Prediction of Cerebral Amyloid Pathology Based on Plasma Amyloid and Tau Related Markers

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Background and Purpose: Pyroglutamate-modified β-amyloid peptide (AβpE3) is crucial for AD pathophysiological process. The potential associations of plasma AβpE3 and total tau (t-tau) with brain Aβ burden and cognitive performance remain to be clarified.

Methods: Forty-six subjects with unimpaired cognition, mild cognitive impairment, or very mild dementia were enrolled. Plasma levels of AβpE3−40, t-tau, and Aβ42 were quantified by immunomagnetic reduction (IMR) assays. We analyzed individual and combined biomarker correlations with neuropsychological scores and Aβ positivity determined by 18F-florbetapir positron emission tomography (PET).

Results: Both plasma AβpE3−40 levels and AβpE3−40/t-tau ratios correlated negatively with short-term memory and global cognition scores, while correlating positively with PET standardized uptake value ratios (SUVRs). Among the biomarkers analyzed, the combination of AβpE3−40 in a ratio with t-tau had the best discriminatory ability for Aβ PET positivity. Likewise, logistic regression analysis showed that AβpE3−40/t-tau was a highly robust predictor of Aβ PET positivity after controlling for relevant demographic covariates.

Conclusion: Plasma AβpE3−40/t-tau ratios correlate with cognitive function and cerebral Aβ burden. The suitability of AβpE3−40/t-tau as a candidate clinical biomarker of AD pathology in the brain should be examined further in larger studies.

Keywords: pyroglutamate, tau, β-amyloid, Alzheimer’s disease, predictor

INTRODUCTION

Alzheimer’s disease (AD) underlies a major unmet medical need in routine clinical practice and casts a considerable burden on patients, caregivers, and societies. The neuropathological characteristics of AD include neuronal loss, β-amyloid (Aβ) plaques, neurofibrillary tangles, and synaptic loss (1, 2).
The main Aβ variants detected in the human brain are Aβ40 and Aβ42. They are found together with N-terminal truncated forms of these variants (Aβn-40/42), which have been shown to have pyroglutamate modifications, yielding a distinct pyroglutamate-modified Aβ peptide (AβpE3) (3–6). The AβpE peptide has been identified in early-stage AD, even before clinical symptoms are apparent, implicating it as a potential seeding molecule that may enable pathological Aβ aggregate formation and triggering hyperphosphorylation of tau (6–11). AβpE is formed when full-length Aβ peptides undergo truncation at the N-terminal glutamate and subsequent dehydration catalyzed by glutaminyl cyclase (12, 13). Compared to full-length peptides, AβpE displays greater β-sheet content, hydrophobicity, aggregation propensity, deposition, amyloidogenicity, resistance to enzymatic degradation, and neuronal toxicity (9, 14–17). In addition, AβpE has been reported to be closely associated with cognitive decline, disease progression, and cerebral amyloidosis in AD (8, 10, 18, 19), suggesting that it may be a key driving force in AD pathogenesis.

Amyloidosis, a primary characteristic of AD pathology, can be detected by positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) measures (20, 21). Both measures possess high diagnostic and prognostic value and may reveal changes many years prior to clinical onset of AD (22–25). Because PET scans are costly and have limited availability, they cannot be incorporated broadly into routine clinical assessments of cognitive impairment before initiation of AD treatment therapies. By contrast, although CSF sampling is less costly and more readily available, it is quite invasive. Neither the PET nor the CSF test is appropriate for population-based screening aimed at identifying high-risk individuals before symptom onset. Moreover, approximately 70% of study participants with clinical syndromes of AD dementia in AD prevention trials and around 25% of participants in AD drug trials did not have brain amyloidosis that was detectable by PET (26, 27). Thus, a minimally invasive measure, such as a blood test, that can reflect cerebral AD pathology accurately and reliably would have critical advantages for supporting clinical decisions regarding AD treatment planning and monitoring of patients during therapeutic trials.

Immunomagnetic reduction (IMR) assay is a relatively new ultrasensitive detection technology capable of quantifying biomarkers down to pg/mL levels. Previously, we observed a positive correlation of IMR-quantified plasma levels of a pyroglutamate cyclization variant of N-terminally truncated Aβ3–40 (AβpE3–40) with cerebral amyloidosis detected by PET imaging (18). In the present study, IMR was implemented to measure plasma levels of AβpE3–40, t-tau, and Aβ42. The primary aim of this work was to investigate associations of these plasma biomarkers, individually and in combination, with cognitive performance and to assess their utility for predicting Aβ PET positivity (+).

