Plasma P-tau181 to Aβ42 ratio is associated with brain amyloid burden and hippocampal atrophy in an Asian cohort of Alzheimer's disease patients with concomitant cerebrovascular disease

Joyce R. Chong Nicholas J. Ashton Thomas K. Karikari Tomotaka Tanaka Francis N. Saridin Anthonin Reilhac Edward G. Robins Ying-Hwey Nai Henri Vrooman Saima Hilal Henrik Zetterberg Kaj Blennow Mitchell K.P. Lai Christopher P. Chen

Abstract

Introduction

There is increasing evidence that phosphorylated tau (P-tau181) is a specific biomarker for Alzheimer's disease (AD) pathology, but its potential utility in non-White patient cohorts and patients with concomitant cerebrovascular disease (CeVD) is unknown.

Methods

Single molecule array (Simoa) measurements of plasma P-tau181, total tau, amyloid beta (Aβ)40 and Aβ42, as well as derived ratios were correlated with neuroimaging modalities indicating brain amyloid (Aβ+), hippocampal atrophy, and CeVD in a Singapore-based cohort of non-cognitively impaired (NCI; n = 43), cognitively impaired no dementia (CIND; n = 91), AD (n = 44), and vascular dementia (VaD; n = 22) subjects.

Results

P-tau181/Aβ42 ratio showed the highest area under the curve (AUC) for Aβ+ (AUC = 0.889) and for discriminating between AD Aβ+ and VaD Aβ− subjects (AUC = 0.903). In addition, P-tau181/Aβ42 ratio was associated with hippocampal atrophy. None of the biomarkers was associated with CeVD.

Discussion

Plasma P-tau181/Aβ42 ratio may be a noninvasive means of identifying AD with elevated brain amyloid in populations with concomitant CeVD.

1 INTRODUCTION

The neuropathological hallmarks of Alzheimer's disease (AD) include the deposition of extracellular amyloid plaques composed of highly aggregated, fibrillar 40- to 42-amino-acid amyloid beta peptides (Aβ40 and Aβ42), as well as intracellular neurofibrillary tangles (NFTs) consisting of paired-helical filaments of hyper-phosphorylated tau protein.[1, 2] After AD, vascular dementia (VaD) has been reported to be the next most common form of dementia in elderly populations.[3] In VaD, there is a temporal relationship between the presence of cerebrovascular disease (CeVD), as evidenced by structural magnetic resonance imaging (MRI) findings, and onset of cognitive impairment.[3] Of interest, MRI markers of CeVD are also frequently observed in AD brains.[4, 5] The presence of vascular lesions and associated disruption of blood-brain barrier may interact with AD pathophysiological processes in an additive or synergistic manner to exacerbate cognitive decline.[3, 5-9] It is important to note that the prevalence of concomitant AD and CeVD may be higher in specific geographic regions, such as Asia, with consequent implications for prevention and treatment strategies.[10] Although current imaging (Aβ-PET [positron emission tomography] radiotracers) and cerebrospinal fluid (CSF; Aβ42 or Aβ42/Aβ40 ratio) biomarkers demonstrated high diagnostic performance, barriers to clinical implementation remain, including high costs, limited accessibility to PET scanners and cyclotrons, as well as the invasiveness of the procedures.[11, 12] In response to this unmet clinical need, research efforts have focused on evaluating blood as an alternative source of viable biomarkers, with the recent use of immunoprecipitation-mass spectrometry, single molecule array (Simoa) immunoassay, and other high-sensitivity platforms showing particular promise. Several studies, including work from our group, have found that plasma P-tau181 strongly associates with PET Aβ load and differentiates AD from non-AD neurodegenerative diseases.[13-20] In contrast, decreases in plasma Aβ42 and Aβ42/Aβ40 ratio variably associated with PET Aβ,[21-25] whereas total tau measures were slightly increased in AD in some studies but not others.[26-28] However, most of the aforementioned biomarker studies were carried out in White populations in North America and Europe with relatively “pure” AD burden. Whether the postulated clinical utility of these blood biomarkers could be applied to non-White cohorts who simultaneously have significant baseline CeVD remains unclear. In this study, we measured plasma P-tau181, T-tau, Aβ40, and Aβ42 in a Singapore-based cohort of AD and vascular cognitive impairment (VCI) patients assessed with Aβ PET imaging as well as MRI measures of hippocampal atrophy and CeVD, and examined the diagnostic value of the plasma markers and their derived ratios.

2 MATERIALS AND METHODS

2.1 Study population

This study adopted a case-control design. Demographic data are presented in Table 1. Patients who were cognitively impaired, no dementia (CIND), AD, and VaD were recruited from National University Hospital (NUH). Control subjects were recruited from both the NUH memory clinic and the community, and are defined as having no cognitive impairment (NCI) based on objective neuropsychological assessments. Diagnosis of clinical dementia was based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) with further etiologic diagnoses following the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for AD, and the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l' Enseignement en Neurosciences (NINDS-AIREN) criteria for VaD. Diagnosis of CIND was determined by impairment in at least one cognitive domain on a locally validated, detailed

neuropsychological test battery,29 which did not meet the DSM-IV criteria for dementia (see Supplementary Data S1). Participants also underwent detailed medical histories, physical, clinical, neuroimaging, and cognitive assessments, including the Mini-Mental State Examination (MMSE).30 Apolipoprotein E genotyping for APOE ε4 carrier status was as described previously.31 Approval for the study was obtained from the Singapore National Healthcare Group Domain-Specific Review Board. Written informed consent was obtained for all participants prior to study commencement.

TABLE 1. Demographic and clinical characteristics of a Singapore-based study cohort

NCI	CIND	AD	VaD	P		
Maximum n	43	91	44	22		
Age, y, mean (SD)		74 (6)	76 (6)	77 (8)	75 (9)	.340
Female, n (%)	27 (63)	45 (50)	35 (80)	8 (36)	.001	
Education, y, mean (SD)		10 (5)	8 (5)	5 (5)*#, *#	5 (4)*	<.001
APOE ε4 carrier, n (%)		9 (21)	24 (26)	19 (43)	6 (27)	.112
MMSE	28 (2)	25 (5)¶	16 (5)¶†, ¶†	18 (7)¶†, ¶†	<.001	
Imaging						
PiB-PET SUVr	1.1 (0.2)	1.2 (0.4)	1.9 (0.6)¶†, ¶†		1.1 (0.3)‡	<.001
Positive Aβ PET read, n (%)		5 (12)	29 (32)	30 (68)	3 (14)	<.001
Hippocampus volume, mL, mean (SD)			7.2 (0.8)	6.3 (1.1)*	5.1 (0.9)*#	6.1 (0.8)*§, *§ <.001
Presence of ≥2 lacunes, n (%)		4 (9)	14 (15)	3 (7)	12 (55)	<.001
Presence of cortical infarct, n (%)			2 (5)	14 (15)	6 (14)	8 (36) .009
White matter hyperintensities volume, mL			1.4 (4.2)	3.6 (10.7)¶	5.3 (10.6)¶	14.6 (18.1)¶ <.001
Plasma biomarkers						
P-tau181, pg/mL		9.6 (6.1)	12.3 (8.4)¶	16.9 (11.4)¶	12.0 (7.9)	<.001
T-tau, pg/mL	3.7 (1.4)	3.2 (1.8)	3.6 (2.4)	4.3 (1.8)†	.041	
P-tau181/T-tau ratio		2.8 (1.8)	3.6 (3.1)¶	4.8 (3.4)¶	2.7 (1.8)	<.001
Aβ42, pg/mL	12.9 (4.5)	12.1 (5.5)	12.7 (7.3)	15.4 (6.2)†	.021	
Aβ40, pg/mL	285 (79)	313 (97)	309 (92)	372 (123)¶†‡-	¶†‡	.001
Aβ42/Aβ40 ratio		0.046 (0.01)	0.039 (0.01)¶	0.040 (0.01)¶	0.041 (0.007)¶ <.001	
P-tau181/Aβ42 ratio		0.75 (0.5)	1.08 (0.9)¶	1.36 (1.4)¶	0.77 (0.6)	<.001

