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RESEARCH ARTICLE

Plasma amyloid beta biomarkers predict amyloid positivity and longitudinal clinical progression in mild cognitive impairment

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Abstract

INTRODUCTION: Previous studies have examined the predictive accuracy of plasma amyloid beta (Aβ) biomarkers in clinical cohorts. However, their accuracy for predicting amyloid-positive patients in community-based cohorts is unclear. This study aimed to determine the predictive accuracy of Aβ precursor protein 669-711/Aβ1-42, Aβ1-40/1-42 and their composite biomarkers for brain amyloid deposition or the clinical progression in community-dwelling older adults with mild cognitive impairment (MCI).

METHODS: This prospective cohort study was conducted from August 2015 to September 2019. Subsequently, the participants underwent follow-up cognitive assessments up to 8 years after the start of the study. Blood samples were collected from older adults aged $\geq$ 65 years with MCI at baseline. Plasma Aβ biomarkers were analyzed using immunoprecipitation-mass spectrometry. The accuracy of plasma biomarkers for brain amyloid status was evaluated using receiver operating characteristic curve analysis. Relationships between comorbidities and plasma Aβ markers were examined using multiple linear regression analysis. Associations of plasma biomarkers with clinical conversion to Alzheimer's disease (AD) dementia were evaluated using Kaplan–Meier curves.

RESULTS: The participants included 107 patients (57 [53.3%] females, median age: 76.0 [72.0–80.0] years). Plasma biomarkers correlated with cortical amyloid uptake (ρ = 0.667–0.754). The composite biomarker had the best area under the curve (0.943, 95% confidence interval [CI]: 0.901 to 0.985) for predicting amyloid positivity. Apolipoprotein ε4 status showed significant correlations with increased plasma amyloid biomarker levels. Participants with high composite biomarker levels at baseline had a greater risk of conversion to AD dementia (hazard ratio 10.74, 95% CI: 3.51 to 32.84, $P < 0.001$). The higher composite biomarker was associated with a faster rate of cognitive decline ($\rho = -0.575$, $P < 0.001$).

DISCUSSION: Plasma Aβ composite biomarker may serve as a surrogate measure for amyloid deposition and a predictor of disease progression in a community-based cohort.

KEYWORDS
amyloid, Alzheimer's disease, composite biomarker, mild cognitive impairment, older adult

Highlights

- Plasma amyloid beta (Aβ) biomarkers correlated with 11C-Pittsburgh compound B uptake, mainly in the frontal/parietotemporal cortices and posterior cingulate gyrus.
- The amyloid composite biomarker can predict amyloid positron emission tomography positivity with a high area under the curve of 0.943 in a community-based mild cognitive impairment cohort.
- The higher amyloid composite biomarker at baseline was significantly associated with worsening Mini-Mental State Examination score and a high risk for developing Alzheimer's disease (AD) dementia over 8 years.
- The amyloid composite biomarker can predict clinical progression to AD dementia with a high area under the curve of 0.860.
- Apolipoprotein E ε4 status influenced the plasma Aβ biomarker levels.

1 | BACKGROUND

The prevalence of mild cognitive impairment (MCI) in Japanese adults aged ≥ 65 years is 17%; 10% to 34% of adults with amnestic MCI develop Alzheimer's disease (AD) dementia annually.[1,2] Recently, the US Food and Drug Administration approved a new disease-modifying therapy targeting amyloid beta (Aβ) for adults with MCI/mild dementia due to AD.[3,4] Accurate detection of amyloid pathology is crucial for enhancing the benefit of disease-modifying therapies in future clinical practice.

Cerebrospinal fluid (CSF) analysis and positron emission tomography (PET) are well-established methods for detecting brain amyloid deposition. However, PET is expensive and not widely available. CSF analysis further requires invasive lumbar puncture. Therefore, these methods are unsuitable as screening tools owing to their high costs. In the future, widely available and minimally invasive blood-based biomarkers for AD are required for prescreening patients with amyloid-positive MCI[5,6] and augmenting PET/CSF analysis.[7,8] Advanced technologies, such as mass spectrometry and immunoassays, can measure plasma Aβ levels with high precision and reproducibility.[9,10] Immunoprecipitation-mass spectrometry (IP-MS) has been proven to be superior to immunoassays in identifying amyloid-positive patients.[11] Most studies have examined the predictive accuracy of plasma Aβ biomarkers measured using IP-MS in clinical cohorts.[12–22] It remains unclear whether these biomarkers can identify amyloid-positive patients with high accuracy in community-based cohorts, which are more diverse in terms of demographic characteristics, lifestyles, and comorbidities than clinical cohorts. Moreover, the amyloid positivity rate in community-based MCI cohorts is lower than that in clinical MCI cohorts.[23] Therefore, this study aimed to determine the predictive accuracy of these Aβ biomarkers for amyloid positivity on PET and the predictive ability of a baseline composite biomarker for clinical progression to AD dementia with 8 years of follow-up in individuals with MCI recruited from a community-based cohort.

2 | METHODS

2.1 | Study design

The Usuki study was designed as a prospective cohort study in Usuki, Japan exploring lifestyle risk factors for dementia/imaging biomarkers of AD, with outcomes preregistered in UMIN Clinical Trials Registry 000017442.[24] This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines. This study was approved by the ethics committee of Oita University Hospital (2346-C43). A total of 855 non-demented community-dwelling adults aged ≥ 65 years enrolled in the Usuki study from August 2015 to March 2016 with continuous follow-up. Of the 855 adults, 118 were diagnosed with MCI.