MATERIALS AND METHODS

Subjects

Eligible subjects at Taipei Veterans General Hospital, Linkou Chang Gung Memorial Hospital, and Kaohsiung Chang Gung Memorial Hospital were enrolled through the Alzheimer’s Disease Neuroimaging Initiative in Taiwan (T-ADNI). The local institutional review boards and the ethics committee of the three hospitals approved the data collection protocol. T-ADNI study was approved by the ethics committees of the three hospitals. The inclusion criteria were an age of 55 to 90 years and educational attainment of at least 6 years. Prior to testing, written informed consent was obtained from all participants and/or their legal guardians. All methods were performed in accordance with the relevant guidelines and regulations.

All participants were interviewed by experienced neurologists to collect information about their demographics, family history, and physical/neurological condition. Additionally, for each participant, neurologists obtained Hachinski ischemic score and vital signs. Blood was drawn for a hematology/chemistry panel and for vitamin B12s, thyroid-stimulating hormone, free thyroxine, and rapid plasma reagin syphilis tests.

A standard battery of neuropsychological tests was administered, including the Geriatric Depression Scale, Mini-Mental State Examination (MMSE) (28), Chinese Version Verbal Learning Test (29), Logical Memory subscale (story A) of the Chinese version of the Wechsler Memory Scale-III (30), 30-item Boston Naming Test (31), categorical Verbal Fluency Test (29), Trail-Making Test A and B (line) (32), and Clinical Dementia Rating Scale (CDR) (33). Only subjects with non-impaired cognition, defined by CDR score of 0, and patients in the prodromal stage or very mild dementia stage, defined by CDR score of 0.5, were invited to participate in this study.

Signs of cognitive impairment were assessed and non-AD etiologies (e.g., tumors, strokes, severe white matter disease, or inflammation) were excluded through a series of clinical interviews, a physical examination, screening laboratory tests, neuropsychological assessments, and brain magnetic resonance (MR) imaging. Exclusionary criteria included any history of major brain trauma, brain tumor, stroke, epilepsy, alcoholism, major psychiatric illness, or any other systemic diseases that might affect cognitive function. Diagnosis of amnestic mild cognitive impairment and probable AD were made on the basis of the core clinical criteria developed by the National Institute on Aging and the Alzheimer’s Association’s 2011 workgroup (NIA-AA) (34). The demographic characteristics of the study cohort, dichotomized according to Aβ PET results, are summarized in Table 1.

Collection and Preparation of Human Plasma Samples

An 8-ml non-fasting venous blood sample (K3 EDTA, lavender-top tube) was collected from each subject and then centrifuged (1,500–2,500 ×g for 15 min) within 1 h of the draw. The plasma was then aliquoted into cryotubes and stored at −20°C.

IMR Measurements

Detailed descriptions of the IMR platform and validation of its accuracy have been published previously (18, 35). IMR reagents were selected based on epitope antigen/antibody affinity, ability to conjugate with MagQu magnetic Fe₃O₄ nanobeads, and ability to produce linear standard curves of quantitated magnetic signal reduction. Each type of reagent consists of
magnetic nanoparticles dispersed in phosphate buffered saline (pH 7.2). By immobilizing functionalized monoclonal antibodies against Aβ37–42 (ABCAM, Cambridge, UK; ab34376) and t-tau protein (Sigma Aldrich; T9450) on the magnetic nanoparticles, two types of reagents were obtained. The antibody against Aβ_{pfE3-40} was developed by Biogen Inc. The mean diameter of the antibody-functionalized magnetic nanoparticles was 50–60 nm. The magnetic concentration of each type of reagent was 12 mg-Fe/mL. Duplicated/paired measurements of Aβ42, Aβ_{pfE3-40}, and t-tau were performed for each plasma sample. We mixed 60-µL plasma samples with 60 µL of reagent (MF-AB2-0060 or MF-DEX-0060, respectively; MagQu) at room temperature in the Aβ assay, 40 µL of reagent was added to the t-tau assay, 40 µL of plasma with 80 µL of reagent (MF-TAU-0060; MagQu) and then incubated at room temperature in the Aβ assay. Ammonium carbonate and sodium ascorbate were added to the plasma sample for dissolution and reduction, respectively. After incubation, the IMR was detected by an IMR reader (XacPro-S, MagQu) and expressed as percentage reductions in magnetic nanoparticles were detected by an IMR reader

