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Diagnostic value of isolated plasma biomarkers and its combination in neurodegenerative dementias: A multicenter cohort study

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ABSTRACT

Background: Plasma amyloid-β (Aβ), phosphorylated tau-181 (p-tau181), neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) potentially aid in the diagnosis of neurodegenerative dementias. We aim to conduct a comprehensive comparison between different biomarkers and their combination, which is lacking, in a multicenter Chinese dementia cohort consisting of Alzheimer's disease (AD), frontotemporal dementia (FTD), and progressive supranuclear palsy (PSP).

Methods: We enrolled 92 demented patients [64 AD, 16 FTD, and 12 PSP with dementia] and 20 healthy controls (HC). Their plasma Aβ, p-tau181, NfL, and GFAP were detected by highly sensitive-single molecule immunoassays. Aβ pathology in patients was measured by cerebrospinal fluid or/and amyloid positron emission tomography.

Results: All plasma biomarkers tested were significantly altered in dementia patients compared with HC, especially Aβ42/Aβ40 and NfL showed significant performance in distinguishing AD from HC. A combination of plasma Aβ42/Aβ40, p-tau181, NfL, and GFAP could discriminate FTD or PSP well from HC and was able to distinguish AD and non-AD (FTD/PSP).

Conclusions: Our results confirmed the diagnostic performance of individual plasma biomarkers Aβ42/Aβ40, p-tau181, NfL, and GFAP in Chinese dementia patients and noted that a combination of these biomarkers may be more accurate in identifying FTD/PSP patients and distinguishing AD from non-AD dementia.

1. Introduction

Current technological advances allow an ultrasensitive measurement of molecules, which significantly identify the multiple blood biomarkers associated with Alzheimer's disease (AD) and other neurodegenerative dementias like frontotemporal dementia (FTD) and progressive supranuclear palsy (PSP) [1,2]. However, to accurately benefit clinical diagnostics and clinical trials, the diagnostic value of plasma biomarkers

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; AUC, Area-under-the-curve; CDR, Clinical Dementia Rate; CSF, cerebrospinal fluid; ERPs, event-related potentials; FTD, frontotemporal dementia; GFAP, glial fibrillar acidic protein; HC, healthy control; MMSE, Mini-Mental State Examination; MoCA, Chinese version of Montreal Cognitive Assessment; NfL, neurofilament light; PSP, progressive supranuclear palsy; p-tau, phosphorylated tau; PET, positron emission tomography; RF, random forest; ROC, receiver operating characteristic; SUVR, standardized uptake value ratio; SIMOA, sensitive-single molecule immunoassays; t-tau, total tau.

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requires further multicomponent and confirmatory evaluation in both isolated and combined ways.

AD is the most common cause of dementia in older adults. It is pathologically characterized by extracellular deposition of amyloid-β (Aβ) peptides and intracellular aggregation of phosphorylated tau protein. Early diagnosis is critical for effective therapy. A noninvasive, readily available, and inexpensive diagnostic method, such as the detection of biomarkers in blood, to screen potential AD patients is desirable. To achieve this goal, a lower ratio of Aβ42 and Aβ40 (Aβ42/40) [2,3], and a higher level of phosphorylated tau, especially threonine 181 (p-tau181), in blood have been shown to identify dementia patients even with similar diagnostic accuracy as measuring these markers in the cerebrospinal fluid (CSF) [1,4–6]. Released neurofilament light chains (NfL) as a biomarker for axonal injury [7–9], and free glial fibrillary acidic protein (GFAP) as a biomarker for reactive astrocytosis have also been detected in blood, with elevated levels in AD patients [1,10]. However, in most studies, the clinical significance of each biomarker in diagnosing AD has been investigated separately, and the relative diagnostic power of these biomarkers remains unclear. Between different studies, the results are sometimes heterogeneous and even controversial. Furthermore, it is still uncertain whether a combination of multiple biomarkers could offer a more robust diagnostic value compared to any individual biomarker.

Moreover, AD shares pathological changes such as neurodegeneration and neuroinflammation with other dementias like FTD and PSP [11]. Initial studies showed that CSF NfL elevated in different FTD phenotypes and PSP patients [12–14]. Recently, plasma biomarkers NfL rose twice in PSP than controls, and even showed higher values in FTD than in AD [1,15]. Similarly, p-tau181 and GFAP were promising candidates for tauopathies, including FTD and PSP [16]. These indicated that the mentioned plasma biomarkers were not specific to one certain dementia. Therefore, the differential diagnosis between AD and non-AD dementia should be noted in the blood screening.

In this study, we recruited 112 subjects from a multicenter cohort in eastern China and analyzed the performance of the blood biomarkers Aβ42, Aβ42/Aβ40, p-tau181, NfL, and GFAP individually and in combination in the diagnosis of AD, FTD, and PSP. We also analyzed the correlation between these peripheral biomarkers and cognitive function, CSF biomarkers, and Aβ deposition levels.

2. Methods

2.1. Participants and study design

In this retrospective multicenter study, we finally recruited 92 patients [64 AD, 16 FTD, and 12 PSP with dementia] from memory clinics (the First Affiliated Hospital, Zhejiang University School of Medicine; the Second Affiliated Hospital, Zhejiang University School of Medicine; Sir Run Run Shaw Hospital, Zhejiang University School of Medicine; and Affiliated Zhejiang Hospital, Zhejiang University School of Medicine). All the participants were aged 55–80 years and were educated for at least 3 years. 12 patients were excluded due to other definite causes like a history of significant neurological disease, psychiatric disorders, alcoholism, drug abuse, or head trauma. Besides, 15 participants were excluded because they lacked Aβ pathological inspection by PET or/and CSF. The diagnosis of probable AD was made by experienced neurologists following the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria [17,18]. 64 individuals with AD were Aβ positive confirmed by 18F-florbetapir PET or/and CSF Aβ42/40. FTD and PSP participants (non-AD dementia) were recruited following the consensus criteria established by expert agreement [19–22], all recruited FTD patients were behavioral variant FTD and all PSP patients have cognitive impairments. Participants with FTD or PSP were Aβ negative as shown by 18F-florbetapir PET or CSF Aβ42/40.