2.2 | Participants

The present study included 118 older adults with MCI aged ≥ 65 years. MCI was diagnosed according to a global rating of 0.5 on the Clinical Dementia Rating scale. All participants underwent blood sampling

at baseline and annual evaluations of cognitive function and amyloid PET at Oita University Hospital. Trained medical staff collected demographic information (age, sex, years of education, body mass index [BMI], and medical history). Cognitive function was assessed using the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment-Japanese version (MoCA-J), and Wechsler Memory Scale-Revised (WMS-R) Logical Memory II Test. Liver and renal functions were assessed by measuring alanine aminotransferase/aspartate aminotransferase/gamma-glutamyl transpeptidase levels and the estimated glomerular filtration rate. Plasma Aβ biomarker levels in blood samples were measured. No participants were taking medication for dementia at baseline. We collected follow-up data regarding dementia diagnosis, determined by a neurologist according to cognitive and clinical data or medication for AD, from November to December 2023.

2.3 | IP-MS

Blood samples were collected during the morning hours after an overnight fast. After centrifugation (1800 × g for 10 minutes at 4°C), plasma was separated and stored at −80°C. Plasma Aβ1-40, Aβ1-42, and amyloid precursor protein (APP)669-711 levels were measured using IP-MS based on matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF MS). Aβ1-40/1-42 and APP669-711/Aβ1-42 were calculated as the ratios of the normalized intensities of Aβ1-40 and APP669-711 to that of Aβ1-42, respectively. The composite biomarker was computed by averaging the normalized scores of Aβ1-40/1-42 and APP669-711/Aβ1-42.[12] We assessed inter-assay precision over 5 days by measuring quality control sample spiked with stable isotope-labeled Aβ1-40, Aβ1-42, and APP669-711 in this study. The inter-assay coefficient of variation of stable isotope-labeled Aβ1-40/1-42 and APP669-711/Aβ1-42 was 1.8% and 6.2%, respectively.

2.4 | PET

^{11}C-Pittsburgh compound B (PiB) PET was conducted using a Biograph mCT PET/computed tomography scanner (Siemens). A 20 minute static PET image was acquired 50 minutes after an intravenous bolus of 543 ± 57 MBq ^{11}C-PiB was injected with a saline flush. The standardized uptake value ratio (SUVR) was calculated to evaluate ^{11}C-PiB uptake. Statistical Parametric Mapping 8 (Wellcome Trust Center for Neuroimaging) implemented in MATLAB 7.9.0. (R2009b; MathWorks) was used for spatial normalization of PET images to a customized PET template in the Montreal Neurological Institute reference space. The SUVR for ^{11}C-PiB PET was calculated as the ratio of the voxel number-weighted average of the mean uptake in the frontal/temporoparietal/posterior cingulate cortices to that in the cerebellar cortex. The global mean SUVR combined single mean values for all regions. ^{11}C-PiB PET positivity was defined according to the global cortical SUVR of ≥ 1.2.[25]

RESEARCH IN CONTEXT

1. **Systematic review**: Plasma amyloid biomarkers measured by mass spectrometry or immunoassays can predict amyloid positivity on positron emission tomography (PET). It remains unclear whether plasma amyloid biomarkers can predict the future development of Alzheimer's disease (AD) dementia in a community-based cohort of individuals with mild cognitive impairment (MCI).
2. **Interpretation**: The amyloid composite biomarker measured by a Shimadzu analytical platform can predict amyloid PET positivity with a high area under the curve of 0.943. Clinical progression to AD dementia over 8 years of follow-up was significantly associated with amyloid biomarker levels in participants with MCI at baseline.
3. **Future directions**: Further validation studies with heterogeneous and diverse populations are needed to determine the usefulness of plasma amyloid biomarkers in routine clinical practice. In addition, longitudinal studies are necessary for establishing the prognostic utility of these biomarkers.

2.5 | Apolipoprotein E ε4 isoform

Apolipoprotein E (apoE) phenotyping was performed using a human apoE enzyme-linked immunosorbent assay kit (MBL Co.),[26] which can identify individuals with an apoE 4/apoE ratio of ≥ 0.3 as having at least one *APOE* ε4 allele.

2.6 | Statistical analysis

Participants were classified into Aβ-negative ($n = 71$) and Aβ-positive ($n = 36$) subgroups according to the SUVR cutoff of ≥ 1.2. Sex, *APOE* ε4 status, and medical history were compared using the chi-square test; age, education level, BMI, MMSE/MoCA-J/WMS-R Logical Memory II Test scores, cortical ^{11}C-PiB uptake values, aspartate aminotransferase/alanine aminotransferase/gamma-glutamyl transpeptidase levels, estimated glomerular filtration rates, and plasma Aβ biomarkers were compared using the Mann–Whitney *U* test. Correlations between plasma biomarkers and cortical ^{11}C-PiB uptake, and plasma biomarkers and MMSE change were assessed using Spearman correlation coefficients. A voxel-wise linear regression analysis was performed using Statistical Parametric Mapping 8 to determine the spatial association between plasma Aβ biomarkers and brain amyloid deposition. MMSE was used to examine the yearly rate of change in cognitive function, which was calculated using the difference in MMSE score between the first visit and the last visit. The data with a follow-up period of ≥ 4 years was used ($N = 60$). A P value < 0.05 was considered statistically

significant in all analyses. Benjamini–Hochberg correction was used for the multiple comparisons of three Aβ biomarkers.

