Performance of Plasma Amyloid β, Total Tau and Neurofilament Light Chain Levels for Alzheimer's Disease Identification

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Research

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Abstract

Background

Robust studies have focused on blood-based biomarkers for diagnosis of Alzheimer's disease (AD), while the results were still controversary and failed verified in different cohorts. The aim of this study was to detect the levels of plasma amyloid β (Aβ), total tau (t-tau), and neurofilament light chain (NfL) in patients with AD and cognitive normal (CN) subjects, and clarify their associations with Aβ, t-tau, and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF) as well as brain amyloid PET, and calculate the diagnostic efficiency of these characteristics regarding AD.

Methods

Plasma Aβ42, Aβ40, t-tau and NfL levels were detected by single-molecule array (Simoa) in 379 AD patients and 153 CN subjects. Additionally, lumbar puncture was conducted in 125 AD patients to detect Aβ42, Aβ40, t-tau, and p-tau levels. Brain amyloid PET was performed in 52 AD patients to identify brain amyloid deposition levels. Correlation analysis were performed between plasma biomarkers and typical biomarkers of AD, including CSF core biomarkers and amyloid PET burden. Finally, the diagnostic value of plasma biomarkers was further assessed by receiver operating characteristic (ROC) curve.

Results

Compared with the CN group, plasma Aβ42 and Aβ42/Aβ40 levels were significantly lower in AD patients, while Aβ40, t-tau and NfL levels were higher in AD patients. Among the AD patients, plasma Aβ42 was positively correlated with CSF Aβ42 ($r = 0.195$, $p = 0.03$) and Aβ42/Aβ40 ($r = 0.208$, $p = 0.04$). Moreover, plasma NfL was positively correlated with age, disease course and severity. The diagnostic model with combined plasma Aβ42, t-tau, and NfL levels controlled for age and *APOE* genotype showed the best performance to identify AD (area under the curve (AUC) = 0.88, sensitivity = 82.84%, specificity = 81.69%, cutoff value = 0.64).

Conclusions

Trends revealed by core biomarkers were generally consistent in AD patients' plasma and CSF. Combining plasma biomarkers can provide comparatively high AD diagnostic performance.

Background

Alzheimer's disease (AD) is the most common neurodegenerative disease in the elderly population, characterized by progressive cognitive decline and behavioral defects with a complex and heterogeneous pathophysiology. It has been reported that there are approximately 50 million people with dementia, 60%-80% of them are AD, and the newly increased number of dementia cases is nearly 10 million each year[1].

A preclinical phase of about 20 years or more may occur before the clinical diagnosis of AD, in which no or only subtle symptoms appear[2-3]. It gives the opportunity to delay the onset of AD or even prevent the occurrence of disease by suitable intervening on the preclinical stage. Nowadays, the most well-established AD biomarkers are mainly based on the core pathological features, including amyloid β (Aβ) deposition (cerebrospinal fluid (CSF) Aβ42 and amyloid PET) and neurodegeneration (CSF t-tau, p-tau, structural MRI, and hypometabolism on FDG PET)[4-6].

The clinical application of the above biomarkers is always limited by high invasiveness, expensive costs or unavailability. Therefore, noninvasive and cost-effective methods are desperately needed for early intervention.

Robust studies have focused on blood-based biomarkers for diagnosis of AD, but they have failed to verify these results in independent studies. To date, somewhat promising results have been demonstrated for Aβ, tau and neurofilament light chain (NfL). More than two independent groups demonstrated the high performance of plasma Aβ-related peptides for AD prediction via different methods[7-8]. Plasma t-tau is a promising candidate marker, because it is a brain-specific protein mainly expressed in central nervous system (CNS) neurons, which is considered to indicate neuronal damage and to be derived from brain parenchyma to the CSF and blood[9]. The levels of plasma t-tau have been shown to improve the prediction of dementia and are suggested to as a biomarker for risk stratification in dementia prevention trials[10]. NfL is the main component of the axonal cytoskeleton and maintains the neuronal structure, which is mainly expressed in large-caliber myelinated axons and is released into CSF following neuroaxonal injury[11]. In recent years, various studies have shown that the NfL level in peripheral blood is a promising biomarker that can track neurodegenerative changes in AD patients, and increased levels are related to brain atrophy, brain hypometabolism and cognitive function decrease[12-14].

However, despite great efforts, the results are still controversial in different cohorts, and no efficient blood-based biomarker has been validated in clinical applications until now. Considering the heterogeneity and complexity of the etiology of AD, it is difficult for a single biomarker to reflect comprehensive pathological changes and contribute to the disease diagnosis. Moreover, although recent progress in Aβ, tau and NfL analysis in the blood has been reported, there are few studies that have integrated these indicators simultaneously to evaluate their diagnostic efficacy for AD and assess their consistency with classic core biomarkers, including CSF biomarkers and amyloid PET.

Thus, in this study, we combined these plasma biomarkers, including Aβ42, Aβ40, t-tau and NfL, detected their levels in plasma simultaneously between AD patients and cognitive normal (CN) individuals, and assessed their performance for discriminating AD from CN subjects.

Methods

Participants

A total of 532 individuals, including 379 AD patients and 153 CN subjects, were enrolled from the Department of Neurology, Xiangya Hospital, Central South University between March 2017 and December 2019. The inclusion criteria for AD participants were probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke-AD and Related Disorders Association (NINCDS-ADRDA) criteria made by two or more experienced neurologists from Xiangya Hospital. The inclusion criteria for the control group were as follows: (1) did not have memory complaints, (2) no evident cognitive impairment, and (3) matched with AD patients regarding age and sex.

The study protocol was approved by the Institutional Review Board of Xiangya Hospital of Central South University in China. Written informed consent was obtained from each participant or guardian.