\[ \text{IMR(%) = } \frac{X_{ac, o} - X_{ac, f}}{X_{ac, o}} \times 100\% \]

where \( X_{ac, o} \) and \( X_{ac, f} \) are the ac magnetic signals of reagent before and after incubation. For each reported IMR (%) in this study, an averaged value of duplicated IMR measurements was calculated. The standard deviations of all duplicated plasma analyte measurements were <15%. The reported analyte concentrations for each sample are means of the paired measurements.

### Analysis of ApoE Genotypes

ApoE genotyping was determined by polymerase chain reaction amplification and DNA sequencing (36). Participants with one or two e4 alleles were defined as e4 carriers.

### Aβ PET Imaging Data Acquisition

All Aβ PET imaging scans were performed on a Biograph mCT PET/CT scanner (GE Healthcare, Milwaukee, WI) at a single site (Linkou Chang Gung Memorial Hospital) as described in detail elsewhere (37, 38). Briefly, the scan commenced with a 10-min acquisition period (two 5-min frames) beginning 50-min after a 10-mCi injection of ^18^F-florbetapir tracer. Each image was obtained with the application of a three-dimensional ordered subset expectation maximization reconstruction algorithm (four iterations, 24 subsets; Gaussian filter: 2 mm; zoom: three) with computed tomography-based attenuation correction, as well as scatter and random corrections, with a matrix size of 400 × 400 × 148 and a voxel size of 0.68 × 0.68 × 1.5 mm³. To achieve useful anatomical information and facilitate co-registration with PET images, structural MR scans were obtained for all subjects with a uniform scanning protocol that minimizes and accounts for between-site differences in MR imaging systems.

### Aβ PET Imaging Processing

PET imaging data were processed and analyzed in PMOD software (version 3.7, PMOD Technologies Ltd., Zurich, Switzerland), including MR-based spatial normalization to the Montreal Neurological Institute MRI template. We selected seven volumes of interest: frontal, anterior cingulate, posterior cingulate, precuneus, parietal, occipital, and temporal cortical areas. We calculated regional standardized uptake value ratios (SUVRs) for each volume of interest, using the whole cerebellum as a reference region, and then averaged the SUVRs for the seven volumes of interest to yield an estimated global cortical SUVR value for further analysis.

All the PET images were interpreted by an experienced nuclear medicine physician (Kun-Ju Lin) who did not have access to clinical data. Aβ burden was graded on a five-point visual scale, from 0, indicating no tracer retention in cortical gray matter, to 4, denoting high levels of cortical amyloid accumulation. Scores of 0 or 1 were categorized as Aβ PET negativity (−) and scores of 2–4 were categorized as Aβ PET+ (39).

### Statistical Methods

All statistical analyses were performed in SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). A \( P < 0.05 \) was considered significant. All variables were analyzed by non-parametric methods. For continuous variables, differences between Aβ PET− group and Aβ PET+ group were detected with Mann-Whitney U tests. For categorical variables, Chi-square
tests were used. We generated neurocognitive numeric composite z-scores by calculating individual z-scores for each test and then averaging them across the cognitive test set. The constituents of the composite z-scores were as follows: short-term memory [Chinese Version Verbal Learning Test and Logical Memory subscale (story A) of the Chinese Version Wechsler Memory Scale-III], semantic memory [Boston Naming Test (total)]; executive function [Trail-Making Test-A/B (line) and categorical Verbal Fluency Test (animal)]; global cognition [short-term memory test, semantic memory test, executive function test, and the MMSE]. Spearman’s rank coefficients were calculated to determine correlation of plasma biomarker levels with estimated global cortical SUVr and domains of cognitive performance. Receiver operating characteristic (ROC) and area under the curve (AUC) analyses were performed to define cut-off points for each biomarker analyte or their ratios to further characterize discriminatory properties between the Aβ PET- and Aβ PET+ groups. Finally, logistic regression modeling was performed to investigate the predictive power of biomarker levels for Aβ PET+, in terms of odds ratios (ORs) and 95% confidence interval (CIs), with and without adjusting for age, sex, and ApoE ε4 carrier status.