2.6.1 | Logistic regression with receiver operating characteristic curve analysis

The accuracy of plasma Aβ biomarkers for predicting amyloid positivity and AD conversion status was assessed using the area under the receiver operating characteristic curve values within a binary logistic regression model. Amyloid positivity was indicated by amyloid positivity on PET. AD conversion status categorized participants based on whether they did or did not convert to dementia. Further details are provided in the supporting information. The cutoff values for predicting the amyloid status were determined at the values with ≥ 90.0% for sensitivity and specificity.

2.6.2 | Reweighting for 60% prevalence of amyloid positivity

We estimated the negative and positive predictive values by assuming that the prevalence of amyloid positivity ranged from 33.6% to 60.0%. Detailed calculations are described in supporting information.

2.6.3 | Effects of simulated bias on sensitivity and specificity

We added different bias percentages to the measured values of plasma APP669-711/Aβ1-42 and Aβ1-40/1-42. Using APP669-711/Aβ1-42 and Aβ1-40/1-42 with bias, we evaluated the sensitivity and specificity at dual cutoff values of ≥ 90.0% for sensitivity and specificity, respectively.

2.6.4 | Multiple linear regression analysis

Multiple linear regression was used to determine the associations of plasma Aβ biomarkers with BMI; *APOE* ε4 status; cortical ^{11}C-PiB uptake; medical history; aspartate aminotransferase/alanine aminotransferase/gamma-glutamyl transpeptidase levels; and estimated glomerular filtration rate, controlling for age and sex. Plasma Aβ biomarkers were *z* scored relative to the entire sample to compare coefficients.

2.6.5 | Kaplan–Meier curves

Kaplan–Meier curves were generated to analyze the time to AD dementia progression in the three groups categorized by plasma composite biomarker levels. The overall difference between the groups was calculated using the log-rank test. Cox proportional hazards regression analysis was performed to investigate the hazard ratio for the conversion from MCI to AD dementia with adjustment for MoCA-J score and WMS-R Logical Memory II Test score. All statistical analyses were conducted using SPSS 25.0 (IBM Corp.) and R 4.2.3 (R Foundation).

3 | RESULTS

3.1 | Clinicodemographic characteristics

Eleven plasma samples were excluded from the current study owing to the failure of analysis of Aβ biomarkers; therefore, the final cohort included 107 participants who underwent assessment of Aβ biomarkers and PET. The median age was 76.0 (range: 72.0–80.0) years; 46.7% of patients were male, and 15.9% were *APOE* ε4 carriers (Table 1). The Aβ-positive subgroup was older ($P = 0.001$), was more likely to be female ($P = 0.048$), had a greater incidence of heart disease ($P = 0.028$), had greater ^{11}C-PiB uptake ($P < 0.001$), and had lower scores on the MoCA-J ($P = 0.006$) and WMS-R Logical Memory II Test ($P < 0.001$) than the Aβ-negative subgroup. APP669-711/Aβ1-42, Aβ1-40/1-42, and the composite biomarker were used as plasma Aβ biomarkers for the validation of the performance shown in our previous report.[12] The Aβ-positive subgroup had greater plasma levels of these Aβ biomarkers than the Aβ-negative subgroup (all $P < 0.001$; composite biomarker: Cliff $d = -0.886$ [95% confidence interval (CI): −0.944 to −0.771]; APP669-711/Aβ1-42: Cliff $d = -0.824$ [95% CI: −0.950 to −0.468]; Aβ1-40/1-42: Cliff $d = -0.748$ [95% CI: −0.855 to −0.581]).

3.2 | Correlations between plasma Aβ biomarkers and ^{11}C-PiB uptake

Among the three plasma Aβ biomarkers, the composite biomarker showed the strongest correlation with the ^{11}C-PiB SUVR ($\rho = 0.754$ [95% CI: 0.649 to 0.829]; $P < 0.001$; Figure S1 in supporting information). Statistical parametric mapping analysis revealed a significant correlation between plasma Aβ biomarkers and ^{11}C-PiB uptake, mainly in the frontal/parietotemporal cortices and posterior cingulate gyrus (Figure S2 in supporting information).

3.3 | Predictive accuracy of plasma Aβ biomarkers for amyloid PET

Models predicting amyloid positivity on PET based on plasma Aβ biomarker levels had areas under the curves of 0.943 (95% CI: 0.901 to 0.985), 0.912 (95% CI: 0.857 to 0.968), and 0.874 (95% CI: 0.806 to 0.942) for the composite biomarker, APP669-711/Aβ1-42, and Aβ1-40/1-42, respectively (Figure 1A). At the single cutoff determined by the Youden index, the composite biomarker had a sensitivity of 88.9% (95% CI: 73.9% to 96.9%), specificity of 87.3% (95% CI: 77.3% to 94.0%), positive predictive value of 78.0% (95% CI: 62.4% to 89.4%), and negative predictive value of 93.9% (95% CI: 85.2% to 98.3%). Combining the composite biomarker with *APOE* ε4ε status, age, and sex

TABLE 1 Clinicodemographic characteristics of all participants.