Neuropsychological assessment

Neuropsychological tests were performed on patients. The general cognitive function was assessed with the Mini-Mental State Examination (MMSE), and Clinical Dementia Rating Scale (CDR). The MMSE was also conducted with healthy controls who met the inclusion criteria.

APOE genotyping

Venous blood was collected from all participants using ethylenediaminetetraacetic acid (EDTA) tubes. Genomic DNA was extracted using the standard phenol-chloroform extraction method. All DNA samples were diluted to 50 ng/μl. A 581-bp fragment was amplified using the following primers: forward 5'-CCTACAAATCGGAACTGG-3' and reverse 5'- CTCGAACCAGCTCTTGAG -3'. Polymerase chain reaction (PCR) was performed as previously described[15]. Each PCR product was sequenced on an ABI 3730xl DNA analyzer (ABI, USA).

CSF collection and measurements

CSF was obtained from lumbar puncture samples from the participants. Briefly, the subjects were positioned in a left lateral position for the lumbar puncture procedure. Approximately fifteen milliliters of CSF collected from each subject, centrifuged at 2000 g for 10 minutes at room temperature and stored in a polypropylene tube at -80°C. The levels of Aβ42, Aβ40, t-tau and p-tau in CSF were measured with an enzyme-linked immunosorbent assay (ELISA). All measurements were performed in a blinded manner.

Amyloid PET scan acquisitions and processing

Amyloid PET imaging was performed using a bonus injection of C^{11}-PiB (15 mci/kg) on the day of the examination. After a rest period of approximately 50 minutes, brain metabolic tomography was performed with a collection time of 15 minutes. Multiple regions of interest (ROIs) were labeled, including the frontal lobe, temporal lobe, parietal lobe, occipital lobe and cingulate gyrus, and the maximal standardized uptake value (SUV_{max}) was measured with the cerebellar gray matter as the reference region. Data were converted to the SUV_{max} relative to the cerebellar gray matter ($SUVR_{max}$).

Plasma protein qualification

Venous blood was collected into EDTA tubes and centrifuged at 2000 g for 10 minutes at 4°C. The obtained plasma was portioned into approximately 500 μl aliquots and frozen at -80°C, and samples were thawed for analysis. All samples underwent no more than 3 cycles of freezing and thawing. Samples were quickly thawed at room temperature and centrifuged at 10000 g prior to analyses to prevent any sample debris from interfering with the measurements. The concentrations of plasma Aβ42, Aβ40, t-tau and NfL were measured simultaneously on the single-molecule array (Simoa)-HD1 platform (Simoa; Quanterix, USA), which employed an automated Simoa principle. Aβ42, Aβ40, and t-tau were measured via multiplex array (Neurology 3-Plex A Advantage Kit, N3PA, catalog number: 101995), and NfL was measured using a single analyte array (NF-light; catalog number: 103186). The samples were measured by a two-step immunoassay. All analytical procedures were performed by well-trained technicians who were blinded to the participant's state and clinical data, according to the manufacturer's published protocol. Samples with coefficients of variance >20% were excluded from analyses. Final data were also examined for extreme outliers.

Statistical analysis

The normality of the variables was checked. Categorical data were analyzed using the χ^2 test. Continuous variable comparisons between two independent samples were conducted via t test or the Mann-Whitney U test, and differences between multiple independent samples were compared using the Kruskal-Wallis H test. Correlations between plasma biomarkers and demographic characteristics and clinical data were assessed with Spearman correlation coefficients. Diagnostic accuracy was assessed with receiver operating characteristic (ROC) curve analysis and logistic regression models. The area under the curve (AUC) and the representative best values for sensitivity and specificity were used to evaluate the performance of the models. Statistical differences in AUCs between two different data sets were analyzed using Delong's test for two uncorrelated ROC curves.

All tests were two-tailed, and $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS v.24 (IBM). Data were visualized using Prism 8 (GraphPad).

Results

Participants

The demographic and clinical characteristics of AD patients and CN subjects are summarized in Table 1**,** including 379 AD and 153 CN subjects. In 532 individuals aged 38 to 92 from AD cohort, the mean age of onset was 61.9 years old, and the mean disease course was approximately 33 months. The sex ratio (F/M) of AD subjects was 239/140, which was matched by the CN group (99/54) ($p > 0.05$). In parallel, 192 (50.7%) patients were *APOE4* allele carriers.

Plasma biomarker differences

All detected plasma biomarkers, including Aβ42, Aβ40, Aβ42/Aβ40, t-tau and NfL, between AD patients and CN subjects showed significant differences before adjusting for age, sex and *APOE* genotype. Among them, compared with controls cohort, plasma Aβ42 was significantly lower in the AD cohort than in the CN group ($p = 0.008$). In parallel, the levels of Aβ40, Aβ42/40, t-tau and NfL were higher in AD patients ($p < 0.05$) (Table 1).However, there was a large overlap area of these plasma biomarker between AD patients and CN subjects(Fig.1). After adjusting for age, sex and *APOE* genotype, the level of Aβ42 showed no significant difference between the AD and CN groups ($p = 0.224$), while Aβ40, Aβ42/40, t-tau and NfL were still presented statistically significant (Table 1).

Correlation between plasma biomarkers and demographic characteristics

Next, we analyzed the correlations between plasma biomarkers and demographic data, including age, disease course and education attainment. Meanwhile, we compared the difference of plasma biomarkers between different sexes, family histories and *APOE4* allele carrier distributions (Table 2). First, the results of the correlation between plasma biomarkers and age indicated that in AD group, plasma biomarkers, including Aβ40, Aβ42/Aβ40, t-tau and NfL, were significantly associated with age ($p<0.05$). Then, subgroup analysis based on sex revealed that in AD group, male patients had higher plasma NfL levels ($p<0.05$), while other plasma biomarkers in AD group showed no significant difference. In addition, the levels of plasma Aβ40 and NfL showed a positive correlation with disease course, while the levels of plasma Aβ42/Aβ40 and t-tau showed a negative correlation with disease course in our study. After conducting subgroup analysis according to family history, the results showed that both Aβ42 and Aβ42/Aβ40 showed a significant difference between family history-positive and -negative groups, and the levels were higher in the group with a positive family history. In addition, for *APOE4* genotypes, the results indicated that

Aβ42/Aβ40 was significantly higher in *APOE4* allele carrier patients (p = 0.028), while other indicators were not significantly different between the two subgroups.