RESULTS

Demographic Data
A total of 46 subjects were enrolled in the study and divided into Aβ PET- and Aβ PET+ groups. Their demographic characteristics and neurocognitive scores are presented in Table 1. There were no significant between-group differences in age, sex, years of education, clinical stage or CDR scores, nor in plasma biomarker levels of t-tau, Aβ42, or Aβ42/t-tau. Compared to the Aβ PET- group, the Aβ PET+ group had more ApoE ε4 carriers (25% vs. 7.7%, p = 0.038), poorer performance on MMSE (23.5 vs. 27.5, p = 0.001), higher Aβ PET SUVr (1.5 vs. 1.1, p < 0.001), and higher levels of plasma AβpE3–40 (65.7 vs. 42.3, p = 0.003) and AβpE3–40/t-tau ratio (4.39 vs. 1.72, p = 0.001).

Association of Plasma Biomarkers With Aβ Burden and Cognitive Performance
AβpE3–40 level (r = 0.343, p < 0.005), and AβpE3–40/t-tau ratio values (r = 0.305, p < 0.005) correlated directly with Aβ PET SUVr (Table 2: Figure 1). Short-term memory scores correlated negatively with AβpE3–40 (r = −0.481, p < 0.001) and AβpE3–40/t-tau values (r = −0.483, p < 0.001), but positively with t-tau (r = 0.3, p < 0.005) and Aβ42 levels (r = 0.391, p < 0.001). Semantic memory scores were found to correlate positively with Aβ42 levels (r = 0.353, p < 0.005), while executive function scores correlated positively with AβpE3–40/t-tau values (r = 0.359, p < 0.005). Global cognition scores correlated negatively with AβpE3–40 (r = −0.337, p < 0.005) and AβpE3–40/t-tau values (r = −0.343, p < 0.005) while correlating positively with Aβ42 levels (r = 0.379, p < 0.005).

ROC Analysis for Prediction of Aβ PET Positivity
ROC-AUC analyses aimed at determining plasma biomarker discriminatory cut-off values yielded the following optimal significant cut-off values for the differentiation of Aβ PET- and Aβ PET+ groups: 55.45 fg/mL for AβpE3–40, 2.85 for AβpE3–40/t-tau; and 0.8 for Aβ42/t-tau. The combination of AβpE3–40 with t-tau in a AβpE3–40/t-tau ratio had a greater AUC than individual biomarkers (i.e., AβpE3–40, t-tau, and Aβ42) and the composite Aβ42/t-tau ratio. Detailed AUCs, cut-off values, sensitivities, and specificities are reported in Table 3.

Association of Plasma Biomarkers With Aβ PET Positivity
The relationship between plasma biomarkers and Aβ PET positivity was examined through a logistic regression analysis with the aforementioned optimal cut-offs. All of the analyzed markers were found to be significantly associated with Aβ PET positivity risk in both the unadjusted and adjusted logistic regression modeling results (Table 4). All adjusted ORs (aORs) became more pronounced after controlling for age, sex, and ApoE ε4 carrier status. Participants with AβpE3–40 > 55.45 fg/mL, t-tau ≤ 19.10 pg/mL, Aβ42 ≤ 16.27 pg/mL, AβpE3–40/t-tau > 2.85, or Aβ42/t-tau > 0.8 were at increased risk for Aβ PET positivity (aOR, 95% CI, and p-values are reported in Table 4).

DISCUSSION
The current study assessed the association of levels of AD-related biomarkers, especially AβpE3–40, quantified by IMR technology with cognitive performance as well as their predictive power in Aβ PET positivity. We found that participants with Aβ PET+ had higher levels of plasma AβpE3–40 and higher AβpE3–40/t-tau ratio values than participants with Aβ PET-.

These elevated values correlated positively with PET analysis findings and correlated negatively with short-term memory and global cognition scores. AβpE3–40 alone had a high discriminatory ability, and consideration of AβpE3–40 together with t-tau (i.e., in AβpE3–40/t-tau) provided greater differential value than any of the individual biomarker values examined and the Aβ42/t-tau ratio. After adjusting for demographic covariates (age, sex, and ApoE ε4 carrier status), AβpE3–40/t-tau proved to be the strongest predictive biomarker for Aβ PET positivity. Collectively, these findings suggest that plasma AβpE3–40/t-tau may indeed be reflective of cerebral amyloid pathology. To the best of our knowledge, the current study is the first to report relationships among plasma t-tau level, plasma AβpE3–40 level, and brain Aβ accumulation revealed by in vivo PET.