Characteristic	All ($n = 107$) Median (IQR)/n (%)	Amyloid negative ($n = 71$) Median (IQR)/n (%)	Amyloid positive ($n = 36$) Median (IQR)/n (%)	P value
Age, years	76.00 (72.00, 80.00)	74.00 (69.00, 79.00)	78.50 (75.75, 81.00)	0.001*
Male sex	50 (46.7%)	38 (53.5%)	12 (33.3%)	0.048*
Education level, years	12.00 (9.00, 12.00)	12.00 (9.00, 12.00)	12.00 (9.75, 12.00)	0.333
BMI, kg/m^2	23.27 (21.39, 24.89)	23.86 (21.43, 24.96)	22.82 (20.56, 24.79)	0.228
MMSE score	26.00 (25.00, 27.00)	26.00 (25.00, 27.00)	26.00 (24.00, 27.00)	0.498
MoCA-J score	22.00 (19.00, 25.00)	23.00 (19.00, 25.50)	20.00 (18.00, 22.25)	0.006*
WMS-R II score	6.00 (2.00, 11.00)	8.00 (3.50, 13.00)	4.00 (0.00, 6.25)	<0.001*
APOE ε4 status	17 (15.9%)	8 (11.3%)	9 (25.0%)	0.066
PiB uptake	0.94 (0.83, 1.36)	0.85 (0.81, 0.94)	1.69 (1.37, 2.25)	<0.001*
Hypertension	59 (55.1%)	37 (52.1%)	22 (61.1%)	0.497
Diabetes mellitus	22 (20.6%)	15 (21.1%)	7 (19.4%)	>0.99
Hypercholesterolemia	34 (31.8%)	19 (26.8%)	15 (41.7%)	0.179
Stroke	7 (6.5%)	3 (4.2%)	4 (11.1%)	0.343
Heart disease	19 (17.8%)	8 (11.3%)	11 (30.6%)	0.028*
Thyroid disease	10 (9.3%)	4 (5.6%)	6 (16.7%)	0.133
Hepatic disorders	2 (1.9%)	1 (1.4%)	1 (2.8%)	>0.99
Malignant tumor	8 (7.5%)	5 (7.0%)	3 (8.3%)	>0.99
AST	22.60 (19.40, 26.05)	22.70 (19.50, 27.15)	22.45 (19.25, 25.03)	0.700
ALT	16.90 (14.00, 21.65)	17.50 (14.35, 21.90)	15.20 (13.33, 21.05)	0.136
γ-GTP	20.70 (14.60, 29.80)	22.20 (14.60, 31.90)	17.60 (13.78, 26.55)	0.379
eGFR	65.70 (56.65, 75.35)	66.30 (58.55, 76.15)	60.05 (53.05, 74.12)	0.150
Composite biomarker	0.45 (−0.14, 1.20)	−0.0037 (−0.31, 0.49)	1.53 (1.08, 1.78)	<0.001*
APP669-711/Aβ1-42	1.00 (0.88, 1.19)	0.93 (0.84, 1.05)	1.26 (1.11, 1.39)	<0.001*
Aβ1-40/1-42	23.06 (20.63, 25.82)	21.65 (20.00, 23.52)	25.90 (25.19, 27.56)	<0.001*

Abbreviations: Aβ, amyloid beta; ALT, alanine aminotransferase; *APOE*, apolipoprotein E; APP, amyloid precursor protein; AST, aspartate aminotransferase; BMI, body mass index; eGFR, estimated glomerular filtration rate; γ-GTP, gamma-glutamyl transpeptidase; IQR, interquartile range; MMSE, Mini-Mental State Examination; MoCA-J, Montreal Cognitive Assessment-Japanese version; PiB, ^{11}C-Pittsburgh compound B; WMS-R II, Wechsler Memory Scale-Revised Logical Memory II Test.
*$P < 0.05$ indicates statistical significance.

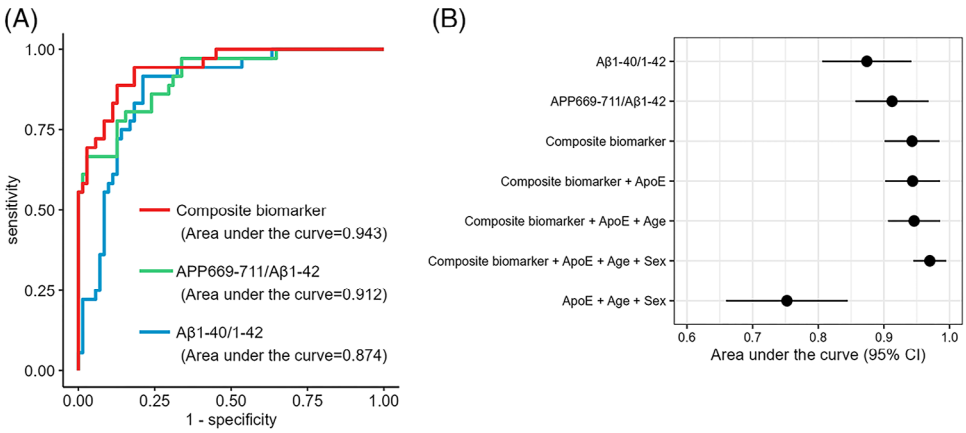

FIGURE 1 Receiver operating characteristic curve analysis for discriminating amyloid PET status (A). The red line shows the composite biomarker, the green line shows APP669-711/Aβ1-42, and the blue line shows Aβ1-40/1-42. Prediction accuracy for amyloid PET status among biomarkers and combinations with *APOE* ε4 status, age, and sex (B). Aβ, amyloid beta; *APOE*, apolipoprotein E; APP, amyloid precursor protein; CI, confidence interval; PET, positron emission tomography

TABLE 2 Performance of amyloid biomarkers using other dual cutoff criteria.