Healthy controls (HC) were non-related members of the patients' families or relatives of caregivers to the older adult, and were included on the basis of the following criteria: (1) absence of cognitive symptoms as assessed by a specialized neurological physician with a special interest in cognitive disorders; and (2) did not fulfill the criteria for mild cognitive impairment or any dementia disorder. The exclusion criteria were: (1) significant unstable systemic illness that made it difficult to participate in the study, (2) current significant alcohol or substance misuse, and (3) significant neurological or psychiatric illness. (Supplementary Fig. 4)

Ethics approval of this study was approved by the institutional review boards of each participating center and was in accordance with the Helsinki Declaration of 1975. Informed consent was obtained from all individual participants included in the study.

2.2. Neuropsychological assessment

All participants underwent general cognitive performance assessment by a battery of standardized neuropsychological tests, including MMSE [23], the Chinese version of the Montreal Cognitive Assessment (MoCA) [24], and the Clinical Dementia Rate (CDR) [25].

2.3. ^{18}F-AV-45 PET scan

Amyloid PET imaging was performed with florbetapir (^{18}F-AV45) and was acquired on PET machines (Siemens and GE Healthcare). Participants (26 AD, 2 FTD, and 1 PSP]) were given an injection of between 7.4 and 11.3 (mean 10.0) mCi, and followed immediately by a low-dose computed tomography scan and PET scan from the 50- to 70-minute post-injection window. The data, processed by an in-house fully automated image processing pipeline, was converted to a standardized uptake value ratio (SUVR) with the cerebellar cortex used as a reference region. The diagnosis of Aβ PET positive or negative was made by consensus of two professional physicians in the PET centers according to the previous study [26]. Amyloid deposition was quantified with the average across the cerebellar cortex, temporal region, and global brain level.

2.4. Cerebrospinal fluid processing and measurements

Lumbar puncture was performed in part of patients (40 AD, 14 FTD, and 11 PSP) in accordance with a standardized protocol, and cerebrospinal fluid concentrations of amyloid (Aβ42 and Aβ40), total tau (t-tau), and phosphorylated tau (p-tau181) were analyzed by enzyme-linked immunosorbent assays. Detailed information on CSF biomarker assay performance is provided in Supplementary Table 1.

2.5. Blood samples processing and plasma biomarkers measurement

At the time of neuropsychological assessment, blood samples were collected into ethylenediaminetetraacetic acid tubes. Samples were then centrifuged at 2000 g for 10 min at 4 ℃. Plasma supernatant was collected and frozen at −80 ℃ until use. Because of suspicious hemolysis, there were several blood samples [1 AD, 2 FTD, and 1 PSP] were excluded when analysis. These four blood samples were normal when they were collected and stored. Plasma Aβ42, Aβ40, NfL, and GFAP were simultaneously measured using the single-molecule array (SIMOA) Human Neurology 4-Plex E assay kit (Quanterix, Billerica, MA, USA, lot#103670), while, p-tau measured using the SIMOA Human pTau-181 V2 assay kit (Quanterix, Billerica, MA, USA, lot#103714) on the board of automated SIMOA HD-X analyzer.

We also searched the data from Alzheimer's Disease Neuroimaging Initiative (ADNI), which includes the plasma biomarkers Aβ42, Aβ40, NfL, GFAP or/and p-tau181 measured by SIMOA. After screening, there are two data sources: Blennow lab and Foundation for the National Institutes of Health (FNIH). The individuals with abnormal sample or suspiciously abnormal value are excluded. Due to the small AD cohort

(n = 7) in FNIH SIMOA data, we only included the data of Blennow lab as validation queue.

2.6. Statistical analyses

Demographic, clinical, and biochemical characteristics were compared among groups using Mann-Whitney, Kruskal-Wallis, or Chi-squared analyses. Logistic regression analysis was used to study the relationships between plasma markers and diagnostic types. Area-under-the-curve (AUC) values from receiver operating characteristic (ROC) analyses were performed in IBM SPSS, Statistics 26.0 and used to assess the diagnostic accuracy of plasma markers (AD vs HC, FTD vs HC, PSP vs HC, dementia vs HC, AD dementia vs non-AD dementia). The diagnostic ability of combinations was evaluated by logistic regression and ROC analysis. Logistic regression was used to assess the performance of all kinds of plasma combinations (Aβ42/40 + p-tau181, Aβ42/40 + NfL, Aβ42/40 + GFAP, Aβ42/40 + p-tau181 + NfL, Aβ42/40 + p-tau181 + GFAP, Aβ42/40 + p-tau181 + NfL + GFAP) at differentiating different groups. The parameters of the combined model were verified linearity assumptions. Models' AUC are plotted by GraphPad Prism version 8.0. Random forest (RF) is an algorithm of recursive partition based on the construction of binary tree. We tested the plasma biomarkers with the R package "randomForest" [27]. We reported the ranking of variables' importance in the final models in terms of mean decrease in Accuracy index and Gini index. Spearman correlation analysis was used to study associations between plasma markers and clinical assessments, plasma markers and CSF markers, and plasma markers and PET SUVR. All hypothesis testing was two-sided, and the level of significance was set at $p < 0.05$. These analyses were performed in IBM SPSS, Statistics 26.0. Figures were generated using GraphPad Prism version 8.0 and R package "ggplot2" [28].

3. Results

3.1. Participants

One hundred and twelve participants were included: 20 HC, 64 AD, 16 FTD, and 12 PSP patients with dementia. Demographic and clinical characteristics for the four groups are summarized in Table 1. PSP patients were significantly older than AD patients. AD and PSP patients had a significantly lower education level compared with the other two groups. There was no significant difference in gender. There was no significant difference in neuropsychological test performance among the dementia groups. However, compared with the HC group, the AD, FTD, and PSP dementia groups showed worse neuropsychological test performances with lower MMSE and MoCA scores, and increased CDR scores. There was no significant difference in the carrier rate of APOE-ε4 after being corrected by sex and age. The relationships between the carrier rate of APOE-ε4 and sex or age were no significance.

3.2. Biomarker concentrations among diagnostic groups

After excluding low-quality blood samples, the plasma of 108 participants [20 HC, 63 AD, 14 FTD, and 11 PSP patients] were analyzed. As shown in Fig. 1 and Table 1, group comparisons revealed lower levels of Aβ42 ($p < 0.05$) and Aβ42/Aβ40 ($p < 0.05$) in AD compared with FTD patients, and lower levels of Aβ42/Aβ40 in patients with AD, FTD, and PSP compared with HC ($p < 0.001$). Plasma GFAP was significantly higher in patients with AD ($p < 0.001$) and FTD ($p < 0.05$) compared with HC. NfL was significantly higher in patients with AD, FTD, and PSP compared with HC ($p < 0.001$). There were no significant differences in Aβ42/Aβ40, NfL, and GFAP levels among the patient groups. Though there were no statistically significant differences between HC and FTD or HC and PSP, group comparisons indicated that plasma p-tau181 was significantly higher in patients with AD than in those with FTD ($p < 0.05$) or PSP ($p < 0.01$), and in HC ($p < 0.001$).