In a previous study, we observed a positive association of AβpE3–40 levels with Aβ PET SUVr (18). In this study, we confirmed those prior findings and further showed that AβpE3–40-related biomarkers were more closely related to brain Aβ accumulation revealed by Aβ PET than were Aβ42, t-tau, and Aβ42/t-tau values. These results are discordant with previous data obtained with ultrasensitive analytical assays—such as,
TABLE 2 | Correlation of plasma biomarkers with Aβ burden and cognitive performance (N = 46).

| Aβ PET biomarker | SUVR | Short-term memory | Semantic memory | Executive function | Global cognition |
|------------------|------|-------------------|-----------------|--------------------|------------------|
| Aβ_{pE3-40}  | 0.343* | −0.481** | −0.084 | 0.268 | −0.337* |
| t-tau           | −0.041 | 0.300* | 0.286 | −0.248 | 0.305 |
| Aβ42            | −0.007 | 0.391** | 0.353* | −0.216 | 0.379* |
| Aβ_{pE3-40}/t-tau | 0.305* | −0.483** | −0.140 | 0.359* | −0.343* |
| Aβ42/t-tau      | 0.073 | −0.261 | −0.236 | 0.229 | −0.287 |

SUVR, standardized uptake value ratio.

*p < 0.05, **p < 0.01.

FIGURE 1 | Scatterplots of the associations between Aβ PET SUVR and plasma biomarker levels (pg/mL) of (A) Aβ_{pE3-40} and (B) Aβ_{pE3-40}/t-tau.

TABLE 3 | ROC analysis for identifying Aβ PET positivity (N = 46).

| Variable   | AUC      | (95% CI)          | P      | Optimal cutoff | Sensitivity (%) | Specificity (%) |
|------------|----------|-------------------|--------|----------------|----------------|-----------------|
| Aβ_{pE3-40}| 0.81     | (0.61–0.87)       | 0.001  | >55.45         | 83.33          | 71.43           |
| t-tau      | 0.66     | (0.50–0.79)       | 0.056  | ≤19.10         | 83.33          | 57.14           |
| Aβ42       | 0.64     | (0.48–0.78)       | 0.098  | ≤16.27         | 77.78          | 60.71           |
| Aβ_{pE3-40}/t-tau | 0.83 | (0.64–0.89) | <0.001 | >2.85 | 83.33 | 75.00 |
| Aβ42/t-tau | 0.67 | (0.51–0.80) | 0.046 | >0.8 | 77.78 | 60.71 |

AUC, area under the curve; ROC, receiver operator characteristic curve.

xMAP, stable isotope labeling kinetics-mass spectrometry, single-molecule array, and immunoprecipitation mass spectrometry approaches—showing that lower plasma Aβ42 levels and Aβ42/Aβ40 ratios were associated with a higher brain Aβ burden revealed by Aβ PET over the normal–mild cognitive impairment–AD cognitive spectrum (40–48). This divergence of findings could be consequent to the critically discrepant roles of Aβ_{pE} and Aβ42 in AD pathophysiological processes. Aβ_{pE} has been considered to be a principal initiator of early-stage AD pathogenesis and has been shown to correlate with tau pathology and cognitive decline in AD (10, 12, 15, 19, 49), whereas plasma Aβ42 was found to be inversely correlated with cortical Aβ burden, likely due to impaired Aβ plaque clearance from the brain or sequestration and deposition of Aβ species within the brain. A recent animal study showed the evidence that impaired meningeal lymphatic drainage can exacerbate the neuroinflammatory response and enhancing meningeal lymphatic function could achieve better ability of monoclonal antibodies to clear Aβ aggregates (50). Together with a phase 2 trial revealing that donanemab, a monoclonal antibody against
Aβ\text{pE} epitope, could curb cognitive decline in early AD (51), we speculate that Aβ\text{pE3−40} is a better plasma biomarker than Aβ42 for detection and monitoring of cerebral amyloidosis.

