The Journal of Prevention of Alzheimer's Disease 12 (2025) 100092

Contents lists available at ScienceDirect

The Journal of Prevention of Alzheimer's Disease

journal homepage: www.elsevier.com/locate/tjpad

Original Article

Diagnostic and discriminative accuracy of plasma phosphorylated tau 217 for symptomatic Alzheimer's disease in a Chinese cohort

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ARTICLE INFO

Keywords:
Phosphorylated tau
Amyloid-β
Plasma biomarkers
Alzheimer's disease
Reference interval

ABSTRACT

Background: Plasma phosphorylated tau at threonine 217 (p-tau217) measured with an ultrasensitive immunoassay method has been demonstrated to be an optimal biomarker for Alzheimer's disease (AD).
Objectives: The aim of this study was to establish the reference interval for plasma p-tau217 in Chinese individuals and evaluate its diagnostic value in symptomatic AD.
Design, setting, participants: We recruited 150 cognitively unimpaired (CU) individuals, 60 patients with AD dementia, 30 patients with mild cognitive impairment (MCI) due to AD, 40 patients with frontotemporal lobar degeneration (FTLD), and 70 patients with subcortical ischaemic vascular dementia (SIVD).
Measurements: The concentrations of plasma p-tau217, total tau, amyloid-beta (Aβ)42 and Aβ40 were measured with a single-molecule array.
Results: Plasma p-tau217 outperformed other biomarkers in discriminating AD patients from CU controls, FTLD patients, and SIVD patients (AUC = 0.983, 0.936, 0.892) and discriminating MCI patients from CU controls (AUC = 0.943). The plasma p-tau217 level was negatively correlated with memory in patients with symptomatic AD.
Conclusion: The diagnostic accuracy of plasma p-tau217 was exceptional for AD, even at early stages, in the Chinese population.

1. Introduction

The increasing prevalence of Alzheimer's disease (AD), which is expected to affect more than 100 million people by 2050 [1], is becoming a major global challenge and a serious threat to society and health care institutions. Since disease-modifying therapies (DMTs) were recently developed, early and accurate diagnosis has become a priority for patients with AD [2]. Amyloid-beta (Aβ) and tau in cerebrospinal fluid (CSF) and measured with positron emission tomography (PET) imaging, reflect the core pathology of AD and are widely recognized diagnostic biomarkers. However, these measurements are either invasive or expensive and inaccessible, particularly for patients in developing countries [3]. In many recent studies, blood-based biomarkers were shown to have high potential in the screening, early diagnosis, tracking progress and, ultimately, monitoring of the effectiveness of AD treatment in affected patients [4,5].

Plasma Aβ peptides and different subtypes of phosphorylated tau protein (p-tau) are of significant interest in AD research, and their accuracy in detecting the pathophysiology of AD is high [6–10]. Compared with that of other plasma biomarkers, such as Aβ, t-tau and neurofilament light chain (NfL), the performance of plasma p-tau protein, which is usually measured with ultrasensitive immunoassays, e.g., single-molecule array (Simoa) and electrochemiluminescence (Eli Lilly and Company), is better in identifying patients with AD whose diagnosis is supported by CSF or PET biomarkers or postmortem findings) [11–13]. Increased levels of plasma p-tau correlate with hallmark pathologies of AD, including Aβ deposition and neurofibrillary tangles, as measured either in postmortem brain tissue [14,15] or in vivo with PET [16].

Among the various p-tau proteins, tau phosphorylated at threonine 217 (p-tau217) has demonstrated the greatest potential for distinguishing AD patients from patients with other neurodegenerative disorders

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https://doi.org/10.1016/j.tjpad.2025.100092
Received 18 December 2024; Received in revised form 5 February 2025; Accepted 5 February 2025
Available online 12 February 2025

and detecting AD pathology at the mild cognitive impairment (MCI) stage [8,17]. Notably, p-tau217 exhibited superior diagnostic accuracy compared with p-tau181, p-tau231 and NfL, with areas under the curve (AUCs) exceeding 0.90 [15,18]. Plasma p-tau217 alone or in combination with demographic variables (age, sex, and APOE status) outperformed other plasma biomarkers, such as p-tau181, p-tau231, the Aβ42/40 ratio, glial fibrillary acidic protein (GFAP), NfL, and their optimal combinations, for predicting both amyloid and tau status(13). Moreover, compared with these biomarkers, p-tau217 longitudinally exhibited significant amyloid-dependent changes over time in both the preclinical and symptomatic stages of AD and was associated with clinical deterioration and brain atrophy in the preclinical stage of AD(4).

The Simoa method, characterized by exceptional sensitivity and precision, can accurately quantify trace plasma biomarkers. However, the diagnostic value and cut-off point of plasma p-tau217 measured with Simoa have not been validated in a Chinese population. Hence, the aim of this study was to 1) establish the reference intervals (RIs) of plasma biomarkers, particularly p-tau217, in a Chinese population with a wide age range; 2) determine the diagnostic performance of plasma p-tau217 and other biomarkers, including Aβ40, Aβ42, and t-tau, in the AD continuum from MCI to dementia and their value in differentiating AD from other common types of dementia; and 3) analyse the correlation between plasma biomarkers and cognitive function in patients with the AD continuum.