Table 1
Demographic characteristics and group differences of plasma biomarker measurements.

	Total (n = 112)	HC (n = 20)	AD (n = 64)	FTD (n = 16)	PSP (n = 12)
Gender, M/F	54/58	10/10	33/31	6/10	5/7
Age, Years, Mean ± SD	64.4 ± 10.0	65.7 ± 9.1	62.8 ± 10.7[d]	64.3 ± 9.00	71.8 ± 5.9[b]
Education, Years, Median (P25, P75)	8 (5, 12)	12 (9, 16)[b,d]	8 (5, 11)[a]	9 (5, 11)	5 (4, 8)[a]
MMSE, Median (P25, P75)	20 (14, 26)	29 (27, 29)[b,c,d]	18 (12, 21)[a]	8 (3.5, 21.5)[a]	19 (18, 25)[a]
MoCA, Mean ± SD	14.6 ± 7.9	25.9 ± 2.3[b,c,d]	12.4 ± 6.1[a]	8.7 ± 8.0[a]	15.2 ± 4.1[a]
CDR, Median (P25, P75)	1 (0.5, 1)	0 (0, 0)[b,c,d]	1 (1, 1)[a]	2 (1, 3)[a]	1 (0.5, 1)[a]
APOE-ε4 (carriers), n (%)	42 (37.5 %)	4 (20 %)	28 (43.8 %)	5 (31.3 %)	5 (41.7 %)
Age at onset, Mean ± SD	61.6 ± 1.1	/	60.5 ± 10.3[d]	60.7 ± 8.4[d]	69.3 ± 6.2[b,c]
	Total (n = 108)	**HC (n = 20)**	**AD (n = 63)**	**FTD (n = 14)**	**PSP (n = 11)**
Plasma Aβ42 (pg/ml), Mean ± SD	5.9 ± 2.4	6.0 ± 1.5	5.3 ± 2.3[c]	7.7 ± 2.5[b]	6.7 ± 2.7
Plasma Aβ40 (pg/ml), Mean ± SD	90.3 ± 36.1	52.2 ± 18.04[b,c,d]	96.8 ± 32.5[a]	102.9 ± 25.2[a]	105.9 ± 46.0[a]
Plasma Aβ42/Aβ40, Median (P25, P75)	0.06 (0.05, 0.08)	0.11 (0.1, 0,14)[b,c,d]	0.06 (0.05, 0.06)[a]	0.07 (0.06, 0.08)[a]	0.07 (0.06, 0.08)[a]
Plasma GFAP (pg/ml), Median (P25, P75)	168.3 (113.3, 216.4)	96.1 (66.5, 135.2)[b,c]	188.2 (142.7, 257.5)[a]	202.02 (177.1, 240.5)[a]	145.90 (120.0, 177.4)
Plasma NfL (pg/ml), Median (P25, P75)	21.2 (14.9, 28.5)	12.9(8.6, 15.8)[b,c,d]	21.5 (16.4, 28.0)[a]	22.2 (21.7, 30.7)[a]	34.5 (21.7, 69.8)[a]
Plasma p-tau181 (pg/ml), Median (P25, P75)	4.0 (2.5, 6.1)	1.8 (1.4, 2.8)[b]	5.2 (3.8, 7.1)[a,c,d]	2.9 (2.2, 5.0)[b]	2.9 (1.5, 3.2)[b]

Healthy controls, HC; Alzheimer's disease, AD; FTD, frontotemporal dementia; PSP, progressive superanuclear palsy; Mini-Mental State Examination, MMSE; Montreal Cognitive Assessment, MoCA; Clinical Dementia Rate, CDR. Amyloid beta, Aβ; glial fibrillary acidic protein, GFAP; neurofilament light, NfL; phosphorylated tau, p-tau; total tau, t-tau. a: Significant value versus HC; b: Significant value versus AD; c: Significant value versus FTD; d: Significant value versus PSP.

CSF biomarker comparisons are also shown in Supplementary Table 2. CSF Aβ42 ($p < 0.05$) and Aβ42/Aβ40 ($p < 0.01$) were lower in AD compared with PSP patients. There were no significant differences in CSF t-tau and p-tau levels among the patient groups.

The screened ADNI data, which belongs to Blennow lab, shows significant difference in NfL levels between HC and MCI, or HC and AD; when significant difference in p-tau181 between HC and AD, or MCI and AD. (Supplementary Table 3).

3.3. Diagnostic performance of plasma and CSF biomarkers

As depicted in Fig. 2, binary logistic regression analyses indicated that dementia (vs controls) was associated with higher odds ratios of plasma GFAP, NfL and p-tau181 (GFAP: OR = 1.023, 95 % CI = 1.011–1.026, $P < 0.001$; NfL: OR = 1.269, 95 % CI = 1.126–1.430, $P < 0.001$; p-tau181: OR = 2.023, 95 % CI = 1.379–2.968, $P < 0.001$), and a lower odds ratio of plasma Aβ42/Aβ40 (OR = 0.051, 95 % CI = 0.014–0.180, $P < 0.001$). AD dementia (vs non-AD dementia) was associated with a higher odds ratio of plasma p-tau181 (OR = 1.484, 95