We found that Aβ\text{pE3−40}, Aβ42, and Aβ\text{pE3−40}/t-tau correlated significantly with short-term memory and global cognition performance measures, whereas t-tau and Aβ42/t-tau did not correlate well with any cognitive performance measures in our study. Aβ\text{pE3−40} has specifically been found to be more cytotoxic to hippocampal and cortical neurons than Aβ40 and Aβ42 in animal studies (52, 53), highlighting the close relationship to hippocampal-dependent cognitive impairment. In addition, Aβ\text{pE3−40} and Aβ\text{pE3−40}/t-tau showed modestly positive correlation with Aβ PET SUVRs. In a previous study, Aβ42/t-tau ratios were also reported to have no correlation with Aβ PET SUVRs, but have positive correlation with brain tau accumulation and longitudinal changes in hippocampal volume and cerebral glucose metabolism (54). Unlike Aβ42/Aβ40 and Aβ42/t-tau in other studies, Aβ\text{pE3−40}/t-tau modestly paralleled with the presence of cerebral amyloidosis in this study. Our findings of a positive relationship between Aβ42 level and cognitive function measures are consistent with the findings of prior studies in which ultrasensitive detection methods were used, affirming the supposition that cognitive decline may be associated with low Aβ42 levels (55, 56), but not t-tau levels. There have been discordant findings regarding Aβ42 and t-tau levels correlating closely or not at all with cognitive test scores (35, 55, 57–66), perhaps due to methodological differences related to study design and the quantitative methods used. Aβ\text{pE} has been reported to trigger AD-related neuronal loss, neurodegeneration, neurological deficits, and cognitive decline and has been shown to have differential expression patterns between AD progression and normal aging (19, 67–69). Similar to the finding indicating that a modified version of tau (i.e., ultrasensitive blood immunoassay-detected tau phosphorylated at threonine 181) (70) appears to be a better marker of disease progression than t-tau, Aβ\text{pE}-related marker is a better biomarker of brain Aβ burden and cognitive decline than Aβ42 or t-tau, and may thus provide useful insights into brain functioning in the AD continuum.

Our ROC-AUC analyses indicated that Aβ\text{pE3−40} (p = 0.001), Aβ\text{pE3−40}/t-tau (p < 0.001), and Aβ42/t-tau (p = 0.046) can be used to differentiate between individuals with Aβ PET+ and individuals with Aβ PET−. Among the singular biomarkers, only Aβ\text{pE3−40} had a significant and moderate-to-high predictive ability. Between the two aforementioned composite biomarkers, Aβ\text{pE3−40}/t-tau yielded a higher AUC (0.83) as well as greater sensitivity (83.33%) and specificity (75.00%). Among the five biomarkers analyzed, Aβ\text{pE3−40}/t-tau exhibited the best discriminatory power. Combining markers reflective of two distinct underlying pathophysiological derangements did indeed lead to an incremental benefit to between-group differentiation. There are differential time courses of Aβ and tau changes in AD progression (24, 62). The better correspondence to Aβ PET findings for Aβ\text{pE}, relative to other singular markers, may reflect Aβ\text{pE} being an earlier marker than Aβ42 or tau in AD pathogenesis (9). Although subjects with an early disease stage (i.e., healthy controls with Aβ PET+) were not enrolled in this study, it may be reasonable to pursue Aβ\text{pE3−40}/t-tau as a potential predictor of cerebral Aβ deposition in population-based screening for high-risk individuals before AD symptom onset.

After controlling for demographic covariates, all of the presently examined putative biomarkers had some ability to predict Aβ PET positivity, with the combination of Aβ\text{pE3−40} with t-tau appearing to be particularly useful for reflecting disease state along the AD continuum. Our ROC-AUC analyses indicated that Aβ\text{pE3−40}/t-tau possessed higher AUC, sensitivity,