All data are median (interquartile range) unless otherwise specified. SD, standard deviation. P-values are derived from Chi-square tests for categorial variables, and from one-way ANOVA or Kruskal-Wallis ANOVA for normally distributed or skewed continuous variables, respectively. Amyloid PET status was based on visual read of [11C]PiB (n = 170) or [18F]Flutafuranol (n = 30) imaging. Among subjects who underwent PiB-PET imaging, SUVr data were not available for one participant. Hippocampal volumetry was not available for 31 participants. White matter hyperintensity data were not available for two participants. Plasma P-tau181 was not available for six participants. Plasma T-tau, Aβ42, Aβ40, and Aβ42/Aβ40 ratio were not available for three participants. Plasma P-tau181/T-tau and P-tau181/Aβ42 ratios were not available for nine participants. NCI indicates no cognitive impairment; CIND, cognitive impairment no dementia; AD, Alzheimer's disease; VaD, vascular dementia.

*Significantly different from NCI (one-way ANOVA with post hoc Bonferroni tests P < .05).

Significantly different from CIND (one-way ANOVA with post hoc Bonferroni tests P < .05).

§ Significantly different from AD (one-way ANOVA with post hoc Bonferroni tests P < .05).

¶ Significantly different from NCI (Kruskal-Wallis ANOVA with post hoc Dunn tests P < .05).

† Significantly different from CIND (Kruskal-Wallis ANOVA with post hoc Dunn tests P < .05).

‡ Significantly different from AD (Kruskal-Wallis ANOVA with post hoc Dunn tests P < .05).

2.2 Blood biomarker measurements

Non-fasting blood was drawn from study participants and processed for plasma extraction before storage at −80°C until use. All biomarkers were measured with evaluators blinded to clinical information at the Sahlgrenska Academy at University of Gothenburg in Sweden on the Simoa HD-1 or HD-X platforms (Quanterix, Billerica, MA, USA). Measurements of plasma P-tau181 with the AT270 mouse monoclonal antibody (MN1050; Invitrogen, Waltham, MA, USA) specific for the threonine-181 phosphorylation site was based on an ultrasensitive Simoa immunoassay as described previously-16 with satisfactory cross-site and test-retest reliabilities (Supplementary Data S2). Plasma Aβ42, Aβ40, and total tau (T-tau) were measured using the Neurology 3-plex A assay kit from Quanterix (Billerica, MA, USA). Plasma P-tau181 was not available for six participants, and plasma T-tau, Aβ42, and Aβ40 were not available for three participants due to limited sample availability.

RESEARCH IN CONTEXT

Systematic review: Alzheimer's disease (AD) is characterized neuropathologically by deposition of amyloid beta (Aβ40 and Aβ42)–containing plaques and neurofibrillary tangles consisting of hyperphosphorylated tau. Recent studies have demonstrated the potential utility of plasma phosphorylated tau (P-tau181) as a specific biomarker for AD pathology. However, these studies were conducted in White populations with relatively low cerebrovascular disease (CeVD) burden, and the postulated clinical utility of the biomarker in non-White cohorts with significant baseline CeVD remains unknown. Using a Singapore-based cohort of AD and vascular cognitive impairment (VCI) patients, we investigated associations between plasma biomarkers (P-tau181, total tau [T-tau], Aβ40, and Aβ42, as well as their derived ratios) and with PET Aβ burden, MRI measures of hippocampal atrophy and CeVD, and cognitive function.

Interpretation: In this study, we found that plasma P-tau181, P-tau181/T-tau, and P-tau181/Aβ42 ratios were significantly increased in AD, but not in vascular dementia (VaD). Furthermore, P-tau181 and P-tau181/Aβ42 ratio were increased in AD patients with elevated PET amyloid (Aβ+). Among the investigated biomarkers, plasma P-tau181/Aβ42 ratio showed the strongest associations with Aβ burden, brain atrophy, and cognitive scores, and was the best biomarker in identifying elevated Aβ PET and in differentiating between AD Aβ+ and VaD Aβ− subjects. None of the plasma biomarkers was associated with CeVD.

Future directions: The current findings suggest that the P-tau181/Aβ42 ratio may be a clinically useful, noninvasive means of identifying individuals with amyloid and brain atrophy in populations with high baseline CeVD. These findings warrant further validation in larger independent cohorts. The potential clinical applications of P-tau181/Aβ42 ratio for prognosis and longitudinal monitoring should also be examined in future studies.

2.3 Neuroimaging

2.3.1 Amyloid PET-MRI acquisition and quantification

Amyloid PET imaging was conducted at the Clinical Imaging Research Centre of the National University of Singapore (CIRC, NUS) using either the [11C]Pittsburgh Compound B (PiB) or [18F]Flutafuranol amyloid tracer radioligands. One hundred seventy subjects underwent a 30-minute brain PET scan on an mMR synchronous PET/MR scanner, 40 minutes after intravenous injection of 370 (+/−15%) MBq of [11C]PiB. In addition, 30 subjects underwent a 20-minute brain PET scan on an mCT PET-CT scanner (Siemens Healthcare GmbH), 50 minutes after intravenous injection of 185 MBq of [18F]Flutafuranol (range 166-203 MBq). Details on the synthesis of [18F]Flutafuranol are in Supplementary Data S3. All images were reconstructed using ordinary Poisson ordered–subsets expectation maximization with all corrections applied. Global standardized uptake ratio (SUVr) values were derived from the [11C]PiB scans and individually parcellated MRI for reference and target region definition and using an in-house developed automated pipeline.32

In addition, amyloid PET images were independently visually interpreted by three raters blinded to the clinical diagnosis of each subject and following the criteria described previously.33-35 Binary criteria were derived by merging the equivocal scans with the positive scans (Aβ+).34, 35

2.3.2 MRI markers of brain atrophy and CeVD

MRI scans were performed on a 3T Siemens Magnetom Trio Tim scanner using a 32-channel head coil, at CIRC, NUS. The sequences included T1-weighted, fluid-attenuated inversion recovery (FLAIR), and T2-weighted Imaging sequences as described previously36 (see Supplementary Data S4 for details).