Correlation between plasma biomarkers and cognitive function

Subsequently, we analyzed the correlation between plasma biomarkers and neuropsychological assessments, including the MMSE and CDR. The MMSE showed a negative correlation with plasma NfL and a positive correlation with Aβ40, indicating that worse cognitive function was accompanied by higher NfL levels and lower Aβ40 levels. After adjusting for age, sex, disease course, *APOE* genotype and education attainment, the results showed that MMSE scores were weak but significantly correlated with plasma Aβ40 (r = -0.114, p = 0.027) (Table 2).

Besides, to further analyze the correlations between plasma biomarkers and disease severity, AD patients were divided into four subgroups according to CDR score. As a result, only plasma NfL level was increased along with clinical severity (Fig.2). Therefore, we speculated that the plasma NfL level is a potential plasma biomarker to monitor disease progression.

Correlation between plasma and CSF biomarkers

To assess the efficacy of plasma biomarkers to reflect brain pathological changes, the correlations between CSF and plasma biomarkers of AD patients (n = 125) were performed, as displayed in Table 3. Plasma Aβ42 was positively correlated with Aβ42, Aβ42/Aβ40 and p-tau in CSF (r = 0.195, p = 0.03; r = 0.208, p = 0.04; r = -0.254, p = 0.004). After adjusting for age, sex and *APOE* genotype, we found that plasma Aβ42 was positively associated with CSF Aβ42/Aβ40 and negatively associated with CSF p-tau (r = 0.227, p = 0.03; r = -0.215, p = 0.03 respectively). Similarly, plasma Aβ40 showed negative correlation with CSF Aβ42/Aβ40 (r = -0.240, p = 0.02), while plasma Aβ42/Aβ40, t-tau and NfL showed no correlations with CSF biomarkers.

Correlation between plasma biomarkers and amyloid PET burden

In addition, we conducted correlation analyses between plasma biomarkers and amyloid PET burden in 52 AD patients. The results showed that only NfL was correlated with the SUVRmax left anterior cingulate gyrus (r = 0.293, p = 0.035). However, after age, sex and APOE genotype adjusted all the plasma biomarkers showed no correlation with SUVRmax of each ROI (Table 4).

Performance of plasma biomarkers for AD diagnosis

Finally, ROC curves were generated to evaluate the utility of the plasma biomarkers to discriminate AD and healthy controls (Fig. 3). The cutoff value and its corresponding sensitivity and specificity were calculated using the maximum Youden index. The results showed that the NfL level in plasma had the best diagnostic efficacy as a single indicator (AUC = 0.83, sensitivity = 84.43%, specificity = 67.97%). Meanwhile, the results showed that the combined diagnostic efficacy, including Aβ42, t-tau and NfL, was superior to other plasma biomarker combined models (AUC = 0.86, sensitivity = 79.41%, specificity = 81.05%). Furthermore, based on logistic regression analysis, after age and *APOE* genotypes were included in the combined model, diagnostic performance reached the maximum value (AUC = 0.88, sensitivity = 82.84%, specificity = 81.69%) (p < 0.05).

Discussion

Currently, numerous studies have focused on exploring the efficacy of plasma biomarkers in AD prediction, diagnosis and monitoring. However, it is still difficult to draw definite conclusions about the changes in plasma biomarkers due to the inconsistency in different studies. In the present study, we found that plasma biomarkers, including Aβ42, Aβ40, Aβ42/Aβ40, t-tau and NfL showed significant differences between AD patients and healthy controls. Interestingly, our most striking finding was that the plasma NfL level was positively correlated with CDR score, indicating that NfL was probably the most promising candidate biomarker to reflect disease severity and monitor the disease progression, although it may not be a specific marker for AD. To our knowledge, there are few domestic studies detecting these plasma biomarkers together in a large cohort, especially in analyzing their correlations with AD typical biomarkers[16-17]. In the present study, a comparatively large-scale AD patients of blood biomarkers, as well as CSF core biomarkers and amyloid PET were analyzed, and interestingly, we found the plasma Aβ42 and Aβ40 were positive associated with CSF Aβ42/Aβ40, suggesting that plasma Aβ can partly reflect brain Aβ status and be superior to other plasma biomarkers. Besides, we compared the efficacy of plasma biomarkers for the diagnosis of AD by plotting ROC curves. According to logistic regression analysis, after introducing age and *APOE* genotype into the combined model, including Aβ42, t-tau and NfL, the diagnostic performance reached the maximum value. It is encouraging the model can be applied to perform initial screening in population with a high risk of AD, which might effectively reduce the application of lumber puncture and PET examinations in clinical practice.