2. Methods

2.1. Study population

All patients, including 60 with AD dementia, 30 with MCI due to AD, 40 with frontotemporal lobar degeneration (FTLD), and 70 with subcortical ischaemic vascular dementia (SIVD), were recruited from the Memory Clinic of Tianjin Medical University General Hospital and were diagnosed by dementia specialists according to specific diagnostic criteria; all patients were aged 42–84 years. Specifically, patients with MCI due to AD met the National Institute on Ageing and the Alzheimer's Association (NIA-AA) criteria (2011) [19] and the International Working Group (IWG)-2 criteria [20] and had positive Aβ PET results, a Mini-Mental State Examination (MMSE) score of 20-30 and a Clinical Dementia Rating (CDR) scale score of 0.5 [21]. Patients with dementia met the diagnostic criteria for major neurocognitive disorder according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), with an MMSE score of 10–26 and a CDR scale score of 1–2. AD patients met the IWG-2 criteria [19,20] and had positive Aβ PET results, FTLD patients met the revised Frontotemporal Dementia Consensus criteria for behavioural variant frontotemporal dementia [22] or the classification recommendations for semantic variants or nonfluent variants of primary progressive aphasia [23], and SIVD patients met the diagnostic criteria for vascular dementia according to the International Society of Vascular Behavioural and Cognitive Disorders [24].

One hundred and fifty cognitively unimpaired (CU) healthy individuals who had no complaints of subjective cognitive decline and normal neurological or neuropsychological examination results were recruited from our centre located in North China and from two other centres located in Southeast China (Hainan General Hospital) and Southwest China (The Affiliated Hospital of Guizhou Medical University). CU individuals had MMSE scores > 26, CDR scale scores of 0, and ages ranging from 21 to 85 years. Sixty CU individuals who were enrolled from our centre and age- and sex-matched with AD patients were classified as CU controls (CUCs) in the analysis of group differences and diagnostic performance for AD.

The exclusion criteria for both patients and CU healthy individuals included active substance abuse, alcohol abuse, recent head trauma, recent major surgery, tumour, multiple sclerosis, hydrocephalus, schizophrenia, thyroid dysfunction, vitamin B12 deficiency, renal function abnormality, syphilis or HIV infection, severe depression, or visual/auditory disability. Patients who received DMTs or who were participating in clinical trials were also excluded. The study was approved by the Medical Research Ethics Committee at Tianjin Medical University General Hospital. All participants provided written informed consent at the time of recruitment.

2.2. Neuropsychological assessment

Neuropsychological assessment was conducted within one week before or after blood sample collection. In addition to the MMSE, CUCs and patients with cognitive impairment underwent a comprehensive cognitive assessment, including the Auditory Verbal Learning Test (AVLT), Brief Visuospatial Memory Test-Revised (BVMT-R), Verbal Fluency Test (VFT), Boston Naming Test (BNT), Controlled Oral Word Association Test (COWAT), Symbol Digit Modifications Test (SDMT), Stroop Colour and Word Test, Trail Making Test-A (TMT-A) and TMT-B, and Benton Judgement of Line Orientation (JLO), as previously described [25,26]. Six patients with AD only completed the MMSE and refused further cognitive assessment.

Z scores for all tests were calculated by utilizing the means and standard deviations of all the CUCs included in this study. The z scores of the TMT-A and TMT-B were then multiplied by –1 to be consistent with the other tests, in which a higher score indicated better performance. The following five main cognitive domains were assessed: (1) memory composite, represented by the average z score of total learning, delayed recall and recognition on the AVLT and the BVMT-R;(2) attention and information processing speed composite, calculated as the average z score of the SDMT and the TMT-A;(3) executive function composite, determined by the average z score of the Stroop Colour and Word test and the TMT-B;(4) language composite, indicated by the average z score of the VFT, COWAT and BNT; and (5) visuospatial function, represented by the z score of the JLO.

2.3. Plasma biomarker quantification

Blood was collected in EDTA tubes in the morning after the participants had fasted and subsequently centrifuged within 2 h after extraction (2500 rpm × 15 min, 4 ˚C). The plasma was then aliquoted into polypropylene tubes and stored at −80 ˚C until analysis. All the samples were centrifuged (12,000 rpm × 5 min, 4 ˚C) again before testing. Biomarker concentrations were quantified by means of the Simoa enzyme-linked immunoassay HD-X platform from Quanterix (Billerica, MA) following the manufacturer's protocol [13]. Plasma p-tau217 was detected by means an ALZpath Simoa p-tau217 v2 EQC Kit (Quanterix, 104372). Plasma Aβ42, Aβ40 and t-tau levels were measured by means of the Neurology 3-Plex Assay A Kit (Quanterix, 503203).

2.4. Statistical analysis

Statistical analyses were performed with SPSS 26.0, GraphPad Prism version 8.0 software and R statistical software version 4.3.0. The data are presented as the means and standard deviations (SDs) for continuous variables. Demographic characteristics (continuous variables) were compared by means of one-way analysis of variance (ANOVA) and subsequent post hoc testing, and the chi-square test was used for between-group comparisons of sex distribution. The RIs were calculated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [27]. The lower and upper limits were defined as the means ± 2 × SDs.

Analysis of covariance (ANCOVA) and post hoc tests on the basis of estimated marginal means (Bonferroni method for adjusting for multiple comparisons) were used for between-group comparisons of plasma biomarker levels and neuropsychological scores after adjusting for age, sex, and years of education. Receiver operating characteristic (ROC) curves were used to examine the discriminative performance of plasma biomarkers between AD patients and CUCs and between AD patients and

Table 1
Demographics and biomarker concentrations of all participants.