		Amyloid PET status, 33.6% prevalence, %		Reweighted for 60% prevalence, %	
Amyloid biomarker level	All, *n* (%)	Amyloid negative	Amyloid positive	Amyloid negative	Amyloid positive
Sensitivity/specificity ≥90.0%					
Composite biomarker					
Low (<0.661)	61 (57.0)	95.1	4.9	86.7	13.3
Intermediate (0.661–1.029)	11 (10.3)	54.5	45.5	28.9	71.1
High (≥1.029)	35 (32.7)	20.0	80.0	7.8	92.2
APP669-711/Aβ1-42					
Low (<0.995)	52 (48.6)	94.2	5.8	84.7	15.3
Intermediate (0.995–1.149)	24 (22.4)	62.5	37.5	36.0	64.0
High (≥1.149)	31 (29.0)	22.6	77.4	9.0	91.0
Aβ1-40/1-42					
Low (<24.067)	59 (55.1)	94.9	5.1	86.3	13.7
Intermediate (24.067–25.792)	20 (18.7)	40.0	60.0	18.4	81.6
High (≥25.792)	28 (26.2)	25.0	75.0	10.1	89.9

Abbreviations: Aβ, amyloid beta; APP, amyloid precursor protein; PET, positron emission tomography.

increased the area under the curve from 0.943 to 0.970 (95% CI: 0.945 to 0.995); however, the difference was not significant (Figure 1B).

3.4 | Performance of plasma Aβ biomarkers in classifying brain amyloid status

To enhance the accuracy of plasma Aβ biomarkers in classifying brain amyloid status, two cutoff values were set: a lower cutoff at a sensitivity ≥ 90.0% and an upper cutoff at a specificity ≥ 90.0%. The low-, intermediate-, and high-level groups were categorized by the dual cutoffs of three Aβ biomarkers (Table 2). According to the composite biomarker, 10.3% of participants were classified into the intermediate-level group. The composite biomarker had a 95.1% (95% CI: 86.3% to 99.0%) negative predictive value and an 80.0% (95% CI: 63.1% to 91.6%) positive predictive value for amyloid positivity on PET. Increasing the prevalence of amyloid positivity on PET to 60% decreased the negative predictive value (86.7%) and increased the positive predictive value (92.2%).

We further evaluated the performance of the composite biomarker using other dual cutoff values (Table S1 in supporting information). With the cutoff set at a negative/positive predictive value ≥ 95.0%, the composite biomarker had a 95.1% (95% CI: 86.3% to 99.0%) negative predictive value and a 95.5% (95% CI: 77.2% to 99.9%) positive predictive value. More participants were classified as having an intermediate probability (22.4%) using the cutoff set at a sensitivity/specificity ≥ 90% compared to a negative/positive predictive value ≥ 95.0%. A cutoff set at a negative/positive predictive value ≥ 90.0% decreased the percentage of participants classified into the intermediate-level group (9.3%). To assess the robustness of plasma Aβ biomarkers, we simulated changes in sensitivity and specificity at a cutoff of 90% by adding different bias percentages to APP669-711/Aβ1-42 and Aβ1-40/1-42. Sensitivity and specificity were less affected by bias for APP669-711/Aβ1-42 than for Aβ1-40/1-42 (Figure S3 in supporting information).

3.5 | Associations between plasma Aβ biomarkers and multiple comorbidities

Linear regression analysis adjusted for age and sex revealed that *APOE* ε4 status was associated with increased plasma levels of the composite biomarker, APP669-711/Aβ1-42, and Aβ1-40/1-42. Hepatic disorders were associated with increased composite biomarker and APP669-711/Aβ1-42 levels, although there were only two patients with hepatic disorders in this study (Figure S4 in supporting information). No comorbidity effects beyond the PET uptake value were observed.

3.6 | Baseline plasma Aβ biomarkers and disease progression

We further investigated the effects of plasma Aβ biomarkers in predicting clinical progression. The higher plasma Aβ biomarkers at baseline were significantly associated with worsening MMSE score (Figure 2). The composite biomarker had the highest correlation coefficient (ρ = −0.575, 95% CI: −0.725 to −0.349, $P < 0.001$), followed by APP669-711/Aβ1-42 (ρ = −0.513, 95% CI: −0.710 to −0.278, $P < 0.001$) and Aβ1-40/1-42 (ρ = −0.508, 95% CI: −0.642 to −0.294, $P < 0.001$). Among 107 older adults with MCI, 28 progressed to AD dementia within 7 years; 61 remained stable with MCI at 7-year follow-up. Others converted to non-AD dementia or dropped out of the study. The composite biomarker, APP669-711/Aβ1-42, and Aβ1-40/1-42 at