In this study, before adjusting for age, sex, and *APOE* genotype, plasma Aβ42 levels were significantly lower in AD patients, while after controlling for these factors, the difference became insignificant. Previously, the change in plasma Aβ42 levels in AD patients is still controversial [18-19]. Approximately 30%-50% of blood Aβ comes from a brain-to-blood transport mechanism, and there is a dynamic equilibrium between peripheral blood and the CNS[20]. It is possible to measure the level of Aβ42 in plasma to reflect pathological changes in the brain to some extent. Based on this, we assessed the correlation between plasma Aβ42 levels and brain Aβ pathology, including CSF Aβ42 and amyloid PET. Interestingly, we found the plasma Aβ42 and Aβ40 were positive associated with CSF Aβ42/Aβ40, suggesting that plasma Aβ can partly reflect brain Aβ status and be superior to other plasma biomarkers, which was consistent with some previous results[21]. Moreover, plasma Aβ42 showed negative correlation with CSF p-tau, suggesting that, to some extent, plasma Aβ can partially reflect the tau pathological changes of brain, which was consistent with the current research view that there lies in close relationship between Aβ and tau pathology In addition, we found that plasma Aβ42 was shown no correlation with Aβ deposition upon amyloid PET evaluation in our study, which was different with some previous studies[22]. Given that the results reflected different forms of Aβ, the former reflects Aβ peptide and the latter reflects the fiber form, it may reasonable that Aβ in plasma cannot represent Aβ accumulation in brain parenchyma. Meanwhile, the small sample sizes of CSF and amyloid PET in this study may not reflect the real relationship between plasma Aβ42 and brain pathology. Thus, to confirm the real trend of plasma Aβ42 in AD patients and the utility of monitoring the pathological changes in brain tissue, head-to-head comparisons based on large-scale prospective studies are necessary, and unified and standardized inclusion criteria, detection and analysis methods are urgently needed.

For Aβ40, the results are mixed, and the meta-analysis by Olsson et al. suggested that there was no difference between AD patients and controls[23]. In our study, compared with the CN group, Aβ40 was significantly higher in the plasma of AD patients, contrary to the trend of Aβ42. Until now, the diagnostic utility of CSF Aβ40 alone was limited, while it is usually used as a reference peptide that could explain the difference in CSF concentration between individuals and the difference in preanalytical processing of the samples, which otherwise may lead to false-positive or false-negative results using Aβ42 alone[24]. In addition, after considering sex, age and *APOE*

genotypes, plasma Aβ40 was correlated with CSF Aβ42/Aβ40, which is consistent with previous studies that plasma Aβ40 can reflect Aβ pathological in the brain to some extent[22].

Compared to healthy controls, plasma Aβ42/Aβ40 was significantly decreased in AD patients and was in line with most previous studies, which showed a higher degree of consistency than single Aβ peptide even using different detection methods[7, 25]. Decreased Aβ42/Aβ40 levels demonstrated value in reflecting pathological changes of AD and predicting cerebral Aβ status, whether regarding amyloid PET or CSF Aβ as a positive reference[26-28]. Furthermore, the ratio in plasma appears to be associated with an increased risk of progression to AD dementia[29]. This means that if using the plasma Aβ42/Aβ40 ratio as a prescreening tool for the most likely AD population, the necessity of amyloid PET inspection may be reduced, which is of great significance for clinical application and scientific research. However, these findings should be taken cautiously, in our study, the aforementioned results were not obtained, and we speculated that the sample size and measurement method cannot be ruled out as the reason; thus, further validation based on large-scale AD cohort studies is necessary.

Plasma t-tau was significantly increased in AD patients compared with the healthy controls in this study, similar to most published results [17, 30], while several studies also found that plasma t-tau showed opposite changes or no significant changes between AD patients and controls [29, 31]. Nowadays, the results of plasma t-tau in AD patients were still contradictory. Mattson et al. found that higher plasma t-tau was associated with AD dementia and showed a significant correlation with worse cognition, more atrophy and more hypometabolism during follow-up in the ADNI study[30]. However, in our study, plasma t-tau showed no significant correlation with cognitive function and can not reflect the levels of t-tau in CSF, which is also in line with some previous findings [30, 32].Besides, increasing evidence supports that plasma p-tau is a promising biomarker for AD, which can not only be associated with CSF p-tau but also correlates with amyloid PET uptake and discriminates AD dementia from non-AD neurodegenerative disease, partly reflecting AD pathology and disease severity[33-34]. In this study, due to the low concentration in the samples and methodology limitations, we did not measure p-tau in plasma. In the next study, we will increase the sample size to reevaluate whether plasma t-tau could predict cognitive function and AD pathology, and assess the utility of plasma p-tau for discriminating patients with AD from controls.

Emerging evidence supports that NfL is a sensitive and promising biomarker for neurodegenerative disease, which is released into CSF and plasma after axonal damage [35-36]. In the latest research, Yakeel T Quiroz et al. found that plasma NfL increased with age and began to differentiate PSEN1 E280A mutation carriers from noncarriers as early as age 22 based on a large kindred study of patients with AD[37]. In agreement with these results, we found that the plasma NfL concentration was associated with age, and became significantly elevated in the plasma as the individuals became older. Meanwhile, the results of correlation analysis showed that the level of plasma NfL was not associated with CSF core biomarkers, which is not consistent with some previous study that plasma NfL levels correlated significantly with CSF core markers[38-39]. Based on these results, plasma NfL is a sufficient sensitive marker to detect axonal damage in the early stage of AD, while it is not a specific indicator to reflect the typical pathological changes of AD [40]. Interestingly, according to CDR score, subgroup analysis was performed, it was found that plasma NfL levels increased with the severity of the disease, supporting that the NfL level is a marker of progressive myelinated axonal damage that develops in the mild to late stage of AD. Combined with a recent longitudinal study, which demonstrated that the plasma NfL level in participants who developed AD rose at a constantly higher rate than that in CN subjects and increased as early as 10 years before an AD diagnosis[41], plasma NfL levels may be a stable and useful indicator for disease identification and for monitoring disease progression.

Finally, we compared the efficacy of markers in plasma for the diagnosis of AD by plotting ROC curves. The results showed that the plasma NfL level has the best performance compared with other single indicators. Combined with previous studies, due to the poor specificity of NfL for discriminating AD and other neurodegenerative disorders, we tried to combine multiple indicators to verify whether the diagnostic efficacy of plasma markers could be improved. The results showed that when NfL, t-tau and Aβ42 levels were combined, the AUC reached its maximum (AUC = 0.86, sensitivity = 79.41%, specificity = 81.05%). Moreover, even though all indicators (Aβ42, Aβ40, t-tau, NfL, and ratio of Aβ42/Aβ40) were introduced into the model, the diagnostic efficacy was not significantly optimized. Furthermore, after introducing age and *APOE* genotype into the combined model, the diagnostic efficacy was significantly improved (AUC = 0.88, sensitivity = 82.84%, specificity = 81.69%). This finding is encouraging because the model can be used to perform initial screening in people with a high risk of AD or probable AD, which can effectively reduce lumber puncture and PET examinations.