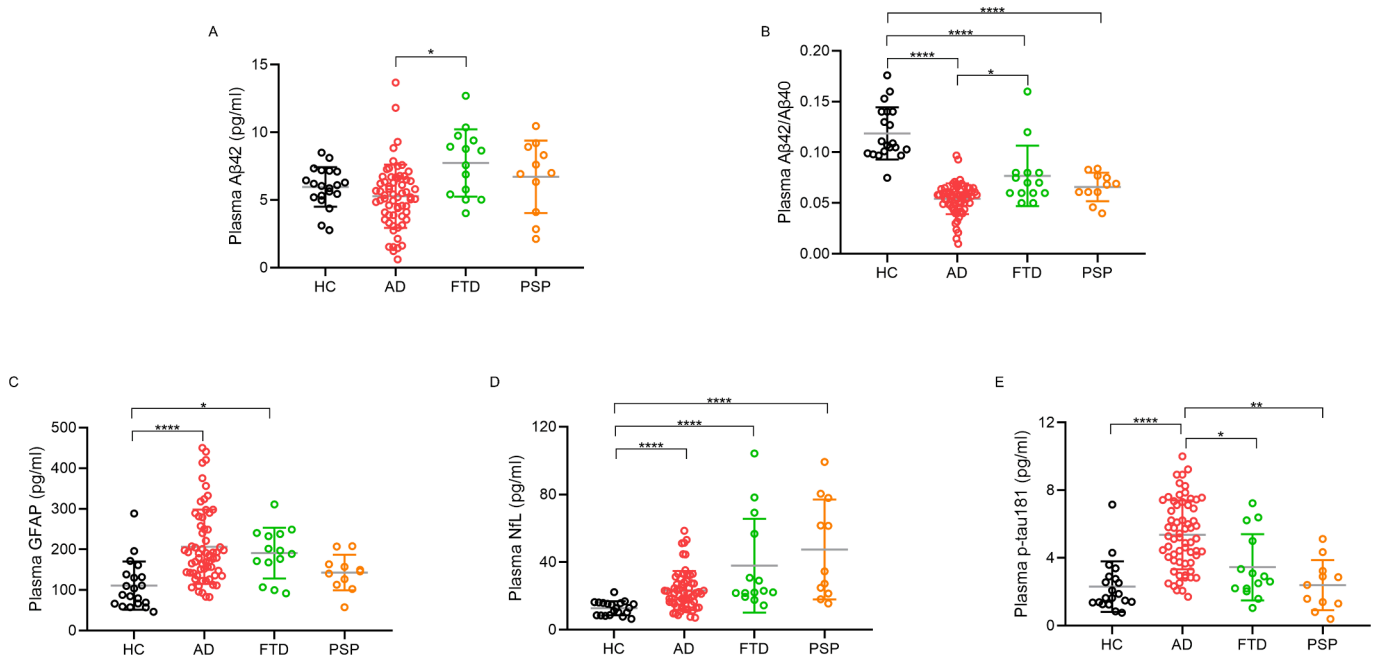

Fig. 1. Group differences of plasma biomarker measurements. Non-parametric test revealed a significant difference between groups. (A) Aβ42 was lower in the AD groups when compared with FTD; (B) The ratio between Aβ42/40 was lower in all diagnostic groups when compared with HC and was lower in the AD groups when compared with FTD; (C) GFAP was elevated in the AD and FTD groups when compared with healthy controls; (D) NfL was elevated across all diagnostic groups when compared with healthy controls; (E) P-tau181 was elevated in the AD group when compared with healthy controls, FTD and PSP. Post-hoc comparisons using the Bonferroni correction are visualized with ****p < 0.001, ***p < 0.005, **p < 0.01, *p < 0.05. Aβ, amyloid beta; AD, Alzheimer's disease; FTD, frontotemporal dementia; GFAP, glial fibrillar acidic protein; HC, healthy controls; NfL, neurofilament light; PSP, progressive supranuclear palsy; p-tau, phosphorylated tau.

% CI = 1.165–1.891, P = 0.001), but lower odds ratios of plasma Aβ42, Aβ42/Aβ40 and NfL (Aβ42: OR = 0.711, 95 % CI = 0.572–0.884, P = 0.002; NfL: OR = 0.970, 95 % CI = 0.950–0.990, P = 0.004; Aβ42/Aβ40: OR = 0.260, 95 % CI = 0.113–0.594, P = 0.001).

Figs. 2 and 3 show the diagnostic accuracy of plasma biomarkers demonstrated by ROC curves, as an isolated marker or in combination, with 95 % Confidence Interval in Supplementary Table 4, and sensitivity and specificity in Supplementary Table 5. Plasma Aβ42/Aβ40, GFAP, NfL, and p-tau181 could discriminate dementias from HC: AD versus HC (Aβ42/Aβ40 AUC = 0.998, 95 % confidence interval [CI] 0.99–1.00; GFAP AUC = 0.829, 95 % CI 0.74–0.95; NfL AUC = 0.836, 95 % CI 0.75–0.92; p-tau181 AUC = 0.882, 95 % CI 0.79–0.97); FTD versus HC (Aβ42/Aβ40 AUC = 0.897, 95 % CI 0.77–1.00; GFAP AUC = 0.831, 95 % CI 0.69–0.97; NfL AUC = 0.953, 95 % CI 0.87–1.00; p-tau181 AUC = 0.761, 95 % CI 0.60–0.92); PSP versus HC (Aβ42/Aβ40 AUC = 0.986, 95 % CI 0.95–1.00; GFAP AUC = 0.723, 95 % CI 0.55–0.92; NfL AUC = 0.959, 95 % CI 0.90–1.00); dementia vs HC (Aβ42/Aβ40 AUC = 0.978, 95 % confidence interval [CI] 0.95–1.00; GFAP AUC = 0.828, 95 % CI 0.72–0.93; NfL AUC = 0.867, 95 % CI 0.80–0.94; p-tau181 AUC = 0.834, 95 % CI 0.74–0.93). Comparison among plasma Aβ42, Aβ42/Aβ40, p-tau181 and NfL could significantly differentiate between AD and non-AD dementia (FTD + PSP): (Aβ42 AUC = 0.740, 95 % CI 0.62–0.86; Aβ42/Aβ40 AUC = 0.768, 95 % CI 0.66–0.88; NfL AUC = 0.704, 95 % CI 0.59–0.82; p-tau181 AUC = 0.805, 95 % CI 0.70–0.91).

When plasma biomarkers combined, the diagnostic accuracy was higher than isolated, especially between AD and non-AD dementia groups: (Aβ42/Aβ40 + p-tau181 AUC = 0.885, Aβ42/Aβ40 + NfL AUC = 0.845, Aβ42/Aβ40 + GFAP AUC = 0.776, p-tau181 + NfL AUC = 0.884, p-tau181 + GFAP AUC = 0.811, Aβ42/Aβ40 + p-tau181 + NfL AUC = 0.937, Aβ42/Aβ40 + p-tau181 + GFAP AUC = 0.887, Aβ42/Aβ40 + p-tau181 + NfL + GFAP AUC = 0.945). Combined plasma biomarker Aβ42/Aβ40 + p-tau181 + NfL shows the highest AUC between FTD versus HC, and PSP versus HC (AUC = 0.979, and AUC = 1, respectively). Besides, the ratio of Aβ42/p-tau in plasma was also evaluated, showing a significant diagnostic performance between dementia groups vs HC (AUC = 0.825); AD vs HC (AUC = 0.915); AD vs non-AD dementia groups (AUC = 0.853). (Supplementary Fig. 1). Supplementary Fig. 2 are based on ADNI_blennow datasets and show the diagnostic performance of plasma NfL, p-tau181, and NfL + p-tau181: MCI vs HC (NfL AUC = 0.6168, p-tau181 AUC = 0.5198, and NfL + p-tau181 AUC = 0.6163), AD vs HC (NfL AUC = 0.7666, p-tau181 AUC = 0.5974, and NfL + p-tau181 AUC = 0.7620).