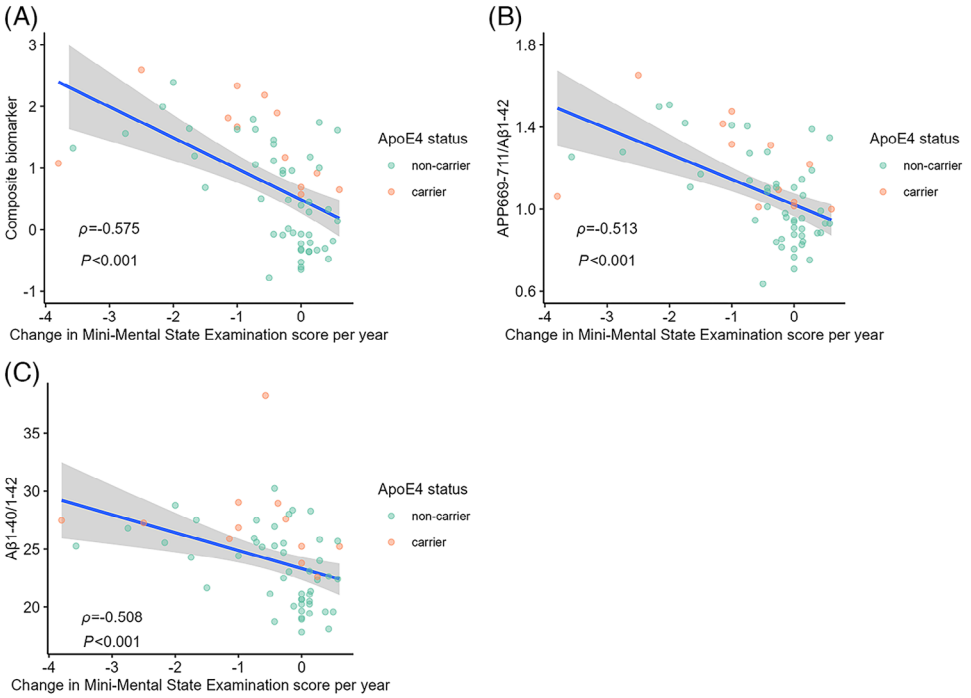

FIGURE 2 Association of baseline plasma composite biomarker (A), APP669-711/Aβ1-42 (B), and Aβ1-40/1-42 (C) with annualized change of Mini-Mental State Examination score. The data are plotted with the 95% confidence interval band (gray band) of the fitted linear regression line (blue line). Spearman rank correlation coefficient is denoted as *ρ*. The green circle shows the *APOE* ε4 carrier, and the orange circle shows the *APOE* ε4. Aβ, amyloid beta; *APOE*, apolipoprotein E; APP, amyloid precursor protein

baseline were higher in the MCI conversion group than in the stable MCI group ($P < 0.001$; Cliff $d = -0.720$ [95% CI: −0.866 to −0.462; Figure 3A–C). Receiver operating characteristic curve analysis for the composite biomarker demonstrated a high area under the curve (0.860 [95% CI: 0.778 to 0.943]; Figure 3D), sensitivity of 0.857 (95% CI: 0.714 to 0.964), and specificity of 0.754 (95% CI: 0.639 to 0.853) at a cut-off of 0.661 for discriminating between MCI conversion and stable MCI. The relationship between the composite biomarker and the time to AD dementia conversion was further analyzed using Kaplan–Meier curves (Figure 4). The risk of AD conversion differed among the low-, intermediate-, and high-level groups when the dual cutoff was set at a sensitivity/specificity ≥ 90.0% (log-rank test, $P < 0.001$). MoCA-J score and WMS-R Logical Memory II Test score differed among the groups ($P < 0.05$; Table S2 in supporting information). Cox regression analysis adjusted for these scores revealed that the risk of AD dementia in the high- and intermediate-level groups was greater than that in the low-level group (intermediate level: hazard ratio, 6.64 [95% CI: 1.74 to 25.31], $P = 0.006$; high level: hazard ratio, 10.74 [95% CI: 3.51 to 32.84], $P < 0.001$).

4 | DISCUSSION

This study provides several novel and interesting insights into the usefulness of plasma biomarkers for predicting brain amyloid burden and future development of AD dementia in a community-based cohort of individuals with MCI. First, the area under the curve values for predicting amyloid positivity on PET were 0.943, 0.912, and 0.874 for the composite biomarker, APP669-711/Aβ1-42, and Aβ1-40/1-42, respectively, in a community-based cohort. Second, a higher plasma composite biomarker at baseline was associated with worsening MMSE score and a high risk for developing AD. Third, *APOE* ε4 status influenced the plasma levels of Aβ. These results suggest the potential for a composite biomarker to supplement PET and CSF tests.

Several studies have examined the predictive accuracy of plasma Aβ biomarkers measured using IP-MS to detect brain amyloid deposition based on PET or CSF analysis in a clinical cohort. Plasma Aβ42/40 or composite biomarkers could predict amyloid positivity on PET, with areas under the curves ranging from 0.793 to 0.954 in cognitively healthy adults and adults with MCI or AD[12–18] and 0.752 to 0.880 in cognitively healthy adults.[19–22] A recent review of plasma amyloid biomarkers reported that Aβ42/40 had a weighted average area under the curve of 0.834 using amyloid PET as a reference standard.[27] Although plasma Aβ42/40 and composite biomarker levels measured using the Shimadzu Analytical Platform could accurately predict amyloid positivity on PET in clinical cohorts,[12] few studies have examined the predictive accuracy of plasma Aβ42/40 and composite biomarkers in community-based cohorts. Our results showed that the areas under the curves for Aβ1-40/1-42 and the composite biomarker were 0.874 and 0.943, respectively, for identifying amyloid positivity on PET in a community-based cohort. These high area under the curve values were similar to our previous results.[12] In general, IP-MS assays are thought to be better than immunoassays in identifying amyloid status.[11] In the IP-MS method used in this study, the Aβs after IP were directly applied