Notably, the results suggested that these plasma indicators were promising for discriminating AD and CN subjects. The use of a peripheral biomarker panel with low cost and invasiveness as an initial screening funnel to identify people who should undergo further examination, such as PET imaging or CSF testing, might be a critical step forward because subjects at an early stage of AD are hard to identify. In addition, given the low concentration of markers in plasma and the limited detection sensitivity, methodological limitations still exist. To minimize possible experimental errors and promote the detection accuracy of plasma markers, we unified the detection method for each marker via Simoa technology.

Of course, the study still has some limitations, including the following aspects. First, the study was a retrospective study without longitudinal follow-up to determine the trajectories of plasma markers from CN individuals to those in different AD stages. Second, in this study, due to the expensive or invasive characteristics of PET or lumbar puncture, we failed to obtain results for all participants; thus, some individuals were not able to contribute standard pathological results to the group data for further analysis. Next, there is a lack of patients with non-AD dementia to compare the difference in plasma markers and verify the ability of the panel to distinguish AD dementia from other forms of dementia.

Conclusions

In conclusion, we found that plasma biomarkers, including Aβ40, Aβ42/Aβ40, t-tau and NfL levels, were significantly different between the AD and CN groups after age, sex and APOE genotype adjusted. The diagnosis model, combined with Aβ42, t-tau and NfL levels and taking age and APOE genotypes into account, showed the best performance to discriminate patients with AD and CN participants. Our findings indicated that the plasma biomarkers panel might partly reflect the change of AD typical biomarkers, and was expected to as a potential biomarker for AD patients.

Abbreviations

Aβ: amyloid β; AD: Alzheimer's disease; AUC: area under the curve; CN: cognitive normal; CSF: cerebrospinal fluid; CDR: Clinical Dementia Rating Scale; CNS: central nervous system; ELISA: enzyme-linked immunosorbent assay; EDTA: ethylenediaminetetraacetic acid; MCI: mild cognitive impairment; MMSE: Mini-Mental State Examination; NfL: neurofilament light chain; PET: position emission tomography; ROC: receive operating characteristic curve; Simoa: single-molecule array; SUVR: standard uptake value ratio; t-tau: total tau

Declarations

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Authors' contributions

BJ and HL carried out the data analysis and interpretation, prepared and wrote the manuscript. LNG, XXL and YFZ analyzed the data and revised the manuscript for intellectual content. LW, XWX, LZ, XW, YLJ, QJY, YZ, WWZ, LZ, XXY and BST participated in the data collection and analysis. LS conceived and designed the study. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Xiangya Hospital of Central South University in China, and all participants provided written informed consent prior to inclusion. Patient recruitment and collection protocols were in accordance with ethical standards according to WMA Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects.

Consent for publication

Consent for publication was provided by every participant of the study.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Demographic and clinical characteristics of AD patients and healthy controls.

	AD (n=379)	Control (n=153)	p value
Demography characteristic			
Age (Mean ± SD)	64.6 ± 10.3	62.2 ± 8.3	0.947
Sex (F/M, n)	239/140	99/54	0.721
Years of education (Mean ± SD)	7.36 ± 4.33	8.79 ± 3.81	<0.001*
*APOE*4 (+/-, n)	192/187	127/26	<0.001*
Family history (+/-，n)	129/250	-	-
disease course, months, (Mean ± SD)	33.2 ± 24.2	-	-
MMSE (Mean ± SD)	13.2 ± 6.4	27.7 ± 2.3	<0.001*
Plasma biomarkers (Mean ± SD)			
Aβ42 (pg/ml)	14.12 ± 3.72	14.58 ± 3.33	0.008* (0.224)
Aβ40 (pg/ml)	275.33 ± 60.74	258.48 ±50.36	0.002* (0.013*)
Aβ42/Aβ40	0.052 ± 0.135	0.057±0.013	<0.001* (0.008*)
t-tau (pg/ml)	4.15 ± 1.24	3.23 ±1.12	<0.001* (<0.001*)
NfL (pg/ml)	26.85 ± 26.83	14.13±10.25	<0.001* (<0.001*)

The p value in brackets is the adjusted p value after controlling age, sex and *APOE* genotype. AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; CDR, Clinical Dementia Rating scale; t-tau, total tau; NfL, neurofilament light chain. *, difference between the groups is statistically significant ($p < 0.05$).

Table 2 Correlations between plasma biomarkers, demographics and cognitive function in AD patients.

	Plasma Aβ42		Plasma Aβ40		Plasma Aβ42/Aβ40		Plasma t-tau		Plasma NfL	
	r	p value	r	p value	r	p value	r	p value	r	p value
Age	0.096	0.062	**0.345**	**<0.001***	**-0.186**	**<0.001***	**-0.144**	**0.005***	**0.276**	**<0.001***
Sex (F/M)	-	0.243	-	0.151	-	0.894	-	0.374	-	**0.028***
Months of disease course	0.78	0.130	**0.314**	**<0.001***	**-0.190**	**<0.001***	**-0.132**	**0.01***	**0.231**	**<0.001***
Family history (+/-)	-	<0.001*	-	0.370	-	<0.001*	-	0.716	-	0.692
*APOE*4 (+/-)	-	0.348	-	0.368	-	**0.028***	-	0.804	-	0.556
Years of education	-0.063	0.218	-0.035	0.494	-0.034	0.515	0.087	0.091	**0.174**	**0.001***
MMSE	0.078	0.130	**0.130**	**0.012***	-0.049	0.341	-0.055	0.284	**-0.121**	**0.019***

AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; t-tau, total tau; NfL, neurofilament light chain. *, difference between the groups or the correlation between indicators is statistically significant ($p < 0.05$).