Quantitative measurements

The image preprocessing and tissue classification algorithms used have been described elsewhere.37 Briefly, a k-nearest-neighbor classifier was used to classify voxels into CSF, gray matter, and normal-appearing white matter, and subsequently volume was calculated from these measurements for the detection of white matter hyperintensity (WMH) using an adapted threshold technique based on the tissue segmentation method.38 Intracranial volume (ICV) was calculated by a non-rigid registration technique, with six atlases (masks) registered to the subject of interest. The volume of the resulting mask is the ICV.

Hippocampal volume was segmented using a model-based automated procedure (FreeSurfer v5.1.0, Harvard University, Cambridge, MA, USA) on T1-weighted images. Segmentation was performed by rigid-body registration and nonlinear normalization of images to a probabilistic brain atlas. In the segmentation process, each voxel of the MRI volumes was labeled automatically as a corresponding brain region based on a parcellation guide.39

Visual gradings

Cortical infarcts were defined as focal lesions involving cortical gray matter, signal following CSF intensity, hyperintense rim on FLAIR images, and tissue loss of variable magnitude, with prominent adjacent sulci and ipsilateral ventricular enlargement. Lacunes were defined as lesions, 3-15 mm in diameter, with low signal on T1-weighted image and FLAIR; a high signal on T2 weighted image; and a hyperintense rim with a center following CSF intensity on FLAIR.

For binary logistic regression analyses, MRI markers of CeVD were transformed into binary variables and recorded as presence/absence of ≥2 lacunes and cortical infarct.

2.4 Statistical analyses

Statistical analyses were performed using SPSS version 26 (IBM SPSS, Armonk, NY, USA). For group comparisons of continuous variables, one-way analysis of variance (ANOVA) with Bonferroni post hoc tests were used for normally distributed data, whereas non-parametric Kruskal-WaIlis ANOVA with Dunn post hoc tests were used for skewed distributed data. Chi-square tests were used for categorical variables. All correlation analyses were performed using Spearman rank correlation. To assess the association between each plasma biomarker and the dichotomous neuroimaging variables, binary logistic regressions with odds ratios (ORs) and 95% confidence intervals (CIs) were computed. To identify the associations between each plasma biomarker and the continuous neuroimaging variables and MMSE, unadjusted and covariate-adjusted linear regression models were performed. The regression models were performed independently for each plasma biomarker. All biomarkers in the regression analyses were standardized to z scores to facilitate comparison between models. Multicollinearity of independent variables was measured with the variance inflation factor (VIF), with VIF >5 indicative of multicollinearity.40 Diagnostic accuracies were assessed using area under the receiver-operating characteristic curve (AUC). Sensitivity and specificity were determined using the Youden index. The AUCs were compared with each other using the DeLong test. Subsampling cross validation was used to evaluate the predictive value of the biomarkers' cut-offs for PET Aβ positivity on test cases. In this Monte Carlo process, 151 cases were

randomly selected as the training data set ("Training") and the cutoff, sensitivity, and specificity determined via ROC and Youden index analyses. The remaining unseen 40 cases ("Test") were classified as PET Aβ+ or Aβ− based on the computed cutoff, and the classification compared to their respective actual PET Aβ status to determine sensitivity and specificity. The average cutoff, sensitivity, and specificity, along with their standard deviations, were reported. P-values < .05 were considered statistically significant.

3 RESULTS

3.1 Participant characteristics

Demographic data, imaging, and plasma marker measurements are shown in Table 1. Cortical Aβ status was based on visual read of PET imaging using [11C]PiB (PiB-PET, n = 170) or [18F]Flutafuranol (FF-PET, n = 30). As expected, in the cohort of 170 subjects with PiB-PET imaging, the AD patients had the highest SUVr values among the diagnostic groups. For brain atrophy markers, CIND, AD, and VaD subjects showed significantly smaller hippocampal volumes compared with NCI (all P < .05). For CeVD markers, the VaD group had the highest proportion of subjects, with CeVD burden like lacunes (55%) and cortical infarcts (36%). Similarly, the VaD group had the highest WMH volumes (median = 14.6 mL), followed by AD, CIND, and NCI (median = 5.3 , 3.6, and 1.4 mL, respectively). Higher P-tau181, P-tau181/T-tau, and P-tau181/Aβ42 ratios (all P < .001), as well as lower Aβ42 and Aβ42/Aβ40 ratios (P = .013 and .030, respectively) were associated with APOE ε4 carrier status. For demographic factors, plasma T-tau and Aβ40 negatively correlated with years of education (r = -0.144, P = .043 and r = -0.149, P = .037, respectively), whereas none of the biomarkers was associated with gender (chi-square tests P > .05). For vascular risk factors, higher plasma Aβ42 and Aβ40 values were associated with presence of hypertension, hyperlipidemia, and diabetes (all P < .05; Supplementary Figure S1). There was no significant difference among the diagnostic groups in the frequency of vascular risk factors (Supplementary Table S1), suggesting relatively high baseline vascular disease burden in our cohort, in line with previous studies.10

3.2 Altered plasma biomarker levels in diagnostic groups

The results for diagnostic group comparisons of plasma biomarkers are shown in Table 1 and Supplementary Figure S2. All plasma biomarkers that included P-tau181 (P-tau181, P-tau181/T-tau, and P-tau181/Aβ42 ratios) were significantly raised in AD but not VaD. In contrast, plasma Aβ42/Aβ40 ratio was significantly decreased in the CIND, AD, and VaD groups. Aβ40 was significantly increased in VaD compared with NCI, CIND, and AD, whereas both T-tau and Aβ42 concentrations were unchanged.

3.3 Associations between plasma biomarkers, neuroimaging, and cognition

Correlations between individual plasma biomarkers, continuous neuroimaging variables, and MMSE scores are shown in Supplementary Table S2. All biomarkers, except T-tau and Aβ40, significantly correlated with brain amyloid burden. In contrast, only P-tau181, P-tau181/T-tau, Aβ42/Aβ40, and P-tau181/Aβ42 ratios correlated with hippocampal volume and MMSE. For WMH volume, only Aβ42 and Aβ40 showed a significant positive correlation.

3.3.1 Associations with brain amyloid burden

Associations between each plasma biomarker and brain Aβ burden are shown in Table 2. After adjustments for covariates, five of the biomarkers, namely P-tau181, P-tau181/T-tau ratio, Aβ42, and Aβ42/Aβ40 and P-tau181/Aβ42 ratios, showed significant associations with elevated brain Aβ (Aβ+, as determined by both PiB-PET and FF-PET visual reads); and PiB-PET SUVr (logistic regression for Aβ+; linear regression for PiB-PET SUVr, all P ≤ .003, Table 2). P-tau181/Aβ42 ratio had the strongest association with PiB-PET SUVr (β = .127). We further compared the five plasma biomarkers by clinical diagnoses and Aβ status (Figure 1). Within CIND and AD, P-tau181, P-tau181/T-tau, and P-tau181/Aβ42 ratios were significantly higher in the Aβ+ subjects compared with Aβ− subjects. In contrast, Aβ42/Aβ40 ratio was significantly lower in the Aβ+ subjects. Compared with the NCI Aβ− subjects, P-tau181, P-tau181/T-tau, and P-tau181/Aβ42 ratios were significantly higher in CIND Aβ+ and AD Aβ+ subjects, whereas Aβ42/Aβ40 ratio was significantly decreased in CIND Aβ−, CIND Aβ+, AD Aβ+, and VaD Aβ+ subjects (Figure 1).