Variable	Diagnostic groups						
	CU (N = 150)	CUC (N = 60)	MCI (N = 30)	AD (N = 60)	SIVD (N = 70)	FTLD (N = 40)	p Value
Sex, F/M	87/63	37/23	19/11	39/21	43/27	24/16	0.988
Age, y	49.54 ± 17.04	65.25 ± 7.37	67.77 ± 6.51	64.52 ± 8.55	67.29 ± 10.28	65.17 ± 7.61	0.237
Age range, y	21–85	51–85	50–82	42–82	42–84	44–76	/
Education, y	14.29 (3.66)	12.00 (3.16)	11.8 (3.23)	10.95 (3.91)	10.89 (3.10)	10.95 (3.67)	0.270
P-tau217 (pg/mL)	0.24 (0.12)	0.28 (0.17)	1.07 (0.49)[a]	1.50 (0.68)[a,b]	0.56 (0.40)[a,b,c]	0.41 (0.39)[b,c]	< 0.001
T-tau (pg/mL)	3.07 (1.19)	3.37 (1.18)	3.36 (1.42)	3.63 (1.64)	3.41 (1.52)	3.41 (1.42)	0.862
Aβ42 (pg/mL)	7.96 (2.03)	8.10 (2.33)	6.99 (1.73)[a]	6.56 (2.45)[a]	7.47 (2.80)[c]	9.39 (1.91)[a,b,c,d]	< 0.001
Aβ40 (pg/mL)	194.69 (44.66)	201.74 (47.12)	218.66 (62.48)	220.99 (62.91)	213.55 (80.65)	237.08 (52.74)[a]	0.100
Aβ42/Aβ40	0.04 (0.01)	0.04 (0.01)	0.03 (0.01)[a]	0.03 (0.01)[a]	0.04 (0.01)[a,c]	0.04 (0.01)[b,c,d]	< 0.001

The data are presented as the mean (SD) unless otherwise indicated.
Statistical analysis was conducted using one-way analysis of variance (ANOVA) for age and education, chi-square test for sex distribution, and analysis of covariance (ANCOVA) for plasma biomarkers controlling for age, sex, and years of education between the five diagnostic groups, including the CUC, MCI, AD, SIVD and FTLD groups.
[a] , vs. CUC, $p < 0.05$
[b] , vs. MCI, $p < 0.05$.
[c] , vs. AD, $p < 0.05$.
[d] , vs. SIVD $p < 0.05$.
Abbreviations: AD, Alzheimer's disease; CU, cognitively unimpaired; CUC, cognitively unimpaired control; SIVD, subcortical ischemic vascular dementia; MCI, mild cognitive impairment; FTLD, frontotemporal lobar degeneration; MMSE, Mini-Mental State Examination; Aβ, amyloid-beta; p-tau217, tau phosphorylated at threonine-217; t-tau, total tau.

patients with other types of dementia. The AUC, cut-off point, and associated sensitivity and specificity were calculated. Differences between AUC values were tested with the Delong test. Pearson and partial correlation analyses were conducted to explore the relationship between plasma p-tau217 levels and cognitive function. $p < 0.05$ indicated statistically significant differences.

3. Results

3.1. Demographics and clinical information

The demographic and clinical features of all participants are presented in Table 1. There were no significant differences in sex, age, or years of education among the CUC, MCI, AD, FTLD and SIVD groups.

3.2. Reference intervals

In total, 240 specimens obtained from participants, including CUs (n = 150), patients with MCI (n = 30) and patients with AD (n = 60), were analysed to establish RIs for plasma biomarkers. The RIs in this study were 0.006 to 0.47 pg/mL for plasma p-tau217 (Fig. 1), 1.320 to 5.678 pg/mL for t-tau, 3.91 to 12.0 pg/mL for Aβ42, 106 to 284 pg/mL for Aβ40, and 0.025 to 0.057 for the Aβ42/40 ratio.

3.3. Differences in plasma biomarker levels between groups

The plasma p-tau217 level significantly differed across the groups (Fig. 2). Subsequent post hoc testing revealed that the plasma p-tau217 level was significantly higher in the AD group and the MCI group than in the CUC group and the other two patient groups (all $p < 0.001$) and that these levels were higher in the AD group than in the MCI group ($p < 0.001$). Additionally, compared with that in the CUC group, the plasma p-tau217 level in the SIVD group was increased ($p < 0.001$).

The Aβ42/Aβ40 ratio was significantly lower in the AD group and the MCI group than in the CUC group (vs. AD, $p < 0.001$; vs. MCI, $p < 0.001$) and the FTLD group (vs. AD, $p < 0.001$; vs. MCI, $p < 0.001$), significantly lower in the AD group than in the SIVD group ($p = 0.005$), and significantly lower in the SIVD group than in the FTLD group ($p = 0.002$) and the CUC group ($p = 0.002$). With respect to the plasma Aβ42 concentration, group differences similar to those for the Aβ42/Aβ40 ratio were evident, and the concentration was higher in the FTLD group than in the CUC group ($p = 0.008$). Furthermore, the concentration of plasma Aβ40 was higher in the FTLD group than in the CUC group ($p = 0.007$) but did not significantly differ among the other groups ($p > 0.05$). With respect to plasma t-tau levels, there were no significant differences among the groups ($p = 0.862$).