The RF analysis showed that the top two important variables when identifying dementia from HC were plasma Aβ42/40 and Aβ42/40 + p-tau181; when identifying AD dementia from non-AD dementia were combination of Aβ42/40 + p-tau181 + NfL and Aβ42/40 + p-tau181 + NfL + GFAP. (Supplementary Fig. 3).

We further tested the diagnostic accuracy of CSF biomarkers. CSF Aβ42/40 and p-tau181 could discriminate AD from non-AD dementia (Aβ42/40: AUC = 0.765, p-tau181 AUC = 0.651), while t-tau was no significance. When combined, both Aβ42/40 + p-tau181 and Aβ42/40 + t-tau could significantly differentiate between AD and non-AD dementia (FTD + PSP) (Aβ42/40 + t-tau: AUC = 0.761, Aβ42/40 + p-tau181 AUC = 0.699). However, there was no significance of the ratio of Aβ42/p-tau or Aβ42/t-tau. (Supplementary Fig. 1).

3.4. Plasma biomarkers and cognitive performances

The associations between plasma measures and MMSE or MoCA scores are displayed in Fig. 4 and Supplementary Table 6. High p-tau181, GFAP, and NfL levels were associated with low MMSE scores, while low Aβ42/Aβ40 levels were associated with high MMSE scores. The MoCA scores presented the same correlations as the MMSE scores. However, we found no significant correlation between plasma measures and PET SUVR or relative CSF biomarkers (Supplementary Table 7).

4. Discussion

The goal of this multicenter clinical cohort study was to investigate the differential levels of plasma Aβ42, Aβ42/40, p-tau181, NfL, and

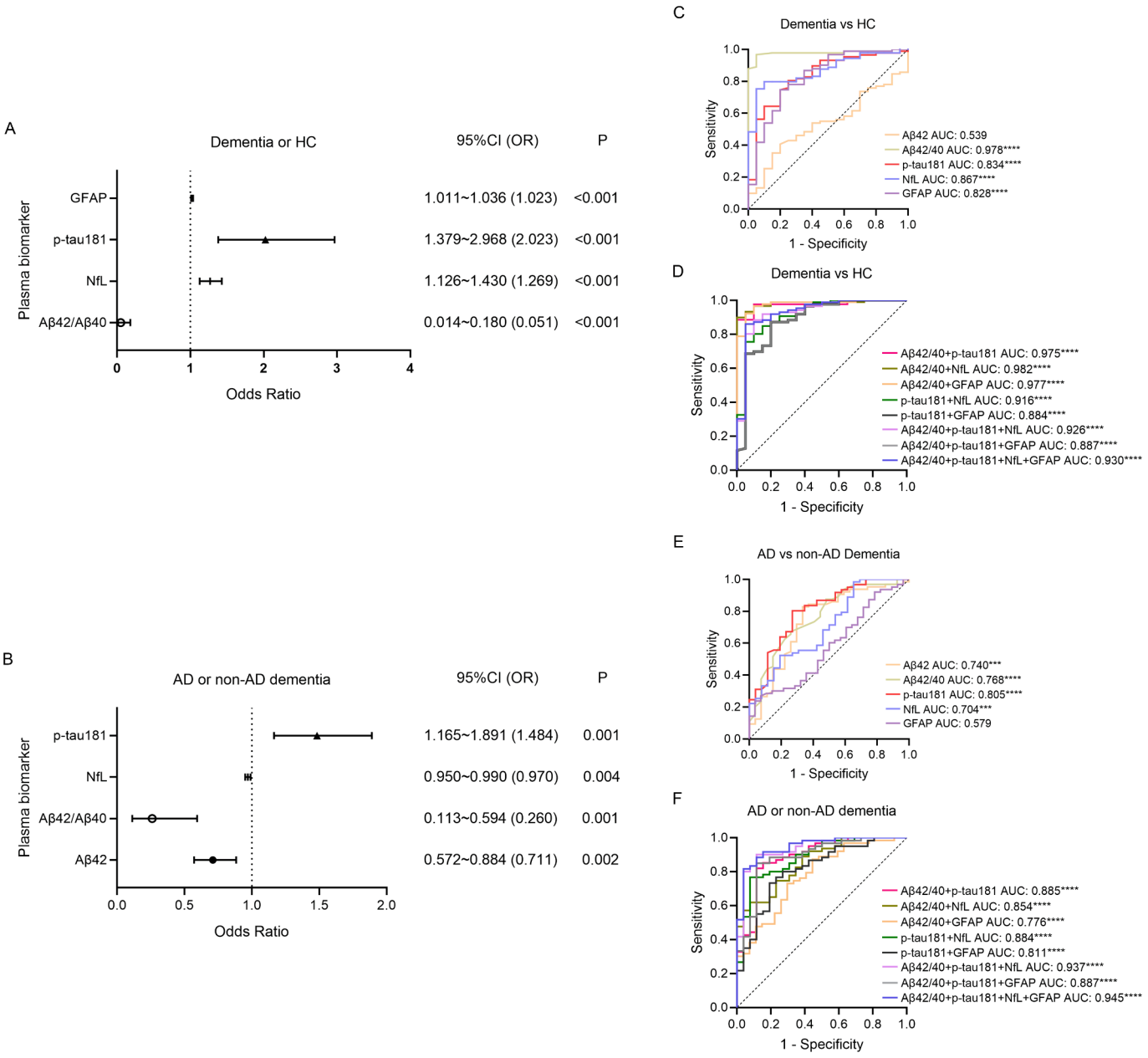

Fig. 2. Logistic regression and diagnostic performance of plasma biomarker in dementia. (A) GFAP, p-tau181, NfL and Aβ42/40 ratio could differentiate dementias from healthy controls. (B) P-tau181, NfL, Aβ42/40 ratio and Aβ42 could differentiate AD dementia from non-AD dementia. (C) ROC curves of single plasma biomarkers in discriminating dementias from HC. (D) ROC curves of combined plasma biomarkers in discriminating dementias from HC. (E) ROC curves of single plasma biomarkers in discriminating AD from non-AD dementia. (F) ROC curves of combined plasma biomarkers in discriminating AD from non-AD dementia. Aβ, amyloid beta; GFAP, glial fibrillar acidic protein; NfL, neurofilament light; PSP, progressive supranuclear palsy; p-tau, phosphorylated tau.