TABLE 2. Associations between individual plasma biomarkers and brain Aβ burden

Dichotomous Outcome (PiB and [18F]Flutafuranol, maximum n = 197)				Continuous Outcome (PiB only, maximum n = 166)	
Model 1	Presence of elevated brain amyloid (Aβ+) odds ratio (OR)	95% CI	P		
	PiB-PET SUVr β coefficient	95% CI	P		
P-tau181	3.913	(2.457, 6.231)	<.001	0.168	(0.106,0.230) <.001
T-tau	0.960	(0.709, 1.298)	.789	0.012	(−0.059, 0.083) .735
P-tau181/T-tau ratio	2.669	(1.653, 4.310)	<.001	0.129	(0.065, 0.192) <.001
Aβ42	0.373	(0.242, 0.576)	<.001	-0.139	(−0.206, -0.072) <.001
Aβ40	0.785	(0.564, 1.092)	.151	-0.063	(−0.129, 0.003) .061
Aβ42/Aβ40 ratio	0.198	(0.111, 0.354)	<.001	-0.132	(−0.193, -0.071) <.001
P-tau181/Aβ42 ratio	8.552	(4.119, 17.756)		<.001	0.181 (0.123, 0.240) <.001

Dichotomous Outcome (PiB and [18F]Flutafuranol, maximum n = 197)				Continuous Outcome (PiB only, maximum n = 166)	
Model 2	Presence of elevated brain amyloid (Aβ+) odds ratio (OR)	95% CI	PValue		
	PiB-PET SUVrβ coefficient	95% CI	Pvalue		
P-tau181	3.964	(2.390, 6.576)	<.001	0.139	(0.081, 0.197) <.001
T-tau	0.924	(0.663, 1.289)	.643	0.004	(−0.062, 0.069) .907

P-tau181/T-tau ratio	2.847	(1.708, 4.746)	<.001	0.121	(0.063, 0.179)	<.001
Aβ42	0.354	(0.220, 0.572)	<.001	-0.115	(−0.179, -0.052)	<.001
Aβ40	0.787	(0.539, 1.151)	.217	-0.048	(−0.110, 0.014)	.126
Aβ42/Aβ40 ratio	0.179	(0.094, 0.341)	<.001	-0.110	(−0.168, -0.053)	<.001
P-tau181/Aβ42 ratio	8.914	(4.093, 19.412)	<.001	0.156	(0.100, 0.211)	<.001

	Dichotomous Outcome (PiB and [18F]Flutafuranol, maximum n = 197)			Continuous Outcome (PiB only, maximum n = 166)		
Model 3	Presence of elevated brain amyloid (Aβ+) odds ratio (OR)	95% CI	Pvalue	PiB-PET SUVrβ coefficient	95% CI	Pvalue
P-tau181	3.456	(2.032, 5.876)	<.001	0.116	(0.058, 0.174)	<.001
T-tau	0.903	(0.629, 1.298)	.583	0.024	(−0.040, 0.088)	.464
P-tau181/T-tau ratio	2.587	(1.583, 4.228)	<.001	0.098	(0.041, 0.155)	.001
Aβ42	0.343	(0.206, 0.572)	<.001	-0.096	(−0.159, -0.033)	.003
Aβ40	0.731	(0.484, 1.104)	.137	-0.033	(−0.096, 0.030)	.305
Aβ42/Aβ40 ratio	0.142	(0.068, 0.300)	<.001	-0.108	(−0.167, -0.048)	<.001
P-tau181/Aβ42 ratio	8.099	(3.569, 18.380)	<.001	0.127	(0.070, 0.183)	<.001

	VIF (lowest, highest)
P-tau181	(1.06, 2.15)
T-tau	(1.04, 2.03)
P-tau181/T-tau ratio	(1.06, 2.05)
Aβ42	(1.05, 2.05)
Aβ40	(1.05, 2.08)
Aβ42/Aβ40 ratio	(1.05, 2.37)
P-tau181/Aβ42 ratio	(1.06, 2.10)

Results from binary logistic regression for dichotomous outcome and linear regression for continuous outcome. The linear regression models were independently performed for each plasma biomarker and adjusted for covariates as stated below. All plasma biomarkers were standardized to z scores to facilitate comparisons between models. Therefore, β coefficients refer to standardized effects (β = 1 implies that 1-SD increase in the plasma biomarker was associated with 1 unit increase in PiB-PET SUVr).

For Model 3, the range of VIF (variance inflation factor) for the independent variables were given, with the lowest and highest VIF values listed as shown in the rightmost column. Indicated VIF of < 5 denotes absence of collinearity of independent variables.

OR = odds ratio.

Model 1: No adjustment.

Model 2: Adjusted for age, gender, education, and APOE ε4 genotype.

Model 3: Adjusted for age, gender, education, APOE ε4 genotype, and diagnosis.

Plasma biomarkers by clinical diagnosis and Aβ status. (a) to (e) Plasma levels of individual biomarker across diagnostic groups, stratified by presence (Aβ+) or absence (Aβ−) of elevated Aβ, as determined by visual read of Aβ-PET. In box-and-whisker plots the central horizontal bar shows the median, and the lower and upper boundaries show the 25th and 75th percentiles, respectively. P-values derived from Kruskal-Wallis test followed by post hoc Dunn test. *P < .05; **P < .01; ***P < .001

3.3.2 Associations with hippocampal atrophy

Associations between each plasma biomarker and hippocampal volume are shown in Table 3. Only the three biomarkers that included P-tau181 (P-tau181, P-tau181/T-tau, and P-tau181/Aβ42 ratios) were negatively associated with hippocampal volume (β = −0.275, −0.249, and −0.305, respectively, all P < .001). Similar to the Aβ-PET findings, P-tau181/Aβ42 ratio was also found to be the strongest influence on hippocampal volume.