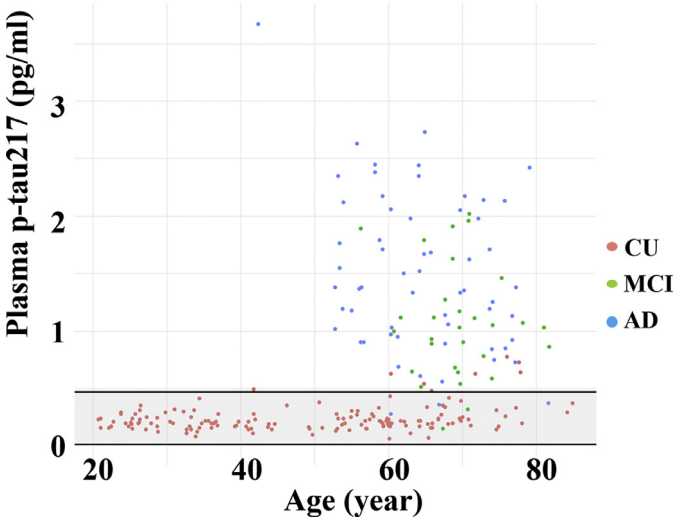

Fig. 1. Reference interval of plasma p-tau217. The shaded regions indicate the reference interval with upper and lower limits. CU, cognitively unimpaired individuals; MCI, mild cognitive impairment; AD, Alzheimer's disease.

3.4. Diagnostic and differential performance of plasma biomarkers

The ROC curves for each biomarker as a function of diagnostic status were generated (Fig. 3) and revealed that plasma p-tau217 had the best diagnostic classification performance in distinguishing AD patients from CUCs (AUC = 0.983, 95 % confidence interval [CI] 0.941–0.998, $p < 0.001$), FTLD patients (AUC = 0.936, 95 % CI 0.869–0.975, $p < 0.001$), and SIVD patients (AUC = 0.892, 95 % CI 0.825–

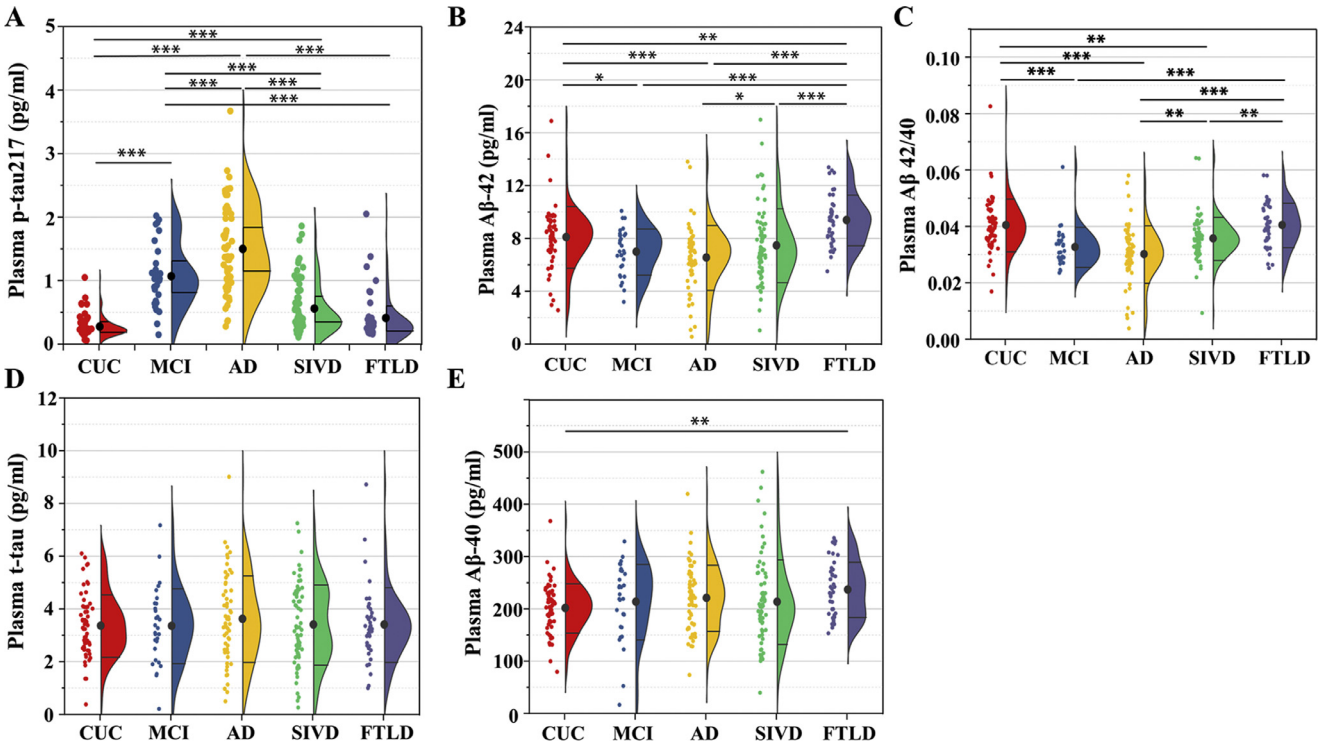

Fig. 2. Plasma biomarker concentrations according to diagnosis. **A.** Plasma p-tau217 levels were increased in the AD group and the MCI group compared with those in the CUC group and the other two dementia groups, with much greater levels in the AD group than in the MCI group. **B and C.** The plasma Aβ42 concentration and Aβ42/Aβ40 ratio were lower in the AD group than in the CUC group and the other two dementia groups and lower in the MCI group than in the CUC and FTLD groups. **D.** There were no significant differences in plasma t-tau levels among the groups. **E.** Plasma Aβ40 levels did not differ between groups except for a higher level in the FTLD group than in the CUC group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. AD, Alzheimer's disease; CUC, cognitively unimpaired control; SIVD, subcortical ischaemic vascular dementia; MCI, mild cognitive impairment; FTLD, frontotemporal lobar degeneration.