Table 3 Correlations between plasma biomarkers and CSF biomarkers in AD patients (n = 125).

CSF biomarkers	Plasma Aβ42		Plasma Aβ40		Plasma Aβ42/Aβ40		Plasma t-tau		Plasma NfL	
	r	p value	r	p value	r	p value	r	p value	r	p value
Aβ42	**0.195**	**0.03***	0.166	0.06	0.047	0.60	**-0.185**	**0.03***	-0.028	0.75
Aβ40	-0.052	0.61	0.044	0.67	-0.001	0.33	-0.074	0.47	-0.122	0.23
Aβ42/Aβ40	**0.208**	**0.04***	0.105	0.31	0.102	0.32	-0.100	0.33	-0.010	0.92
p-tau	**-0.254**	**0.004***	**-0.221**	**0.013***	-0.133	0.14	0.117	0.19	-0.055	0.53
t-tau	-0.156	0.08	-0.120	0.18	-0.007	0.441	0.135	0.13	-0.033	0.71
Adjusted for age, sex and *APOE* genotype										
Aβ42	0.187	0.07	0.161	0.12	0.033	0.75	-0.092	0.38	0.005	0.96
Aβ40	-0.097	0.35	-0.068	0.52	-0.051	0.62	-0.058	0.58	-0.178	0.09
Aβ42/Aβ40	**0.227**	**0.03***	**0.240**	**0.02***	0.029	0.78	-0.055	0.60	0.115	0.27
p-tau	**-0.215**	**0.03***	-0.156	0.13	-0.099	0.35	0.014	0.89	-0.162	0.12
t-tau	-0.044	0.67	-0.063	0.54	-0.027	0.80	0.090	0.39	-0.078	0.46

CSF, cerebrospinal fluid; AD, Alzheimer's disease; p-tau, phosphorylated tau; t-tau, total tau; NfL, neurofilament light chain; *, the correlation between indicators is statistically significant ($p < 0.05$)

Table 4 Correlations between plasma biomarkers and SUVR of regions of interest of AD patients (n = 52)

	Plasma Aβ42		Plasma Aβ40		Plasma Aβ42/Aβ40		Plasma t-tau		Plasma NfL	
Regions of interest	r	p value	r	p value	r	p value	r	p value	r	p value
Left frontal lobe	-0.117	0.408	-0.044	0.756	-0.205	0.144	0.014	0.919	0.235	0.093
Right frontal lobe	-0.149	0.291	-0.070	0.620	-0.201	0.152	0.007	0.961	0.222	0.114
Left temporal lobe	-0.167	0.235	-0.064	0.652	-0.254	0.069	0.040	0.777	0.206	0.143
Right temporal lobe	-0.111	0.434	0.013	0.927	-0.234	0.094	0.050	0.727	0.183	0.195
Left parietal lobe	-0.175	0.213	-0.103	0.466	-0.211	0.133	0.029	0.838	0.178	0.206
Right parietal lobe	-0.174	0.218	-0.043	0.763	-0.258	0.065	0.059	0.677	0.245	0.080
Left occipital lobe	-0.140	0.322	-0.128	0.367	-0.111	0.433	-0.008	0.957	0.148	0.295
Right occipital lobe	-0.136	0.337	-0.083	0.560	-0.139	0.325	0.083	0.557	0.211	0.133
Left anterior cingulate gyrus	-0.169	0.230	-0.118	0.406	-0.218	0.120	0.147	0.299	**0.293**	**0.035***
Right anterior cingulate gyrus	-0.180	0.201	-0.067	0.637	-0.241	0.085	0.129	0.364	0.251	0.073
Left posterior cingulate gyrus	-0.057	0.690	0.046	0.747	-0.175	0.215	0.078	0.585	0.224	0.110
Right posterior cingulate gyrus	-0.076	0.590	0.002	0.986	-0.165	0.243	0.092	0.516	0.176	0.213
Average SUVR	-0.156	0.270	-0.069	0.626	-0.211	0.132	0.029	0.841	0.231	0.099

Correlation between levels of plasma Aβ and SUVR of the ROIs. SUVR, standardized uptake value ratio; AD, Alzheimer's disease.