TABLE 3. Associations between individual plasma biomarkers and hippocampal atrophy

Hippocampal Volume (Maximum n = 166)

Model 1	β coefficient	955 CI	P-value	
P-tau181	-0.472	(−0.636, -0.307)	<.001	
T-tau	-0.018	(−0.210, 0.174)	.852	
P-tau181/T-tau ratio	-0.349	(−0.517, -0.181)		<.001
Aβ42	0.173	(−0.016, 0.363)	.073	
Aβ40	0.002	(−0.180, 0.183)	.987	
Aβ42/Aβ40 ratio	0.309	(0.140, 0.478)	<.001	
P-tau181/Aβ42 ratio	-0.461	(−0.617, -0.305)		<.001

Hippocampal Volume (Maximum n = 166)

Model 2	β coefficient	95% CI	P-value	
P-tau181	-0.392	(−0.545, -0.240)	<.001	
T-tau	0.028	(−0.146, 0.202)	.751	
P-tau181/T-tau ratio	-0.324	(−0.477, -0.172)		<.001
Aβ42	0.144	(−0.029, 0.318)	.103	
Aβ40	0.010	(−0.156, 0.176)	.906	
Aβ42/Aβ40 ratio	0.248	(0.095, 0.402)	.002	

P-tau181/Aβ42 ratio	-0.422	(−0.568, -0.277)		<.001	
Hippocampal Volume (Maximum n = 166)					
Model 3	β coefficient	95% CI	P-value	VIF(lowest, highest)	
P-tau181	-0.275	(−0.413, -0.136)		<.001	(1.07, 2.16)
T-tau	0.025	(−0.133, 0.182)		.759	(1.04, 2.06)
P-tau181/T-tau ratio	-0.249	(−0.384, -0.113)		<.001	(1.06, 2.07)
Aβ42	0.108	(−0.049, 0.265)		.177	(1.05, 2.07)
Aβ40	0.045	(−0.110, 0.199)		.568	(1.05, 2.11)
Aβ42/Aβ40 ratio	0.120	(−0.029, 0.270)		.115	(1.05, 2.41)
P-tau181/Aβ42 ratio	-0.305	(−0.439, -0.170)		<.001	(1.07, 2.11)

Results from linear regression models. The linear regression models were independently performed for each plasma biomarker and adjusted for covariates as stated below. All plasma biomarkers were standardized to z scores to facilitate comparisons between models. Therefore, β coefficients refer to standardized effects (β = 1 implies that 1-SD increase in the plasma biomarker was associated with 1 mL increase in hippocampal volume).

For Model 3, the range of VIF (variance inflation factor) for the independent variables were given, with the lowest and highest VIF values listed as shown in the rightmost column. Indicated VIF of < 5 denotes absence of collinearity of independent variables.

3.3.3 Associations with cognition

Associations between each plasma biomarker and MMSE are shown in Table 4. After adjustment for covariates, only P-tau181/Aβ42 ratio was significantly associated with MMSE (β = −0.556, P < .05), whereas P-tau181 itself showed a non-significant trend (β = −0.518, P = .065). For the neuropsychological cognitive domains, the associations between each plasma biomarker and global cognition or individual cognitive domain are listed in Supplementary Data S1.

TABLE 4. Associations between individual plasma biomarkers and cognition

MMSE (Maximum n = 197)				
Model 1	β coefficient	95% CI	P-value	
P-tau181	-1.743	(−2.566, -0.920)	<.001	
T-tau	-1.046	(−1.864, -0.228)	.013	
P-tau181/T-tau ratio	-0.531	(−1.378, 0.317)		.218
Aβ42	-0.337	(−1.166, 0.493)	.425	
Aβ40	-0.784	(−1.608, 0.040)	.062	

Aβ42/Aβ40 ratio		0.927	(0.106, 1.747)	.027	
P-tau181/Aβ42 ratio		-1.468	(−2.292, -0.643)	.001	
MMSE (Maximum n = 197)					
Model 2	β coefficient	95% CI	P-value		
P-tau181	-1.394	(−2.189, -0.599)	.001		
T-tau	-0.784	(−1.574, 0.006)	.052		
P-tau181/T-tau ratio		-0.463	(−1.272, 0.345)	.260	
Aβ42	-0.313	(−1.115, 0.488)	.442		
Aβ40	-0.668	(−1.466, 0.130)	.101		
Aβ42/Aβ40 ratio		0.775	(−0.009, 1.560)	.053	
P-tau181/Aβ42 ratio		-1.275	(−2.072, -0.478)	.002	
MMSE (Maximum n = 197)					
Model 3	β coefficient	95% CI	P-value	VIF (lowest, highest)	
P-tau181	-0.518	(−1.069, 0.033)	.065	(1.04, 1.99)	
T-tau	-0.198	(−0.729, 0.333)	.463	(1.04, 1.79)	
P-tau181/T-tau ratio		-0.233	(−0.772, 0.305)	.393	(1.04, 1.82)
Aβ42	0.175	(−0.366, 0.715)	.525	(1.06, 1.79)	
Aβ40	0.129	(−0.422, 0.680)	.645	(1.05, 1.80)	
Aβ42/Aβ40 ratio		0.104	(−0.455, 0.664)	.713	(1.04, 2.07)
P-tau181/Aβ42 ratio		-0.556	(−1.108, -0.005)	.048	(1.04, 1.92)

Results from linear regression models. The linear regression models were independently performed for each plasma biomarker and adjusted for covariates as stated below. All plasma biomarkers were standardized to z scores to facilitate comparisons between models. Therefore, β coefficients refer to standardized effects (β = 1 implies that 1-SD increase in the plasma biomarker was associated with 1 unit increase in MMSE).

For Model 3, the range of VIF (variance inflation factor) for the independent variables were given, with the lowest and highest VIF values listed as shown in the rightmost column. Indicated VIF of < 5 denotes absence of collinearity of independent variables.

MMSE = Mini-Mental State Examination.

3.3.4 Association with CeVD

Associations between each plasma biomarker and markers of CeVD are presented in Supplementary Table S3 and Supplementary Figure S3. Although higher plasma Aβ40 was significantly associated with the presence of lacunes (Supplementary Table S3a and Supplementary Figure S3a), the

significance was lost after adjustment for covariates (Supplementary Table S3a). None of the other biomarkers was associated with lacunes, cortical infarcts, or WMH.

3.4 Plasma P-tau181 to Aß42 ratio as a biomarker for elevated brain amyloid

Given the associations between elevated brain Aβ-PET (Aβ+) and plasma P-tau181, P-tau181/T-tau ratio, Aβ42, Aβ42/Aβ40, and P-tau181/Aβ42 ratios (Table 2), we next assessed the utility of these biomarkers in distinguishing between Aβ+ and Aβ− status (Figure 2). In the combined cohort (Figure 2a), the non-dementia (NCI and CIND; Figure 2b) as well as the dementia (AD and VaD; Figure 2c) subgroups, the aforementioned biomarkers showed variable ability in distinguishing Aβ+ from Aβ− subjects (AUC = 0.701-0.932). However, P-tau181/Aβ42 ratio was consistently the best predictor of increased Aβ-PET in the combined cohort and the two subgroups (Delong $P < .05$; Figure 2a). AUC of P-tau181/Aβ42 ratio was also significantly higher than those of all other markers except Aβ42/Aβ40 ratio in the non-dementia subgroup (Figure 2b), and P-tau181 in the dementia subgroup (Figure 2c). For differentiation between AD Aβ+ and VaD Aβ− subjects, P-tau181/Aβ42 ratio showed the highest AUC of 0.903, with a high sensitivity and specificity of 92.6% and 88.9%, respectively (Supplementary Table S4).