GFAP, and directly compare the diagnostic accuracy of combined models generated by these biomarkers and their isolated models in patients with neurodegenerative dementias (AD, FTD and PSP). The associations between these plasma biomarkers and other kinds of indices were also explored. As expected, our cohort showed that plasma levels of p-tau181, NfL, and GFAP were higher and those of Aβ42/40 were lower in patients with AD, FTD, or PSP, compared with controls. Moreover, plasma Aβ42/40 or NfL itself has exhibited efficient diagnostic performances between the dementia group and controls. When compared with FTD or PSP, only plasma p-tau181 was significantly higher in AD and showed a modest value of AUC [0.805]. However, we observed that the combined marker model performed much better than a single biomarker to discriminate FTD or PSP patients from HC. Besides, each kind of combination generated by Aβ42/40, p-tau181, NfL, and GFAP could better differentiate AD from non-AD dementia (FTD/PSP).

Tau-related pathology is a hallmark of neurodegenerative diseases, such as Alzheimer's disease (AD), frontotemporal dementia (FTD) and progressive supranuclear palsy (PSP) [11]. The clinical syndromes of AD, FTD and PSP are heterogeneous and frequently overlap. These overlapping diseases have complex contributions to clinical manifestation and require classification [29]. The plasma biomarkers to differentiate AD dementia from non-AD dementia (FTD and PSP) are under development. We confirmed the overall diagnostic performance and discriminatory power of the core pathological proteins and neurodegenerative proteins in plasma.

The biomarkers including neurodegeneration (t-tau, NFL, neuron-specific enolase, visinin-like protein 1, and heart fatty acid binding protein), amyloid precursor protein (APP) metabolism (Aβ42, Aβ40,

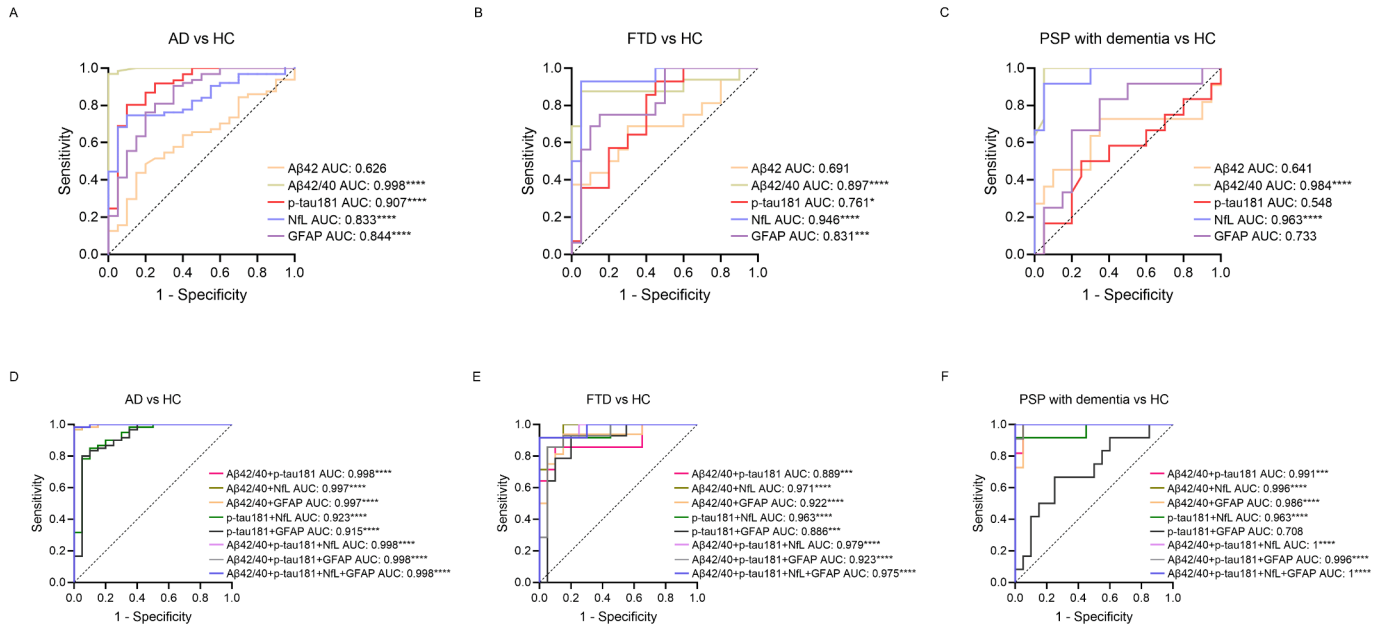

Fig. 3. Classification of three patient groups using area under the curve. (A) ROC curves of single plasma biomarkers in discriminating AD from HC. (B) ROC curves of single plasma biomarkers in discriminating FTD from HC. (C) ROC curves of single plasma biomarkers in discriminating PSP from HC. (D) ROC curves of combined plasma biomarkers in discriminating AD from HC. (E) ROC curves of combined plasma biomarkers in discriminating FTD from HC. (F) ROC curves of combined plasma biomarkers in discriminating AD from HC. Aβ, amyloid beta; AD, Alzheimer's disease; FTD, frontotemporal dementia; GFAP, glial fibrillar acidic protein; HC, healthy controls; NfL, neurofilament light; PSP, progressive supranuclear palsy; p-tau, phosphorylated tau.