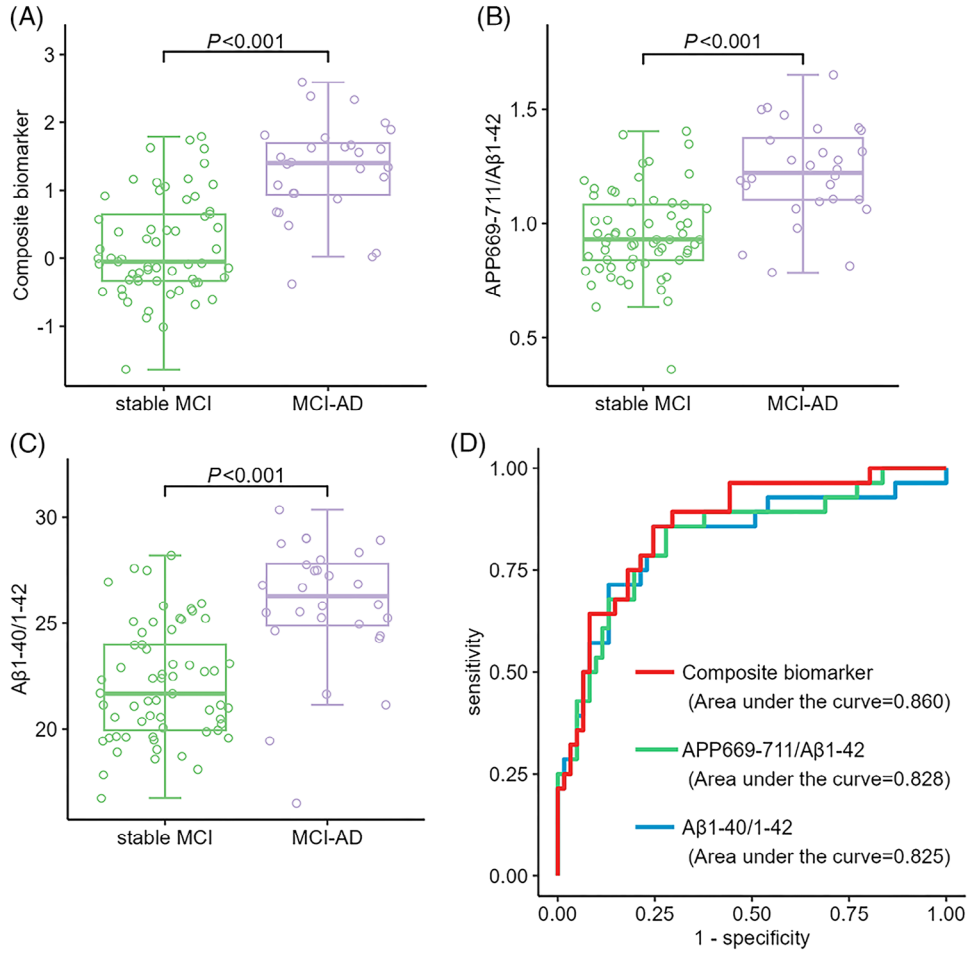

FIGURE 3 Plasma Aβ biomarker levels to predict the conversion to AD dementia. Distribution of baseline plasma levels of the composite biomarker (A), APP669-711/Aβ1-42 (B), and Aβ1-40/1-42 (C) between the MCI converted to AD dementia (MCI-AD) and stable MCI groups. Receiver operating characteristic curve analysis for discriminating between the AD conversion group and stable MCI group (D). Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; MCI, mild cognitive impairment

to MS without any steps such as protease digestion and liquid chromatography because of the use of MALDI-TOF MS. The procedure with fewer steps may contribute to suppressing variation of Aβ ratios during the assay, which could result in higher performance. The areas under the curves of CSF Aβ1-42/1-40 and Aβ1-42/phosphorylated tau (p-tau)181 were 0.94 and 0.95, respectively, in 288 individuals selected from the Amsterdam Dementia Cohort,[28] with a sensitivity/specificity of 94%/84% and 91%/86%, respectively, in 77 individuals selected from BioFINDER.[29] The areas under the curves in our study are similar to those of CSF Aβ1-42/1-40 and Aβ1-42/p-tau181, which have been approved by the Japanese Pharmaceuticals and Medical Devices Agency and the Food and Drug Administration.[28,29] Previous studies have proposed a two-step workflow in which plasma biomarkers are screened for Aβ, with additional confirmatory testing for uncertain cases.[12] In this study, when thresholds were set to satisfy negative/positive predictive values of 90% and 95%, similar to those of PET,[30–32] the intermediate ranges were 9.3% and 22.4%, respectively. Therefore, we suggest that the Shimadzu composite biomarker can predict amyloid positivity on PET with high performance in community-dwelling adults before dementia onset. Assuming that the cost of IP-MS is the same as that previously reported[33] and that the cost ratio of PET to plasma biomarker analysis is 8× or 4×, plasma biomarkers may have a cost benefit for the Japan Universal Health Insurance System. The use of a composite biomarker combined with age and *APOE* ε4 status slightly improved the accuracy of detecting brain amyloid deposition, consistent with previous findings.[11,17,22]

Longitudinal analysis showed that older adults with higher baseline levels of composite biomarkers had a greater rate of conversion to AD dementia and worsening MMSE score than those with lower levels. Moreover, the amyloid composite biomarker can predict clinical progression to AD dementia with a high area under the curve of 0.860. Several studies have demonstrated the usefulness of plasma amyloid biomarkers using enzyme-linked immunosorbent assays or single-molecule arrays for predicting the development of dementia.[34–40] However, few studies have reported the association of Aβ42/40 or composite biomarkers with cognitive function measured using IP-MS, which showed hazard ratios of 1.09 and 1.36 for worsening cognitive status.[41,42] Moreover, the follow-up period for the majority of these studies was < 5 years. Our findings revealed a stronger association between higher plasma composite biomarker levels and a