ROC analyses for distinguishing PET Aβ+ and Aβ- subjects. (A) All subjects (n = 191); (B) Non-dementia subjects only (n = 130); (C) Dementia subjects only (n = 61). Sensitivity and specificity were calculated using the cutoff that produced the highest Youden index (sensitivity + specificity −1). P-values are from the comparison of AUCs (DeLong statistics). AUC = Area under the receiver-operating characteristic (ROC) curve. NA = Not applicable

Finally, we assessed the predictive value of the biomarkers cutoffs for PET Aβ positivity on test cases. As observed in Supplementary Table S5, for P-tau181/Aβ42 ratio and P-tau181, the average cutoff, AUC, sensitivity, and specificity derived from Training or Test were consistent with values obtained for the combined cohort. This suggests the generalizability of their respective cutoff as computed from the combined cohort to test cases. In contrast, there was an adjustment in the cutoffs and decreased specificity in the Training or Test for the other biomarkers.

4 DISCUSSION

In most clinical settings, AD is conceived as a clinical-pathological construct, such that cognitive symptoms define the presence of AD.11 However, the National Institute on Aging–Alzheimer's Association (NIA-AA) research framework has recently suggested that AD should be defined as a biological construct, using biomarkers that are characteristic of AD pathophysiology, such as Aβ and pathologic tau.41, 42 The pathophysiological relevance of Aβ biomarkers for AD is suggested by findings from post-mortem and in vitro studies showing the Aβ42/Aβ40 ratio as an index of increased amyloidogenicity, neurotoxicity, and disease severity,43-45 whereas P-tau181 is one of the sites contributing directly to tau hyperphosphorylation and NFT formation in the brain.46, 47 In addition, hyperphosphorylated tau has been implicated as a principal instigator of degenerative axonal loss in AD.5 Notwithstanding the close mechanistic links between amyloidogenesis and tauopathy,48, 49 Aβ42 and P-tau181 represent distinct pathophysiological processes. Therefore, the present study had a twofold aim: to compare the utility of combined P-tau and Aβ42 with individual markers, and to investigate potential confounding by higher baseline of concomitant CeVD, a known characteristic of

certain populations in Asia, including ours.10. To the best of our knowledge, this is the first study on blood P-tau181 in non-White populations outside of Europe and North America, and the findings support the potential clinical utility of plasma P-tau181/Aβ42 ratio in a number of ways as detailed below.

First, in agreement with previous CSF studies, we showed that plasma P-tau181 concentrations were specifically increased in AD, but not VaD. In addition, P-tau181 was increased in Aβ+ subjects in the CIND and AD groups. We further demonstrated the utility of this biomarker in discriminating PET Aβ+ from Aβ− subjects (AUC = 0.840 for all subjects). Our results are corroborated by recent studies that reported higher plasma P-tau181 in Aβ+ NCI, mild cognitive impairment (MCI), and AD subjects, but not in non-AD neurodegenerative diseases.14, 16, 17 Nonetheless, a direct comparison of the diagnostic performance of CSF versus plasma P-tau181/Aβ42 ratios will be needed to comprehensively evaluate the clinical utility of the plasma biomarkers.

Next, we compared the single biomarkers with their derived ratios in detecting PET Aβ+. Of the five biomarkers studied (P-tau181, P-tau181/T-tau ratio, Aβ42, and Aβ42/Aβ40 and P-tau181/Aβ42 ratios), P-tau181/Aβ42 ratio showed the strongest association with Aβ PET SUVr. In addition, P-tau181/Aβ42 ratio consistently gave the highest AUC for detecting PET Aβ+ in the entire cohort and in subgroups of non-dementia and dementia subjects, and in distinguishing between AD Aβ+ and VaD Aβ− subjects. In contrast, the other biomarkers yielded variable AUCs depending on the clinical subgroup. Of interest, our data suggest that the P-tau181/Aβ42 ratio may be useful in enriching recruitment of NCI and CIND participants who are likely to be Aβ+ on PET (Figure 2b and Supplementary Table S4d) into clinical trials, thus enhancing validity and potential success. Furthermore, Aβ42/Aβ40 ratio yielded a higher AUC than P-tau181 alone (AUC = 0.848 vs 0.807) in the non-dementia subgroup, suggesting that plasma Aβ42/Aβ40 ratio, putatively reflecting Aβ pathology, may become abnormal earlier during the course of AD than P-tau, in line with current understanding.50 Although further studies in larger cohorts are warranted to assess the diagnostic performance of these plasma biomarkers across the AD continuum, our findings on the generalizability of the P-tau181/Aβ42 ratio cutoff to cases (Supplementary Table S5) provides some reassurance on the validity of this biomarker.

The superiority of P-tau181/Aβ42 ratio over other single biomarkers and ratios may be due to a number of reasons. First, the P-tau181/Aβ42 ratio combines measures of two different pathophysiological processes into a single variable, thus improving its ability to distinguish AD from controls and VCI. Second, given that P-tau181 was elevated in Aβ+ subjects while Aβ42 was decreased, a ratio of the two biomarkers may accentuate differences between the Aβ+ and Aβ− subjects. Third, as mentioned earlier, plasma P-tau and Aβ42 may change at different stages in the disease. Therefore, a combination of both Aβ42 and P-tau may correspond better to Aβ PET burden in all stages of the AD continuum.

Besides Aβ-PET, we reported the association between the biomarkers and other neuroimaging hallmarks of dementia, such as neurodegeneration and CeVD. A recent study on a separate cohort by our team reported significant associations between plasma P-tau181 and hippocampal volume and

MMSE.16 We now show that the P-tau181/Aβ42 ratio demonstrated stronger associations with lower hippocampal volume and poorer cognitive performance compared to P-tau181 alone. Furthermore, after adjustment for covariates, only plasma P-tau181, P-tau181/Aβ42 ratio, and Aβ40 were significantly associated with global cognition. For the individual cognitive domains, whereas plasma P-tau181 was associated with both non-memory (attention, language, visuoconstruction) and memory (verbal memory, visual memory) domains, plasma P-tau181/Aβ42 ratio was associated primarily with the memory domains. This suggests that P-tau181/Aβ42 ratio is a marker of memory function. On the other hand, plasma Aβ40 was associated with visuoconstruction and visual memory (see Supplementary Data S1).

In contrast to markers of brain amyloid burden and neurodegeneration, the plasma biomarkers were generally not associated with markers of CeVD. Whereas higher plasma Aβ40 was associated with the presence of lacunes (Supplementary Table S3a and Supplementary Figure S3a), its statistical significance was lost after adjustment for covariates (Supplementary Table S3a). The putative associations between plasma Aβ40 and WMH51 also remain unclear, as they were not neither observed by us or by Toledo et al.52

Our study's strengths include the use of comprehensive neuropsychological assessments to properly diagnose cognitive impairment and dementia, as well as the availability of both Aβ-PET and MRI data for hippocampal atrophy as well as for a range of CeVD. Furthermore, we have considered possible confounding effects of demographic characteristics and vascular risk factors (Supplementary Table S1) that may affect the results. We also used a state-of-the-art, ultrasensitive Simoa immunoassay platform, with all P-tau181 measurements above the limit of detection, thus offering an advantage over Meso Scale Discovery (MSD) platforms.14 However, several limitations are also apparent. First, we do not yet have tau-PET, and therefore could not determine the association between the investigated plasma biomarkers and cortical tau pathology (eg, NFTs) in our cohort. This would be of interest in follow-up studies in view of work by others reporting significant association between plasma P-tau181 and tau-PET.14, 16, 17 Furthermore, the cross-sectional design of this study does not allow the examination of the temporal association between the plasma biomarkers and the progression of brain Aβ accumulation and atrophy, and cognitive impairment, necessitating follow-up studies using longitudinal data.