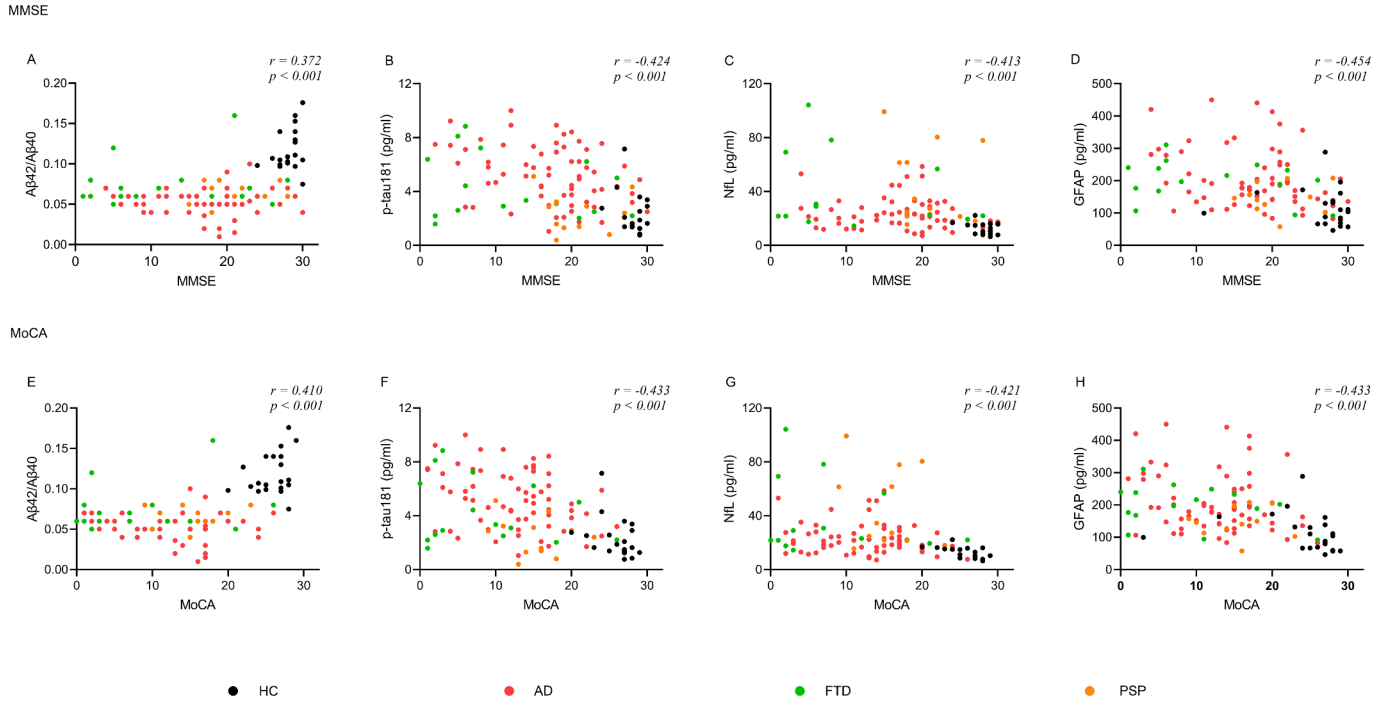

Fig. 4. Correlations between plasma biomarker measurements and global cognitive tests. Spearman correlation revealed that (A) the Aβ42/40 ratio was positively associated with MMSE scores while (B-D) p-tau, NfL and GFAP were negatively associated with MMSE scores; (E) the Aβ42/40 ratio was positively associated with MoCA scores while (F-H) p-tau, NfL and GFAP were negatively associated with MoCA scores. Post-hoc comparisons used the Bonferroni correction. Aβ, amyloid beta; AD, Alzheimer's disease; FTD, frontotemporal dementia; GFAP, glial fibrillar acidic protein; MMSE, Mini-Mental State Examination; MoCA, Chinese version of Montreal Cognitive Assessment; HC, healthy control; NfL, neurofilament light; PSP, progressive supranuclear palsy; p-tau, phosphorylated tau.

Aβ38, and α and β cleaved soluble APP), tangle pathology (p-tau), blood–brain-barrier function (CSF to serum albumin ratio), glial activation (YKL-40, monocyte chemotactic protein 1, and GFAP), chemokines, and metabolites in CSF or blood has been reported to have the diagnostic ability for dementia groups [30–32]. However, the validity of these biomarkers varied greatly in different studies. According to the established *meta*-analysis research, the core biomarkers (p-tau, t-tau, Aβ42, and NfL) differentiated AD from controls with good performance, while other biomarkers above were moderate and discrepant [30]. It is consistent with our results. Our logistic regression results showed that

increasing levels of GFAP, p-tau181, and NfL indicated higher risk of neurodegenerative dementias, while decreasing levels of Aβ42/40 indicated higher risk of dementia. To further explore the risk of AD and non-AD dementia, we found that rising levels of p-tau181 and NfL indicated higher risk of AD, while declining of Aβ42 and Aβ42/40 indicated higher risk of AD. The predictive value of plasma biomarkers hinted that the core biomarkers are apparently promising.

As the determination of core AD biomarkers, the level of plasma Aβ42/Aβ40 is explicitly reduced in AD and has perfect accuracy and sensitivity for AD diagnosis [11]. Our RF analysis shows that when differentiate the dementia from HC, Aβ42/Aβ40 are the top important variable. Meanwhile, the abundance and discrimination of plasma Aβ42/Aβ40 were also significant in FTD and PSP, probably because mixed brain protein pathologies frequently occur in many neurodegenerative dementias [11]. In accordance with previous studies [2,26], our findings indicate that Aβ42/Aβ40 could be used as a biomarker to screen cognitively impaired individuals from those cognitively unimpaired ones with strong sensitivity and specificity, including those with AD dementia and non-AD dementia patients. Our results showed that plasma NfL significantly elevated in patient groups than in HC, which indicated that patients with dementia have more prominent neurodegeneration [33]. Consistent with previous study [7], the highest NfL levels among the three neurodegeneration groups were in FTD in this study. This may result from the intensity of neurodegeneration or the degree of axonal damage. A series of studies has shown that neuron and axon damage in FTD cause NfL-release into body fluids [7,34,35]. Similarly, the result of ADNI data (Blennow lab) also supported that NfL could significantly identify the cognitive impairment individuals with controls. Though plasma NfL level also permitted a practicable classification for dementia from controls, the sensitivity seems to be moderate than Aβ42/Aβ40 in our cohort. Probably because the neurodegeneration in aging is universalization. Besides, both Aβ42/Aβ40 and NfL showed limited ability in differentiating AD dementia from non-AD dementia [36].

Studies based on large cohorts have verified the high accuracy of plasma p-tau181 in distinguishing AD from HC [4,37,38], and a recent study demonstrated that p-tau181 is more suitable than NfL and GFAP for diagnosing AD from other neurodegenerative diseases, including FTD and dementia with Lewy bodies [1]. Plasma p-tau181 in our study was significantly higher in AD patients compared with FTD and PSP patients and also better at discriminating AD from other neurodegenerative dementias (FTD + PSP) (AUC 0.805) compared with Aβ42 (AUC 0.740), Aβ42/40 (AUC 0.768) and NfL (AUC 0.704). Comparatively, the AUC value and the sensitivity and specificity of p-tau181 when differentiating AD from non-AD dementias lack ideality. There is, therefore, now a need to identify which other measures plasma p-tau should be combined with to produce the most accurate differential diagnosis of AD.

