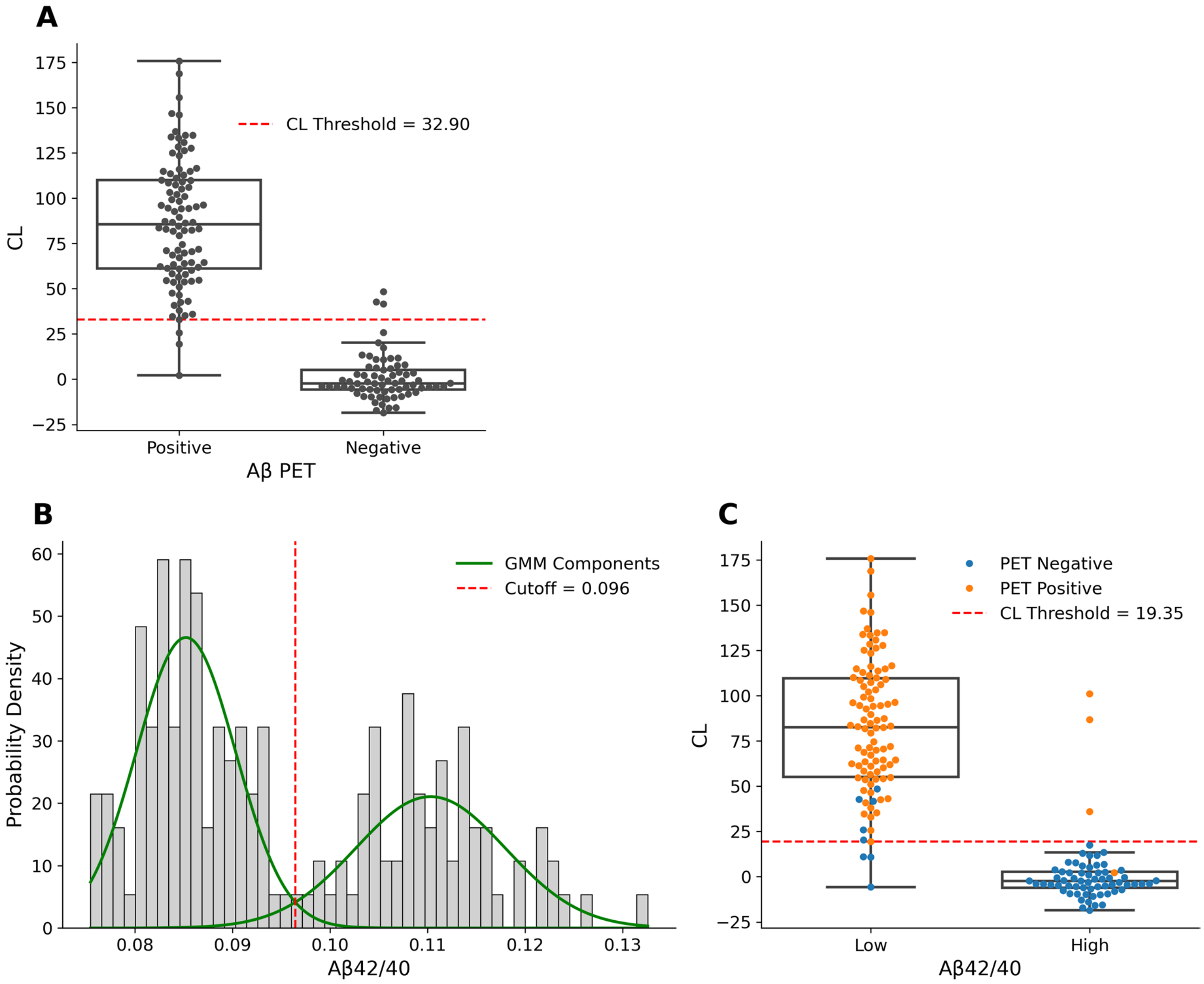

Fig. 5 **A**: Comparison of CL values between Aβ PET-positive and -negative groups. The CL threshold was determined using the Youden index. **B**: Histogram of Aβ42/40 values with probability density functions estimated by a two-component Gaussian mixture model (GMM). The intersection of the two components was used as the threshold to classify values into low and high groups. **C**: Comparison of CL values between the low and high Aβ42/40 groups. The CL threshold was determined using the Youden Index. All analyses were conducted in HC ($n = 69$) + the AD continuum (preclinical AD [$n = 13$], AD-MCI [$n = 38$], and AD-D [$n = 44$])