0.939, $p < 0.001$). Moreover, the plasma Aβ42 concentration and Aβ42/Aβ40 ratio performed well in distinguishing AD patients from CUCs (AUC = 0.715, 95 % CI 0.625–0.793, $p < 0.001$; AUC = 0.760, 95 % CI 0.674–0.833, $p < 0.001$) and from FTLD patients (AUC = 0.842, 95 % CI 0.756–0.907, $p < 0.001$; AUC = 0.780, 95 % CI 0.686–0.857, $p < 0.001$) but not from SIVD patients (AUC = 0.578; AUC = 0.644). The accuracy of plasma p-tau217 was also greater in identifying patients with MCI due to AD from CUCs (AUC = 0.943, 95% CI 0.873–0.981, $p < 0.001$) than the plasma Aβ42/Aβ40 ratio was (AUC = 0.764, 95 % CI 0.662–0.847, $p < 0.001$; Delong test, $p < 0.001$). However, the diagnostic accuracy of both plasma t-tau and Aβ40, whose AUCs were less than 0.70, was poor.

3.5. Cognitive correlations of plasma biomarkers in the AD continuum

There were significant differences in the MMSE scores and z scores for all five cognitive domains among the groups (Table 2). In particular, AD patients had lower scores on the MMSE and on the memory, information processing, executive function, language, and visuospatial function domains than did CUCs (all $p < 0.001$) and MCI patients (all $p < 0.01$). Compared with the CUC group, the MCI group had lower MMSE scores and z scores for memory, information processing, and executive function (all $p < 0.001$).

The plasma p-tau217 level was negatively correlated with memory ($r = -0.291$, $p = 0.007$), information processing ($r = -0.234$, $p = 0.033$) and visuospatial function ($r = -0.259$, $p = 0.017$) in patients with AD and MCI (Fig. 4B, C, F). However, after adjustments were made for age, sex, and education, only the correlation with the memory domain remained significant ($r = -0.218$, $p = 0.047$). There was no significant correlation between cognitive function and plasma Aβ biomarkers or t-tau.

4. Discussion

In this study, the RIs of plasma biomarkers measured with Simoa, particularly p-tau217, were established in a Chinese population. Additionally, plasma p-tau217 was significantly elevated in patients with MCI due to AD compared with CUCs and was further increased in patients with AD dementia. Although there was also a difference in plasma amyloid biomarkers such as Aβ42 and the Aβ42/Aβ40 ratio between AD patients and CUCs and patients with other types of dementia, the diagnostic and differential accuracy of plasma p-tau217 was significantly higher for AD than that of other plasma biomarkers. Moreover, there were correlations between plasma p-tau217 levels and cognitive function, especially in the memory domain, in patients with AD continuum.

The establishment of RIs for biomarkers is crucial for the accurate interpretation of clinical laboratory test results and disease diagnosis. Clinically, the RIs of p-tau217 could help to estimate whether dementia syndromes are caused by abnormal Aβ pathology. Patients with uncertain plasma p-tau217 results are advised to undergo this confirmatory test, and the necessity for advanced testing is determined by the initial screening results and the patient's clinical stage. In the present study, the RI of plasma p-tau217 was established using CU healthy individuals with a wide age range (21 to 85 years old) and patients with AD continuum, including those with MCI and dementia. For the RI of plasma p-tau217 (0.006 to 0.47 pg/mL) measured with Simoa, the lower limit was lower than those (0.4 to 0.63 pg/mL) reported in a previous study of Western cohorts[13]. One possibility for this difference is that the age range of the CU individuals in the present study was more diverse. It is also possible that racial disparities might contribute to variations in plasma biomarker levels, as one previous study revealed that the average p-tau217 concentration was lower among non-Hispanic Blacks than