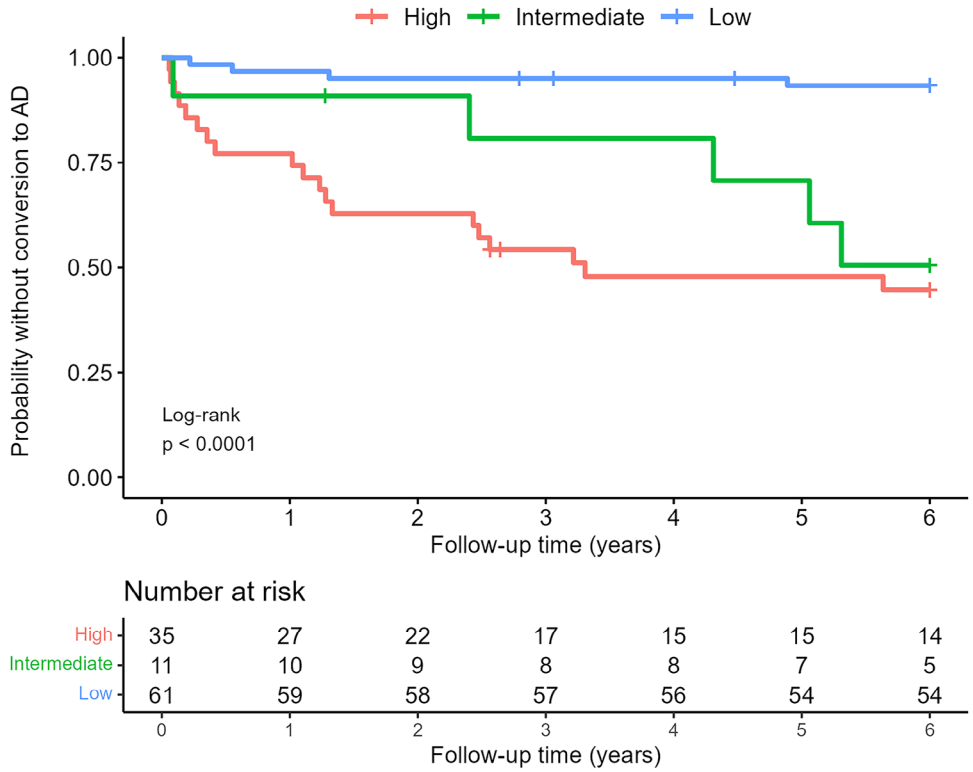

FIGURE 4 Association of plasma composite biomarker at baseline with incident AD at 6-year follow-up. The Kaplan–Meier curve shows the fraction of adults without AD conversion. The log-rank test was used for the statistical comparison between the low- (blue line), intermediate- (green line), and high-level groups (red line). AD, Alzheimer's disease

greater risk of developing AD dementia over 8 years of follow-up in a community-based MCI cohort (hazard ratios of 6.64 and 10.74).

Several factors may affect plasma Aβ biomarkers, influencing the interpretation of results or the development of reference ranges because patient populations are heterogeneous.[43] This study showed that *APOE* ε4 status and hepatic disorders were associated with increased plasma Aβ biomarker levels. Previous studies have reported that age, *APOE* ε4 status, ischemic heart disease, hypertension, diabetes, hepatic disorders, and chronic kidney disease affect plasma Aβ42/40 levels.[44–47] Conversely, sex and BMI are not associated with plasma Aβ42/40.[47,48] Our results are consistent with those of previous studies showing that hepatic disorders are associated with elevated levels of plasma amyloid biomarkers through reduced clearance of Aβ.[44,45] Only two patients had hepatic disorders in this study. One patient had increased levels of APP669-711/Aβ1-42, resulting in increased levels of the composite biomarker. Further studies are needed to confirm this finding.

This study had some limitations. First, the number of participants was small. Second, the number of adults with amyloid positivity on PET was relatively small. Further studies, including larger sample sizes and different sampling techniques, are needed to determine the usefulness of plasma amyloid biomarkers. The standardization of preanalytical, analytical, and standard references was required to use plasma amyloid biomarkers.

In conclusion, the composite biomarker may be suitable for routine clinical practice as a screening tool for adults at risk of AD dementia in a community-based cohort. Our results suggest that the composite biomarker may be a suitable surrogate for PET positivity or CSF Aβ42 levels, indicating the applicability of plasma Aβ biomarkers from clinical to community settings.

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CONFLICT OF INTEREST STATEMENT

Takuya Ataka has no conflicts of interest to declare. Noriyuki Kimura received honoraria from Takeda Pharmaceuticals, Daiichi Sankyo, Eisai, Sumitomo Pharma, PDRadiopharma, and Otsuka Pharmaceutical outside the submitted work. No other disclosures were reported. Naoki Kaneko is an employee of Shimadzu Corporation. Teruaki Masuda has

no conflicts of interest to declare. Yosuke Takeuchi has no conflicts of interest to declare. Kenichi Yabuuchi has no conflicts of interest to declare. Takeshi Mizukami has no conflicts of interest to declare. Tsukasa Takeuchi is an employee of Shimadzu Corporation. Temmei Ito has no conflicts of interest to declare. Hideaki Tasai has no conflicts of interest to declare. Takehiko Miyagawa has no conflicts of interest to declare. Shunya Hanai is an employee of Shimadzu Corporation. Shinichi Iwamoto is an employee of Shimadzu Corporation. Etsuro Matsubara has no conflicts of interest to declare. Author disclosures are available in the supporting information.

CONSENT STATEMENT

This study was approved by the ethics committee of Oita University Hospital (2346-C43). Informed consent was obtained for experimentation with human participants.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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