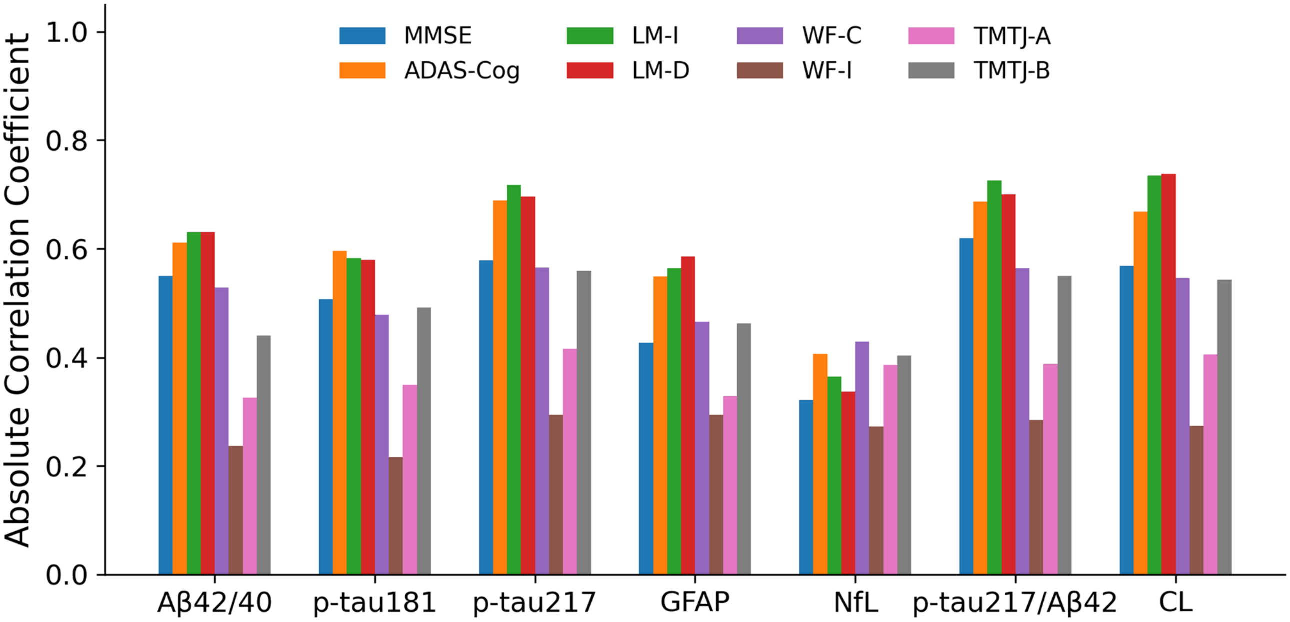

Fig. 6 Absolute values of Spearman's correlation coefficients between biomarkers (including p-tau217/Aβ42 and CL) and cognitive scores were adjusted for sex, age, and years of education using a linear regression model

LM (Table S4, Fig. S3). Additionally, WF correlated more strongly with WF-C than with WF-I, while TMTJ correlated more strongly with TMTJ-B than with TMTJ-A.

Discussion

In this study, we demonstrated that plasma Aβ42/40 and p-tau217 levels accurately predict Aβ PET positivity and negativity in individuals ranging from cognitively normal to mild dementia. Aβ42/40 exhibited a numerically higher AUC than p-tau217 (0.937 vs. 0.926); however, the difference was not statistically significant (DeLong's $p = 0.905$). Aβ42/40 showed high specificity (sensitivity 0.884, specificity 1.000; Table S2) in the cognitively normal group, suggesting its utility in identifying preclinical AD. Plasma Aβ42/40 has already been validated in multiple studies [3–5, 7, 31, 32], establishing its clinical usefulness. Although measurement techniques can influence biomarker performance, the inexpensive and simple HISCL platform used in this study has been reported to offer superior stability and accuracy compared with other methods [6, 7]. Among the single biomarkers, p-tau217 exhibited the highest AUC. A significant difference was observed only in the comparison between p-tau217 and NfL (DeLong's $p = 0.018$), while comparisons between p-tau181 and GFAP were not significant [33]. The AUC values obtained in this study (p-tau217 = 0.926, Aβ42/40 = 0.937) are comparable to those reported for other advanced platforms—Lumipulse® p-tau217 (0.92–0.97) [34–37], IP-MS %p-tau217 (approximately 0.95) [38], and Lumipulse® Aβ42/40 (0.81–0.92) [35, 39]—inidicating that diagnostic accuracy remains clinically similar across different assay technologies. Lumipulse® (Fujirebio, Tokyo, Japan), like HISCL, is a fully automated chemiluminescence immunoassay designed for routine clinical laboratories [34–37, 39], whereas IP-MS combines antibody-based immunoprecipitation with targeted mass spectrometry to achieve high peptide specificity and analytical sensitivity [40]. The cost-effectiveness, feasibility, and practical implementation of these testing approaches, and head-to-head comparisons of their diagnostic accuracy should be assessed in future studies.