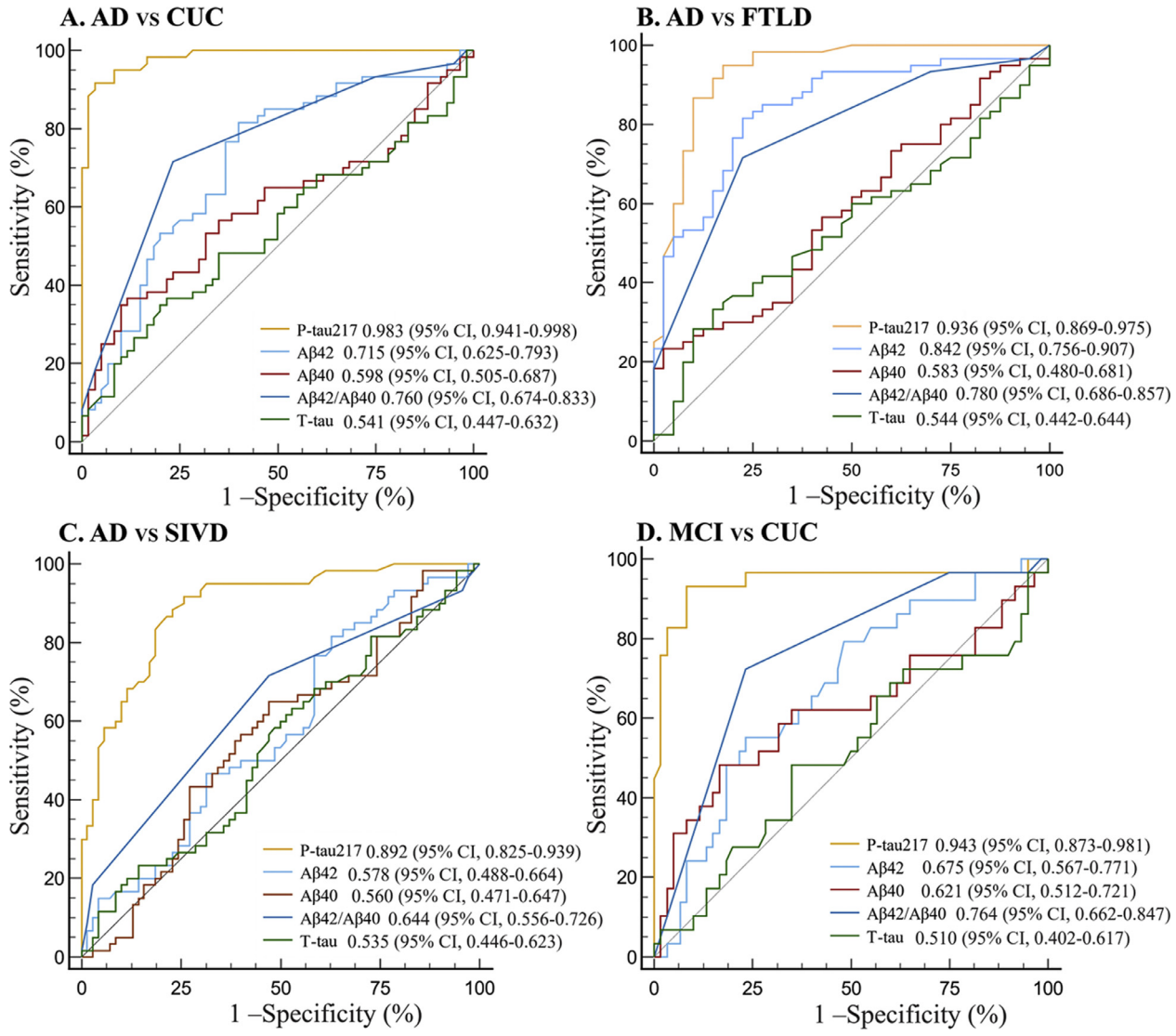

Fig. 3. Diagnostic and differential accuracy of plasma biomarkers for AD and MCI patients. **A.** Plasma p-tau217 and Aβ42 levels and the Aβ42/Aβ40 ratio distinguished AD patients from CUCs ($p < 0.001$; $p < 0.001$; $p < 0.001$). **B.** Plasma p-tau217 and Aβ42 levels and the Aβ42/Aβ40 ratio distinguished AD patients from FTLD patients ($p < 0.001$; $p < 0.001$; $p < 0.001$). **C.** Plasma p-tau217 distinguished AD patients from SIVD patients ($p < 0.001$). **D.** Plasma p-tau217 and the Aβ42/Aβ40 ratio distinguished MCI patients from CUCs ($p < 0.001$; $p < 0.001$). AD, Alzheimer's disease; CUC, cognitively unimpaired control; SIVD, subcortical ischaemic vascular dementia; MCI, mild cognitive impairment; FTLD, frontotemporal lobar degeneration.

Table 2
Cognitive scores of CUCs and dementia patients.

	CUC ($N = 60$)	MCI ($N = 30$)	AD ($N = 60$)	SIVD ($N = 70$)	FTLD ($N = 40$)	p Value
MMSE	28.02 (1.40)	24.23 (3.04)[a]	16.47 (5.70)[a,b]	21.36 (4.29)[a,b,c]	19.13 (6.54)[a,b,c,d]	< 0.001
Memory	1.17 (0.87)	−1.83 (0.96)[a]	−2.41 (0.56)[a,b]	−1.90 (0.82)[a,c]	−2.04 (1.51)[a,c]	< 0.001
Processing speed	−0.32 (0.79)	−1.54 (0.99)[a]	−2.56 (1.31)[a,b]	−2.24 (1.08)[a,b]	−1.99 (1.40)[a,c]	< 0.001
Executive function	−0.33 (0.81)	−1.60 (1.14)[a]	−2.28 (0.89)[a,b]	−1.48 (1.17)[a,b]	−1.71 (1.16)[a,c,d]	< 0.001
Language	−0.30 (0.49)	−0.61 (0.92)	−1.59 (0.75)[a,b]	−1.55 (0.72)[a,b]	−2.31 (1.04)[a,b,c,d]	< 0.001
Visuospatial function	−0.14 (0.99)	−0.83 (1.30)	−2.99 (2.25)[a,b]	−1.84 (1.97)[a,b,c]	−1.03 (1.68)[a,c,d]	< 0.001

The data are expressed as the means (SDs). MMSE scores are presented as raw scores, and cognitive domain scores are presented as z scores. Statistical analysis was conducted using analysis of covariance (ANCOVA) while controlling for age, sex, and years of education. Six patients with AD underwent only the MMSE and refused further cognitive assessment.

[a], vs. CUC, $p < 0.05$.

[b], vs. MCI, $p < 0.05$.

[c], vs. AD, $p < 0.05$.

[d], vs. SIVD, $p < 0.05$.

Abbreviations: AD, Alzheimer's disease; CUC, cognitively unimpaired control; SIVD, subcortical ischemic vascular dementia; MCI, mild cognitive impairment; FTLD, frontotemporal lobar degeneration; MMSE, Mini-Mental State Examination.