Figures

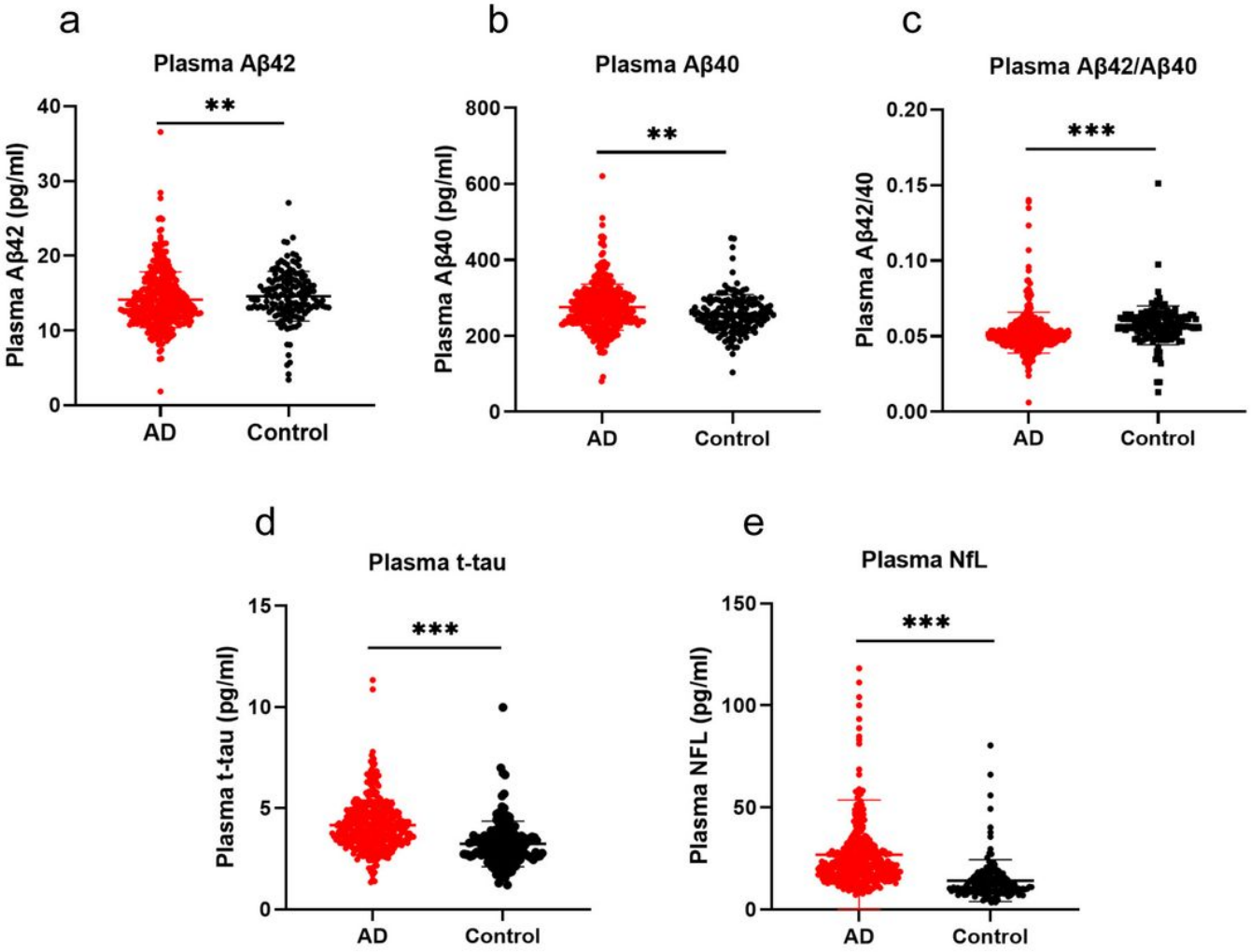

Figure 1

Comparison of plasma biomarkers between AD and CN groups. Plasma biomarkers are presented as the mean ± SD. The significance of differences between groups was determined by the Mann-Whitney U test. AD, Alzheimer's disease; CN, cognitively normal; t-tau, total tau; NfL, neurofilament light chain; *, p<0.05; **, p<0.01; ***, p<0.001.