When we evaluated biomarker combinations, using HISCL-based Aβ42 and Simoa-based p-tau217, the p-tau217/Aβ42 ratio yielded a numerically higher AUC than Aβ42/40 (0.946 vs. 0.937); however, this difference was not statistically significant (DeLong's $p = 0.951$). Since Aβ42/40 provides higher specificity, and p-tau217 offers greater sensitivity (Table S2), their ratio represents a promising index that combines the strengths of both markers. Notably, the fully automated Lumipulse® G p-tau217/Aβ42 plasma ratio assay received FDA clearance in May 2025 as the first blood-based in-vitro diagnostic test for AD [41]. In a 499-patient multicentre study, this assay achieved an AUC of approximately 0.96 for identifying Aβ PET positivity [42], essentially matching the performance of the HISCL and Simoa combination reported in this study, and thereby demonstrating that the composite ratio retains its diagnostic validity across distinct automated platforms.

Aβ42/40 showed little change after the preclinical AD stage, particularly once CL values exceeded 20 (Figs. 3 and 4). This observation suggests that plasma Aβ42/40 reflects Aβ deposition but does not track subsequent disease progression along the AD continuum. The finding is consistent with the established view that Aβ deposition reaches a plateau in the early phase of AD and remains relatively stable after clinical symptoms emerge [43]. Therefore, Aβ42/40 serves as a marker of early Aβ pathology. In contrast, p-tau217 increased in parallel with disease progression across the AD continuum, consistent with previous findings [9]. The largest increase was seen between HC and preclinical AD, while changes from preclinical AD to AD-D were more modest. Although CSF biomarkers were not measured in this study, longitudinal studies show the same pattern: CSF Aβ42/40 declines early and plateaus, whereas CSF p-tau217 rises step-wise with its plasma counterpart as tau pathology advances [44, 45]. Together, these findings indicate that substantial pathological change exists even in the preclinical stage and underscore that plasma Aβ42/40 captures amyloid status, while plasma p-tau217 provides a sensitive readout of disease progression across the AD continuum.

Although previous studies have reported a low fold difference between individuals with and without Aβ pathologic change (only 10%) for plasma Aβ42/40 compared to CSF assays [46–48], in this study, HISCL-based Aβ42/40 acted as a robust binary classifier of Aβ status and exhibited a clear bimodal distribution at a cutoff of 0.096, separating participants into low and high groups (Fig. 6). To rule out chance, we performed extensive bootstrap resampling; the bimodal pattern and its cutoff consistently reappeared, confirming the robustness of our finding. The corresponding CL threshold for this division was 19.35 CL, which was lower than the typical threshold for Aβ PET positivity (32.9 CL). This suggests that Aβ42/40 changes occur earlier and can detect low levels of Aβ accumulation before the PET positivity threshold is reached. Notably, a threshold of approximately CL 20 has been reported to capture moderate to extensive Aβ plaque accumulation in post-mortem findings [49], aligning with the Aβ42/40 cutoff identified here. These findings suggest that plasma Aβ42/40 reflects underlying pathological changes more sensitively than visual reading PET-based thresholds.

Among neuropsychological tests, the LM test showed the strongest correlations with both plasma biomarkers and CL, with no significant differences between LM-I and LM-D. Although delayed recall is often emphasized in the clinical evaluation of AD, previous studies indicate that immediate recall can also decline in early preclinical stages [50], highlighting it as another key measure to monitor. In the WF test, the category version correlated more strongly with biomarkers than the initial letter version. This likely reflects a characteristic decline in semantic retrieval, which is primarily associated with the temporal lobe, rather than executive functioning, which is more closely linked to the frontal lobe [51].

Compared with Aβ PET, plasma biomarkers have a lower physical burden, reduced cost, and greater overall availability. In the 2024 update of the Alzheimer's Association diagnostic guidelines, blood-based biomarkers were included in "Core 1," the category of markers considered sufficient for diagnosis, and a flexible diagnostic approach was proposed in which blood testing may be used for initial evaluation, with CSF or PET recommended for cases with indeterminate results or clinical uncertainty [52]. Moreover, the fully automated Lumipulse® G plasma p-tau217/Aβ1–42 ratio assay received FDA clearance, making blood testing a practical option for routine clinical use [41]. The performance of the HISCL platform reported in this study reinforces this international trend and suggests that blood biomarkers are poised to become the new standard in AD diagnosis.