### TABLE 4 | Association of plasma biomarkers with Aβ PET positivity (N = 48).

| Plasma markers Cut-off groups | Unadjusted | Adjusted* |
|---|---|---|
| | OR | 95%CI | P | OR | 95%CI | P |
| Aβ\text{pE3−40}, fg/mL | | | | | | |
| ≤55.45 | Reference | | | | | |
| >55.45 | 12.36 | (2.36–64.64) | 0.003 | 21.75 | (2.38–198.79) | 0.006 |
| t-tau, pg/mL | | | | | | |
| >19.10 | Reference | | | | | |
| ≤19.10 | 6.66 | (1.56–25.0) | 0.010 | 16.66 | (1.96–100.2) | 0.011 |
| Aβ42, pg/mL | | | | | | |
| >16.27 | Reference | | | | | |
| ≤16.27 | 5.55 | (1.41–20.0) | 0.014 | 16.66 | (1.85–100.1) | 0.012 |
| Aβ\text{pE3−40}/t-tau | | | | | | |
| ≤2.85 | Reference | | | | | |
| >2.85 | 10.50 | (2.58–42.68) | 0.001 | 33.98 | (3.37–342.83) | 0.003 |
| Aβ42/t-tau | | | | | | |
| ≤0.8 | Reference | | | | | |
| >0.8 | 5.41 | (1.41–20.77) | 0.014 | 18.97 | (2.04–176.34) | 0.010 |

CI, confidence interval; OR, odds ratio. *Adjusted for age, sex, and ApoE e4 allele status.
and specificity than Aβ42/t-tau, supporting the notion that utilization of plasma Aβ40−42 and t-tau together is superior to considering absolute levels of individual peptides or Aβ42/t-tau ratio as markers of cerebral amyloidosis. In the face of the high cost and low accessibility of Aβ PET restricting wide use in clinical practice, Aβ40−42/t-tau may help support clinical decisions and be used as a clinical screening tool to rule out individuals who do not need costly Aβ PET scanning in scenarios other than confirmaory diagnosis.

All patients in this study were classified into amnestic-type MCI or AD based on the clinical diagnostic criteria proposed by the 2011 NIA-AA workgroup (3-4). We intended to identify potential plasma biomarkers with proper cutoff values indicating Aβ PET positivity over the normal–mild cognitive impairment–AD cognitive spectrum, rather than to correlate clinical diagnoses with Aβ PET positivity or to differentiate clinical groups by Aβ PET positivity.

The current study had some limitations. First, we could only infer a possible association of plasma Aβ40−42 and t-tau with imminent risk of Aβ PET positivity. Owing to small sample size and lack of Aβ+ healthy controls, further replication, particularly with larger samples and longer follow-up, is warranted to validate the predictive values obtained in this study and to clarify the temporal relationship of these variables with Aβ PET+. Second, the striatum was not included in the SUVR measurements for amyloid PET. Third, we did not account for comorbidity covariates that may affect plasma biomarker levels in our analyses. Finally, the relatively small sample size of our cohort limits the generalizability of the present findings.

CONCLUSION

In conclusion, a dual-factor biomarker consisting of the ratio between Aβ40−42 and t-tau measures, each determined by an ultrasensitive and easy-to-implement IMR assessment, showed superior performance characteristics and was in concordance with PET analysis and cognitive function assessment results. Given that Aβ and tau have long been considered hallmarks of AD pathogenesis, it may be reasonable to pursue an Aβ40−42 and t-tau composite factor as a predictor of cerebral Aβ deposition. Aβ40−42/t-tau might be an effective and feasible candidate for blood-based screening of cerebral AD-related neuropathology aimed at identifying at-risk individuals that should be recommended for CSF and/or PET studies prior to clinical treatment planning. These findings must be interpreted with caution given the relatively small sample size.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Taipei Veterans General Hospital, Linkou Chang Gung Memorial Hospital, and Kaohsiung Chang Gung Memorial Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

T-BC and P-NW contributed to the conception and design of the study, acquisition of data, interpretation of the data, critical revision of the manuscript, and wrote the first draft of the manuscript. K-JL conducted PET imaging analysis. S-YL, Y-JL, Y-CL, and C-YW were involved in the collection and/or analysis of data. J-PC performed statistical analysis. All authors read and approved the submitted version.

FUNDING

This study was carried out with financial support from the Brain Research Center, National Yang-Ming University from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan, the Taiwan Alzheimer’s Disease Neuroimaging Initiative (T-ADNI) group, the National Science Council and the Ministry of Science and Technology, Taiwan (MOST 105-2325-B-182A-005-, MOST 108-2321-B-010-013-MY2, and MOST 110-2321-B-010-007), Taipei Veterans General Hospital (V107C-090, V108C-060), and Chang Gung Memorial Hospital (CMRPG3D1802).

ACKNOWLEDGMENTS

We acknowledge the help of our staff and participants for their efforts and contributions.

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