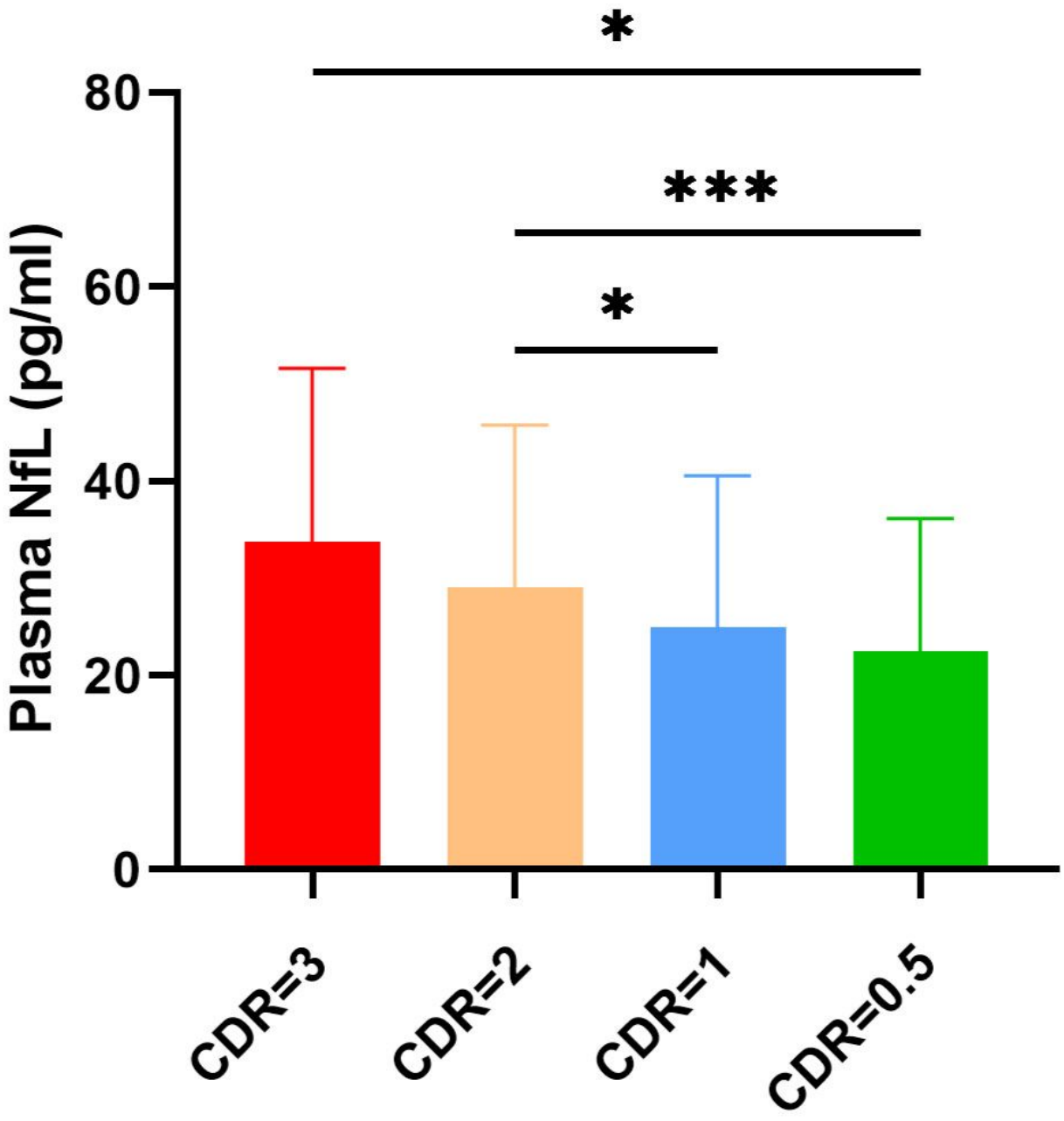

Figure 2

Correlations between disease severity and plasma biomarkers. According to the CDR score classification (CDR = 0.5, 1, 2, or 3), AD patients were divided into 4 groups for subgroup analysis. The Kruskal-Wallis test was used to analyze the difference between groups, and Bonferroni correction was used to correct the P value. CDR, Clinical Dementia Rating scale; AD, Alzheimer's disease; NfL, neurofilament light chain. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

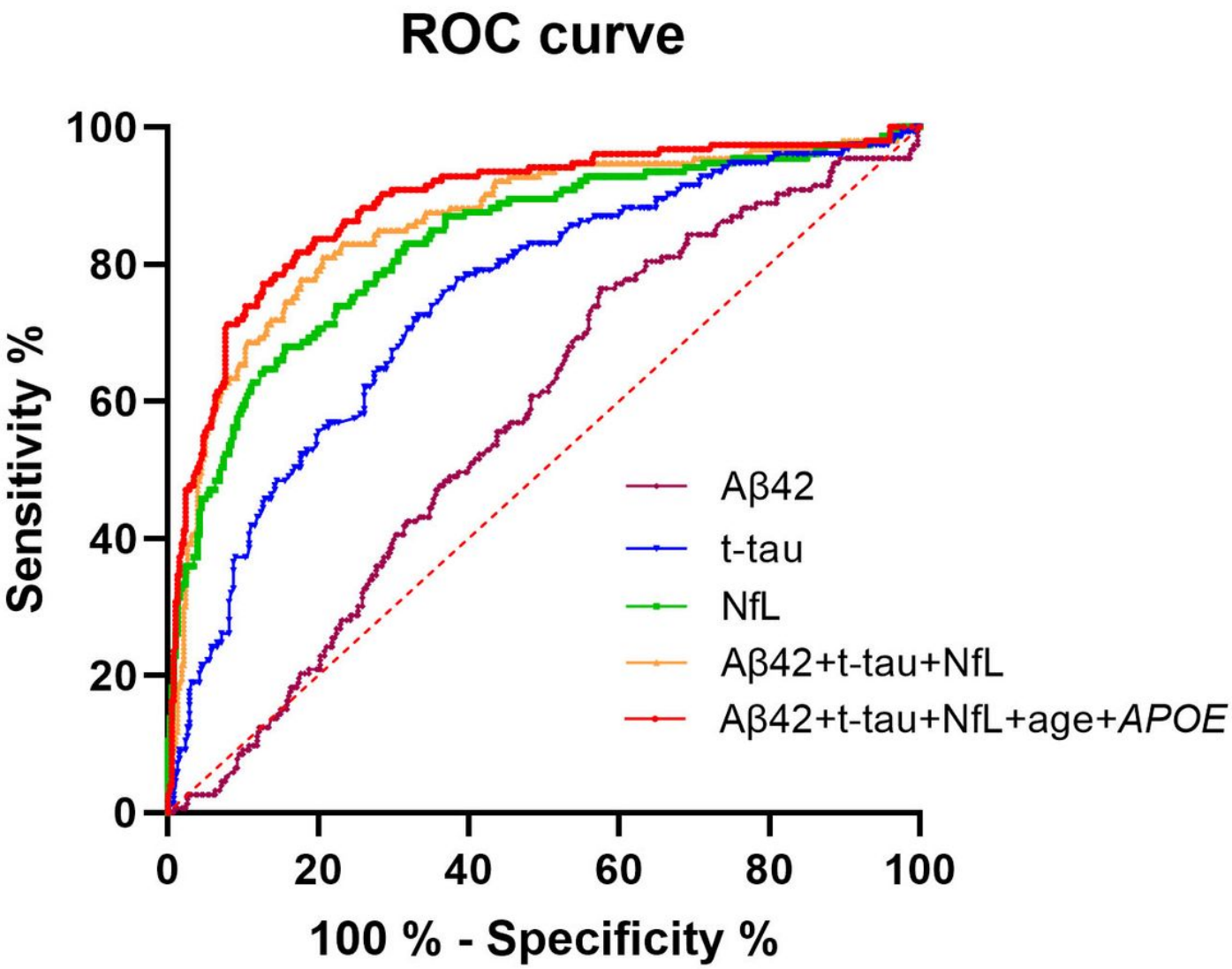

Figure 3

ROC curve analysis of plasma biomarkers for AD diagnosis. NfL levels in plasma had the best diagnostic efficacy as a single indicator (AUC=0.83, sensitivity=84.43%, specificity=67.97%, p<0.001). Aβ42, t-tau and NfL levels in plasma were combined, and the performance was significantly improved (AUC= 0.86, sensitivity=79.41%, specificity=81.05%). After adding age and APOE genotypes into the combined model, including Aβ42, t-tau and NfL levels, the maximum diagnostic efficacy was achieved compared with the diagnostic accuracy of other diagnostic models (AUC = 0.88, sensitivity = 82.84%, specificity = 81.69%, p < 0.05). A Delong test was used. AUC, area under the curve; ROC, receiver operating characteristic curve; NfL, neurofilament light chain; t-tau, total tau.