Finally, with the rapid changing landscape of biomarkers, new P-tau candidates continue to be introduced, including P-tau217, and it remains unclear which P-tau species has long-term clinical utility. Of interest, a study examining P-tau217 in a familial AD (FAD) cohort found significant increases as early as 20 years before the expected year of onset (EYO) of symptoms,53 whereas another study found significant increases in P-tau181 around 16 years before EYO in FAD mutation carriers.54 More recently, head-to-head comparisons of P-tau181 and P-tau217 (together with another candidate, P-tau231) in CSF showed similar performance.55, 56 These findings support the idea that both these plasma P-tau biomarkers reflect AD pathology. However, further studies directly comparing P-tau181 and P-tau217 in plasma using the same assay technology and in the same cohort, are needed to learn whether these biomarkers substantially differ in diagnostic utility in the different phases of the AD continuum.

In conclusion, we showed that plasma P-tau181 and Aβ measurements in an Asian population are broadly in line with findings in Caucasian populations showing clinical utility in detecting cortical amyloid burden and AD (see Introduction). Furthermore, plasma P-tau181 and Aβ42 were unaffected by CeVD status, thereby extending their potential clinical utility to populations with higher baseline vascular risk factors and CeVD burden. Finally, plasma P-tau181/Aβ42 ratio may have superior utility compared to single biomarkers in differentiating Aβ+ AD from Aβ− VaD subjects.

ACKNOWLEDGEMENTS

This study was supported by the National Medical Research Council (NMRC/CSA-SI/007/2016 to CPC and MKPL; and NMRC/CG/M006/2017, NMRC/CG/013/2013, and NMRC/CG/NUHS/2010 to CPC). NJA is supported by the Swedish Alzheimer Foundation (Alzheimerfonden), the Swedish Brain Foundation (Hjärnfonden), the Swedish Dementia Foundation (Demensförbundet), and Gamla Tjänarinnor Foundation. TKK holds a research fellowship from the BrightFocus Foundation (#A2020812F) and is further supported by the Swedish Alzheimer Foundation (Alzheimerfonden; #AF-930627), the Swedish Brain Foundation (Hjärnfonden; #FO2020-0240), the Swedish Dementia Foundation (Demensförbundet), the Swedish Parkinson Foundation (Parkinsonfonden; #1252/20), Gamla Tjänarinnor Foundation, the Aina (Ann) Wallströms and Mary-Ann Sjöbloms Foundation, the Agneta Prytz-Folkes & Gösta Folkes Foundation (#2020-00124), the Gun and Bertil Stohnes Foundation, and the Anna Lisa and Brother Björnsson's Foundation. HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL. KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), and the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236). The authors would like to thank the patients and their families for their participation in this study. We would also like to thank the NUS CIRC radiochemistry and QA team for the production and supply of radioligands, and specifically Dr Akbar Kulasi (quality assurance), David James Green (radioisotope production/cyclotron), Elaine Jia Hui Tan (quality control), and Mohd Fadli Bin Mohammad Noh (manufacturing) for contributing to the different aspects of the synthesis of [18F]Flutafuranol.

CONFLICTS OF INTEREST

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, and CogRx; has given lectures in symposia sponsored by Fujirebio, Alzecure, and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, on advisory boards, or on data-monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. The other authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

All authors have made a substantial intellectual contribution to the conception and design of the study (H.Z., K.B., M.K.P.L., C.P.C.), acquisition of data (J.R.C., N.J.A., T.K.K., T.T., F.N.S., A.R., E.G.R., Y.H.N., H.V., S.H.), analysis and interpretation of data (J.R.C., N.J.A., T.K.K., T.T., F.N.S., A.R., H.V., S.H., H.Z., K.B., C.P.C.), drafting the manuscript (J.R.C., M.K.P.L.), or drafting a significant portion of the manuscript or figures (T.T., F.N.S., A.R., H.V., S.H., C.P.C.). All authors approved the final version of the manuscript for publication. M.K.P.L. and C.P.C. had full access to the data in the study and take responsibility for data integrity and accuracy of data analysis.

REFERENCES

1 2015 Alzheimer's disease facts and figures. Alzheimer's Dement: J Alzheimer's Assoc. 2015; 11: 332-384.

Google Scholar

2Molinuevo JL, Ayton S, Batrla R, et al. Current state of Alzheimer's fluid biomarkers. Acta neuropathologica. 2018; 136: 821- 853.

Google Scholar

3O'Brien JT, Thomas A. Vascular dementia. The Lancet. 2015; 386: 1698- 1706.

Google Scholar

4Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. J Neural Transm (Vienna). 2002; 109: 813- 836.

Google Scholar

5McAleese KE, Alafuzoff I, Charidimou A, et al. Post-mortem assessment in vascular dementia: advances and aspirations. BMC Med. 2016; 14: 129.

Google Scholar

6Pantoni L, Poggesi A, Inzitari D. Cognitive decline and dementia related to cerebrovascular diseases: some evidence and concepts. Cerebrovasc Dis. 2009; 27(Suppl 1): 191- 196.

Google Scholar

7Lee CW, Shih YH, Kuo YM. Cerebrovascular pathology and amyloid plaque formation in Alzheimer's disease. Current Alzheimer research. 2014; 11: 4- 10.

Google Scholar

8Sweeney MD, Sagare AP, Pachicano M, et al. A novel sensitive assay for detection of a biomarker of pericyte injury in cerebrospinal fluid. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2020; 16: 821- 830.

Google Scholar

9Montagne A, Nation DA, Sagare AP, et al. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. Nature. 2020; 581: 71- 76.

Google Scholar

10Chen C, Homma A, Mok VC, et al. Alzheimer's disease with cerebrovascular disease: current status in the Asia-Pacific region. J Internal Med. 2016; 280: 359- 374.

Wiley Online Library Google Scholar

11Hampel H, O'Bryant SE, Molinuevo JL, et al. Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. Nature Rev Neurol. 2018; 14: 639- 652.

Google Scholar

12Ashton NJ, Scholl M, Heurling K, et al. Update on biomarkers for amyloid pathology in Alzheimer's disease. Biomark Med. 2018; 12: 799- 812.

Google Scholar

13Benussi A, Karikari TK, Ashton N, et al. Diagnostic and prognostic value of serum NfL and p-Tau(181) in frontotemporal lobar degeneration. J Neurol Neurosurg Psychiatry. 2020; 91: 960- 967.

Google Scholar

14Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. Nature Med. 2020; 26: 379- 386.