Surprisingly, we found that a combination model of plasma biomarkers [Aβ42/40, p-tau181, NfL, and GFAP] obviously enhanced the diagnostic accuracy than a single one. CSF biomarkers divide AD biomarkers into 3 pathophysiologic categories according to the requirement of the 'A/T/X' diagnostic system [39], and recently, plasma biomarkers have appeared to rival CSF markers in recognizing the pathologic AD under this framework [40]. Current research showed that combined plasma biomarker index improves the diagnostic accuracy of classifying AD and non-AD dementia, with the highest value of Aβ42/Aβ40 + p-tau181 + NfL + GFAP (AUC = 0.945). The result, importance index of RF analysis, also supported that the combined plasma biomarkers were better than single to distinguish AD dementia from non-AD dementia. It has been proved that a combination of plasma p-tau, APOE genotype, and magnetic resonance imaging measures showed higher diagnostic accuracy in AD dementia [41]. However, the plasma biomarkers the combination of various plasma biomarkers was hardly seen. These combined biomarkers allowed assessment of amyloid (A), neurofibrillary degeneration (T), and neurodegeneration (N) aspects of AD, which may thus enhance the discrimination capacity. Concurrently, plasma biomarkers play a vital role in diagnosis of other diseases [42–44]. It seems the combined model is a promising index for differentiation between AD and other neurodegenerative dementias. Regarding NfL, as is not a specific biomarker for AD, its combination with Aβ42/Aβ40 showed satisfactory accuracy between FTD versus HC and PSP versus HC. Besides, our results suggested that the combined Aβ42/Aβ40 + p-tau181 + NfL model was the best one to diagnose both FTD versus HC and PSP versus HC. Nevertheless, its clinical usefulness of FTD and PSP required further analysis in an extensive cohort to validate these results.

The blood samples of the participants were processed and detected uniformly, but the CSF samples of patients were processed and measured separately by each center. Due to the variation of inter-assay between different labs [45], the diagnostic accuracy of CSF biomarkers was moderate whether isolated or combined. Integrated CSF sample including cognitively unimpaired controls was needed to match and assess the combined assay of plasma. According to the published study [40], we verified the diagnostic performance of the ratio of p-tau181/Aβ42. Similarly, it performed better in detecting dementia with AD vs HC. Whereas, the sensitivity was not as good as Aβ42/40. In addition, the combination of plasma biomarkers was still preferable to discriminate AD from non-AD dementia.

In our study, the diagnostic performance of GFAP was not strong compared with other biomarkers. As previously reported, combining GFAP with Aβ42/Aβ40, ApoE4, and p-tau181 did not consistently increase the accuracy of Aβ positivity [46]. Interestingly, several studies have shown GFAP to be a promising marker for AD compared with controls and non-AD dementias [47,48]. Besides, GFAP could distinguish AD vs behavioral variant FTD and Parkinson's disease without cognitive impairment and behavioral variant FTD [49]. These findings suggested that GFAP plays a role in various diseases. Based on these varied results and non-disease-specific nature of neuroinflammation, multi-comparisons of GFAP are required.

Though previous study showed that event-related potentials (ERPs) have shown promise in distinguishing between AD and FTD [50], ERPs serve as sensitive indicators of AD risk even before any cognitive dysfunction manifests when coupled with APOE genotype [51]. Future studies will necessitate and contemplate multidimensional comparisons and diverse combinations of ERPs with other biomarkers. All plasma biomarkers in our research were further supported by associations with MMSE and MoCA scores, with negative correlation of p-tau181, NfL, and GFAP to MMSE/MoCA, and positive correlation of Aβ42/Aβ40 to MMSE/MoCA. Plasma levels of markers change with cognitive impairment and pathological load [7]; however, possibly owing to the smaller number of CSF and PET samples, there were no significant differences between plasma biomarker levels and CSF levels, or plasma biomarker levels and global or regional PET SUVR.

The strengths of our study are that (1) we tested the clinical utility of isolated plasma biomarkers in a pooled multicenter clinical dementia cohort of older participants from Eastern China. (2) We first used the combined model of pathological and non-specific plasma biomarkers in a cohort with overlapping presentations of three different types of tau-related neurodegenerative diseases. (3) The Aβ status for all demented patients was confirmed by CSF and/or PET analysis.

There are also some limitations to our study. (1) There were age and education differences in the cohort caused in part by including neurodegenerative dementias with different epidemiological characteristics. (2) Not all patients underwent both CSF and PET examination, which limited our correlation analysis. (3) We did not have longitudinal data, so we could not observe changes in biomarkers over time, which might vary between the different dementias. (4) The number of FTD and PSP patients was small, and the FTD cohort included both familial and sporadic cases.

5. Conclusions

In summary, ample evidences demonstrated that core AD biomarkers (Aβ42/40 and p-tau) and non-specific biomarkers (NfL and GFAP) showed considerable variation in diagnostic performance. In this representative study, we included a dementia cohort from Eastern China to verify the diagnostic accuracy of isolated plasma biomarkers and tested the diagnostic performance of combination models generated by these markers. We confirmed that plasma biomarkers in neurodegenerative dementias have diagnosis potential in a low-cost, accessible, minimally invasive, and convenient approach. Our findings verified the diagnostic accuracy of isolated plasma biomarkers in the Chinese dementia cohort, and also explored the novel combined model, which highlights that a combined model can more accurately identify the FTD or PSP from HC, as well as AD from non-AD dementia.

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CRediT authorship contribution statement

Yi Chen: Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis. **Yunyun Wang:** Writing – original draft, Resources, Formal analysis. **Qingqing Tao:** Validation, Resources, Project administration, Formal analysis, Data curation. **Peilin Lu:** Formal analysis, Data curation, Project administration, Resources, Validation, Writing – original draft. **Fanxia Meng:** Validation, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources. **Liying Zhuang:** Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Song Qiao:** Validation, Resources, Project administration, Formal analysis, Data curation. **Ying Zhang:** Formal analysis, Data curation, Project administration, Resources, Validation. **Benyan Luo:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation. **Yang Liu:** Writing – review & editing, Resources, Formal analysis, Conceptualization. **Guoping Peng:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

Data will be made available on request.

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Data statement

The related materials, data and associated protocols during the current study are available from the corresponding author on reasonable request without undue delay or qualifications. All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2024.118784.

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