Limitations

This study has several limitations. First, the small sample size limits the generalizability of the results. Particularly, the preclinical AD group included only 13 individuals, which limits the reliability of subgroup findings and requires cautious interpretation. Second, the recently highlighted biomarker p-tau231, which reached abnormal levels at the lowest Aβ burden [10], was not measured. Finally, the absence of an independent validation cohort and longitudinal data underscores the urgent need for replication in independent samples and longitudinal validation studies.

Conclusion

Our findings demonstrate that plasma Aβ42/40, p-tau217, and particularly their ratio (p-tau217/Aβ42) effectively detect and monitor AD pathology. Notably, HISCL-based Aβ42/40 identifies Aβ accumulation at an earlier stage than the conventional PET threshold, while p-tau217 effectively tracks disease progression. The p-tau217/Aβ42 ratio showed comparable or slightly better performance than Aβ42/40 in predicting Aβ positivity. These results underscore the potential of plasma assays for large-scale screening and more accessible disease monitoring, particularly with emerging AD-modifying therapies.

Abbreviations

Aβ	Amyloid Beta
Aβ42/40	Amyloid Beta 42/40 ratio
AD	Alzheimer's Disease
AD-D	Alzheimer's Disease Dementia
AD-MCI	Mild Cognitive Impairment due to Alzheimer's Disease
CL	Centiloid
CDR	Clinical Dementia Rating
FDR	False Discovery Rate
FTLD	Frontotemporal Lobar Degeneration
GFAP	Glial Fibrillary Acidic Protein

HISCL	High-Sensitivity Chemiluminescence Enzyme Immunoassay
IP-MS	Immunoprecipitation-Mass Spectrometry
LM-D	Logical Memory Delayed Recall
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
NfL	Neurofilament Light Chain
p-tau	Phosphorylated Tau
p-tau181	Phosphorylated Tau at Threonine 181
p-tau217	Phosphorylated Tau at Threonine 217
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristic
TOST	Two One-Sided Test
WF-C	Word Fluency Category
WF-I	Word Fluency Initial Letter
WMS	Wechsler Memory Scale
YI	Youden Index
YOE	Year of Education

Supplementary Information

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Additional file 1: Histogram of Ashman's D values calculated from 1,000 bootstrap resamples of the plasma Aβ42/40 dataset. The red dashed vertical line (Ashman's D = 2) denotes the conventional benchmark for clear bimodality.

Additional file 2: Histogram of the Gaussian mixture model (GMM) intersection thresholds for plasma Aβ42/40, derived from the same 1,000 bootstrap resamples.

Additional file 3: Scatter plots between cognitive scores and each biomarker (including p-tau217/Aβ42 and CL), with Spearman's correlation coefficients (ρ) and the best-fit linear regressions.

Additional file 4: Major Criteria for Participant Enrollment

Additional file 5: Performance metrics of single biomarkers and biomarker combinations. Description: This table presents performance metrics, including AUC, sensitivity, specificity, PPV, and NPV for various biomarkers and combinations, as well as the results of pair-wise DeLong tests with FDR correction, across different clinical groups.

Additional file 6: Statistical Tests Comparing Biomarkers Across AD Stages Description: This table shows the results of the Kruskal–Wallis test, polynomial contrast test, and two one-sided test among HC and preclinical AD, AD-MCI, and AD-D individuals.

Additional file 7: Spearman's correlation coefficients between each biomarker and cognitive scores Description: This table shows the correlation between biomarkers such as Aβ42/40, p-tau181, p-tau217, and cognitive scores including MMSE, ADAS-Cog, LM-I, LM-D, and others.

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Author contributions

MK, SB, HT, and DI contributed to study conception (lead contributor was DI). SB, MK, YMomota, YI, TT, MS, YY, RS, SK, YM, and SS contributed to participant recruitment. KT and TH KT, HA, YS contributed to data curation, including activities to clean and maintain research data. NS, AM, AO, and YH contributed to administrative, technical, or material support. All authors interpreted the results and critically reviewed the manuscript. JK and JN, MM supervised the study.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The Certified Review Board of Keio University (#N20170237) approved the study design and protocol. The study was conducted in accordance with the Declaration of Helsinki. All participants (plus their proxies as needed) provided written informed consent for participation in the study. The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; https://www.umin.ac.jp/ctr/index.htm, ID# UMIN000032027) and Japan Registry of Clinical Trials (jRCT; https://jrct.niph.go.jp/, ID# jRCTs031180225).

Consent for publication

Not applicable.

Competing interests

DI has received honorariums from Daiichi Sankyo, Nihon Medi-Physics, Kowa, PDRadiopharma, Otsuka Pharmaceutical, Lilly and Eisai and has a joint research agreement with Sysmex. There are no other relationships or activities that could appear to have influenced the submitted work.

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