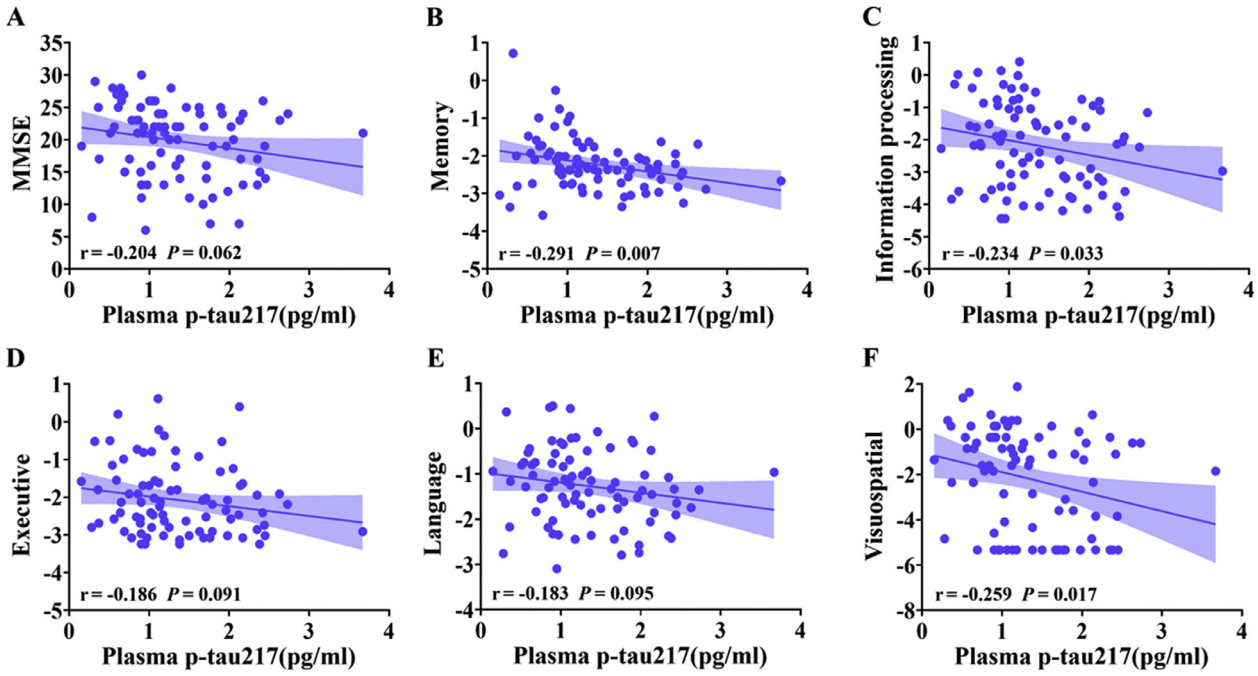

Fig. 4. Correlations between the plasma p-tau217 concentration and cognitive function in patients with AD continuum. Pearson correlation analysis was conducted without adjustment for age, sex, or years of education. MMSE, Mini-Mental State Examination.

among non-Hispanic Whites [28]. In addition, the RIs of Aβ42, Aβ40 and t-tau established in this study differed slightly from previous findings in a Chinese cohort (Aβ42 ranged from 2.72 to 11.09 pg/mL; Aβ40 ranged from 61.4 to 303.9 pg/mL; and t-tau ranged from 0.20 to 3.12 pg/mL) [27], which was based only on middle-aged to elderly CU individuals (50 to 89 years).

The results of the present study revealed a significant increase in the plasma level of p-tau217 and a reduction in the Aβ42/Aβ40 ratio among patients with AD compared with both CUCs and patients with other types of dementia. Consistently, plasma p-tau217 and the Aβ42/Aβ40 ratio detected by various methods are considered valuable biomarkers associated with Aβ pathology that support the clinical diagnosis of AD [6,7,29]. A significant association between the plasma Aβ42/Aβ40 ratio and incident AD and dementia has been demonstrated in previous studies [30], supporting the hypothesis that AD results from an imbalance between Aβ42 and Aβ40 peptides. Consistent with previous findings [30,31], we did not observe differences in plasma Aβ40 or t-tau between patients with AD and either the CUCs or the patients with other types of dementia. CSF t-tau levels are significantly increased in AD patients, plasma t-tau levels change only slightly [32], and there is no correlation between plasma and CSF t-tau levels [33]. Unlike p-tau, nonphosphorylated tau is produced in peripheral nerves or tissue [34], and it is estimated that only approximately 20 % of plasma t-tau comes from the central nervous system [35].

The plasma level of p-tau217 outperformed all other biomarkers across analyses, achieving an AUC of 0.983 overall when distinguishing AD patients from CUCs. Among the currently available plasma biomarkers, the p-tau217 concentration has been demonstrated to have the strongest association with amyloid- and tau-PET positivity [35] and has proven to be valuable in the clinical diagnosis of AD [36]. Moreover, we found that plasma p-tau217 levels showed excellent diagnostic accuracy in discriminating AD patients from FTLD patients, which is consistent with previous findings [15,18]. Taken together, these findings showed that plasma p-tau217, a biomarker reflecting both amyloid and tau pathologies specific to AD, could be applied in a wide range of clinical practices, including both the identification and differentiation of AD patients.

However, SIVD patients also exhibited an increase in plasma p-tau217 and a decrease in the Aβ42/Aβ40 ratio compared with those in the CUC group. This result suggested that AD pathology might be a comorbidity or contribute to the pathogenesis of SIVD. The accumulation of Aβ in brain tissue is attributable to reduced clearance, which depends on stable cerebral blood flow [37]. Conversely, the deposition of Aβ in blood vessels has specific toxic effects, potentially leading to hypoperfusion through the induction of vasoconstriction, elevation of vascular resistance, and initiation of pericyte degeneration [38,39]. SIVD is a main subtype of vascular dementia that manifests as an insidious and gradual clinical progression similar to that of AD, and the prevalence is high in East Asia [40]. However, the comparison of plasma biomarkers between AD patients and SIVD patients has been the focus of only a few studies. Although plasma cyclophilin A (CyPA), placental growth factor (PlGF), and B-type natriuretic peptide (BNP) have been investigated as potential biomarkers for differentiating AD from SIVD, their sensitivity and specificity are relatively low [41–43]. Our results suggest that the levels of Aβ and tau biomarkers should be interpreted cautiously when differentiating AD patients from SIVD patients, although the discriminative performance of plasma p-tau217 is still good (AUC = 0.892).

In the present study, individuals with MCI due to AD had higher plasma p-tau217 levels than did in the CUC group and patients with FTLD or SIVD and had lower Aβ42 concentrations and Aβ42/Aβ40 ratios than did those in the CUC group and those with FTLD. We further demonstrated that plasma p-tau217 outperformed Aβ biomarkers, achieving an AUC of 0.943, for discriminating prodromal AD patients from CUCs. These findings indicate that plasma p-tau217 might be elevated in the early stages of AD and may better reflect the insidious initial AD pathology, which is consistent with published data [44]. A combination of the plasma Aβ42/Aβ40 ratio and p-tau217 level could be used to discriminate Aβ status with relatively high accuracy, with AUCs ranging from 0.83 to 0.86, whereas the associations were strongest between p-tau217 and Aβ pathology in MCI patients (AUCs 0.86–0.88) [45]. Moreover, compared with those of other biomarkers, such as p-tau181, Aβ42/Aβ40, GFAP, and NfL, the plasma level of p-tau217 was elevated even during the presymptomatic stages of AD and may be able to identify preclinical AD [46,47]; in addition, p-tau217 had better sen-

sitivity in capturing the earliest cerebral Aβ alterations in CU individuals, preceding the manifestation of evident Aβ plaque pathology [44]. Although plasma p-tau217 was also positively correlated with pathological changes in cerebral tau, this association was observed only when Aβ pathology was clearly present [48,49]. Thus, plasma p-tau217 is a biomarker for the early stages of AD even when tau pathology cannot be identified via PET, as suggested in the revised version of the NIA-AA guidelines.

Moreover, the plasma level of p-tau217 was further elevated in patients with AD compared with patients with MCI and was significantly correlated with cognitive function in patients with AD continuum. Longitudinal increases in p-tau217 have been reported to be associated with cognitive decline and brain atrophy in AD patients [4]. In addition, plasma p-tau217 levels are highly correlated with the disease progression of AD [13] and can be used to predict the risk of cognitive decline in individuals with MCI [50]. In clinical trials of DMTs, the clearance of Aβ was shown to contribute to significantly slowed clinical progression and decreased amyloid deposition on PET, accompanied by a reduction in plasma p-tau217 levels [51]. Therefore, plasma p-tau217 could also be used as an indicator for disease severity and progression prediction in AD patients, specifically enabling the identification of individuals most likely to experience deterioration during the early clinical stages and being an outcome or indicator of treatment response for DMT.

This study established the first diagnostic and differential values of plasma p-tau217 in the Chinese population, including CU individuals with a wide age range, patients with AD continuum from MCI to dementia, and patients with other types of dementia, such as FTLD and SIVD. However, some limitations should be noted. Although sampling at the individual level was robust, the number of participants was relatively small, e.g., among the CU individuals, only 34 individuals were aged 65–85 years. In addition, although the CU individuals were enrolled from centres in three different regions of China to establish the RIs of plasma biomarkers for the Chinese population, all patients with dementia or MCI were recruited only from our centre to obtain interindividual consistency in cognitive assessment and PET scan procedures. Therefore, the current results need to be validated in future studies with larger sample sizes, including samples with more older individuals and participants with geographical, ethnic and socioeconomic diversity. In addition, we did not account for the potential impact of renal function and BMI on plasma p-tau217 and Aβ in this study, although a recent study has shown that they have only minor effects on the performance of AD-relevant plasma biomarkers [52]. Furthermore, in this study, the correlations between plasma biomarkers and CSF and PET biomarkers for AD neuropathology were not assessed. Additionally, the associations between plasma biomarkers and cognitive function were only cross-sectionally analysed. It will be interesting to further investigate the relationship between longitudinal changes in plasma biomarkers and cognitive function, as this would provide evidence of dynamic changes in p-tau217 and its role in reflecting clinical stages and progression. Taken together, the clinical and pathological associations of plasma biomarkers in patients with AD warrant further investigation.

5. Conclusions

In this study, we demonstrated the excellent diagnostic performance of plasma p-tau217 measured with Simoa for AD in a Chinese cohort. The detection of p-tau217 in blood is expected to become a valuable supplement in clinical practice, serving as a rapid screening test for suspecting or excluding AD pathology and guiding and evaluating the use of DMTs.

Funding

This work was supported by the Science and Technology Innovation 2030-Major Project (2021ZD0201805), the Tianjin Key Medical Discipline (Specialty) Construction Project (TJYXZDXK-004A), the Tianjin Science and Technology Leading Cultivation Enterprise Project (22YDPYSY00220) and the Tianjin Health Research Project (TJWJ 2022QN008).

Ethical standards

The study was approved by the Medical Research Ethics Committee at Tianjin Medical University General Hospital. All the subjects provided written informed consent at the time of recruitment.

Data Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Li-Min Li: Writing – review & editing, Writing – original draft, Software, Methodology, Funding acquisition, Data curation, Conceptualization. **Ping Che:** Writing – original draft, Software, Methodology, Data curation. **Dequan Liu:** Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Yu Wang:** Software, Methodology, Data curation. **Jia Li:** Software, Methodology, Formal analysis, Data curation. **Dian He:** Writing – review & editing, Data curation. **Tao Liu:** Writing – review & editing, Data curation. **Nan Zhang:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Acknowledgments

The authors thank all the volunteers who participated in the study and all the staff involved.

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