Google Scholar

15Karikari TK, Benedet AL, Ashton NJ, et al. Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's Disease Neuroimaging Initiative. Mol Psychiatry. 2020. https://doi.org/10.1038/s41380-020-00923-z. In Press.

Google Scholar

16Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. Lancet Neurol. 2020; 19: 422- 433.

Google Scholar

17Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. Alzheimer's & Dementia: The Journal of the Alzheimer's Association. 2018; 14: 989- 997.

Google Scholar

18Shekhar S, Kumar R, Rai N, et al. Estimation of Tau and phosphorylated Tau181 in serum of Alzheimer's disease and mild cognitive impairment patients. PLoS One. 2016; 11:e0159099.

Google Scholar

19Tatebe H, Kasai T, Ohmichi T, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. Molecular Neurodegeneration. 2017; 12: 63.

Google Scholar

20Yang CC, Chiu MJ, Chen TF, Chang HL, Liu BH, SY Yang. Assay of plasma phosphorylated Tau protein (Threonine 181) and total Tau protein in early-stage Alzheimer's disease. J Alzheimer's Dis : JAD. 2018; 61: 1323- 1332.

Google Scholar

21Ovod V, Ramsey KN, Mawuenyega KG, et al. Amyloid b concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimer's Dement: J Alzheimer's Assoc. 2017; 13: 841- 849.

Google Scholar

22Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-β biomarkers for Alzheimer's disease. Nature. 2018; 554: 249.

Google Scholar

23Kaneko N, Nakamura A, Washimi Y, et al. Novel plasma biomarker surrogating cerebral amyloid deposition. Proceed Japan Acad Series B, Physical Biological Sci. 2014; 90: 353- 364.

Google Scholar

24Verberk IMW, Slot RE, Verfaillie SCJ, et al. Plasma amyloid as prescreener for the earliest Alzheimer pathological changes. Ann Neurology. 2018; 84: 648- 658.

Google Scholar

25Janelidze S, Stomrud E, Palmqvist S, et al. Plasma β-amyloid in Alzheimer's disease and vascular disease. Scientific Reports. 2016; 6:26801.

Google Scholar

26Zetterberg H, Wilson D, Andreasson U, et al. Plasma tau levels in Alzheimer's disease. Alzheimer's Res Therapy. 2013; 5: 9.

Google Scholar

27Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016; 15: 673- 684.

Google Scholar

28Mattsson N, Zetterberg H, Janelidze S, et al. Plasma tau in Alzheimer disease. Neurology. 2016; 87: 1827- 1835.

Google Scholar

29Hilal S, Chai YL, Ikram MK, Elangovan S, Yeow TB, Xin X, et al. Markers of cardiac dysfunction in cognitive impairment and dementia. Medicine (Baltimore). 2015; 94: e297- e..

Google Scholar

30Folstein MF, Folstein SE, McHugh PR. Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975; 12: 189- 198.

Google Scholar

31Chai YL, Yeo H, Wang J, et al. Apolipoprotein ε4 is associated with dementia and cognitive impairment predominantly due to Alzheimer's disease and not with vascular cognitive impairment: a Singapore-based cohort. J Alzheimer's Dis : JAD. 2016: 51.

Google Scholar

32Tanaka T, Stephenson MC, Nai Y-H, et al. Improved quantification of amyloid burden and associated biomarker cut-off points: results from the first amyloid Singaporean cohort with overlapping cerebrovascular disease. Euro J Nuclear Med Mole Imaging. 2020; 47: 319- 331.

Google Scholar

33Ng S, Villemagne VL, Berlangieri S, et al. Visual assessment versus quantitative assessment of 11C-PIB PET and 18F-FDG PET for detection of Alzheimer's disease. J Nuclear Med : official publication, Society Nuclear Med. 2007; 48: 547- 552.

Google Scholar

34Yamane T, Ishii K, Sakata M, et al. Inter-rater variability of visual interpretation and comparison with quantitative evaluation of (11)C-PiB PET amyloid images of the Japanese Alzheimer's Disease Neuroimaging Initiative (J-ADNI) multicenter study. Euro J Nuclear Med Mole Imaging. 2017; 44: 850-857.

Google Scholar

35Tanaka T, Stephenson MC, Nai Y-H, et al. 2019; 47: 319- 31.

Google Scholar

36van Veluw SJ, Hilal S, Kuijf HJ, et al. Cortical microinfarcts on 3T MRI: clinical correlates in memory-clinic patients. Alzheimer's & dementia : the journal of the Alzheimer's. Association. 2015; 11: 1500-1509.

Google Scholar

37Vrooman HA, Cocosco CA, van der Lijn F, et al. Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. NeuroImage. 2007; 37: 71- 81.

Google Scholar

38de Boer R, Vrooman HA, Ikram MA, et al. Accuracy and reproducibility study of automatic MRI brain tissue segmentation methods. Neuroimage. 2010; 51: 1047- 1056.

Google Scholar

39Hilal S, Baaij LGA, de Groot M, et al. Prevalence and clinical relevance of diffusion-weighted imaging lesions: the Rotterdam study. Neurology. 2019; 93: e1058- e67.

Google Scholar

40Kim JH. Multicollinearity and misleading statistical results. Korean J Anesthesiol. 2019; 72: 558-569.

Google Scholar

41Bateman RJ, Blennow K, Doody R, et al. Plasma biomarkers of AD emerging as essential tools for drug development: an EU/US CTAD Task Force Report. J Prev Alzheimers Dis. 2019; 6: 169- 173.

Google Scholar

42Jack CR Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimer's Dement. 2018; 14: 535- 562.

Google Scholar

43Chen YR, Glabe CG. Distinct early folding and aggregation properties of Alzheimer amyloid-beta peptides Abeta40 and Abeta42: stable trimer or tetramer formation by Abeta42. J Biol Chem. 2006; 281: 24414- 24422.

Google Scholar

44Klein AM, Kowall NW, Ferrante RJ. Neurotoxicity and oxidative damage of b amyloid 1-42 versus b amyloid 1-40 in the mouse cerebral cortex. Ann NY Acad Sci. 1999; 893: 314- 320.

Google Scholar

45Hellström-Lindahl E, Viitanen M, Marutle A. Comparison of Ab levels in the brain of familial and sporadic Alzheimer's disease. Neurochem Int. 2009; 55: 243- 252.

Google Scholar

46Duka V, Lee JH, Credle J, et al. Identification of the sites of tau hyperphosphorylation and activation of tau kinases in synucleinopathies and Alzheimer's diseases. PLoS One. 2013; 8:e75025.

Google Scholar

47Kimura T, Sharma G, Ishiguro K, Hisanaga SI. Phospho-Tau bar code: analysis of phosphoisotypes of Tau and its application to Tauopathy. Front Neurosci. 2018; 12: 44.

Google Scholar

48Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. Arch Neurol. 2004; 61: 378- 384.

Google Scholar

49He Z, Guo JL, McBride JD, et al. Amyloid-beta plaques enhance Alzheimer's brain tau-seeded pathologies by facilitating neuritic plaque tau aggregation. Nat Med. 2018; 24: 29- 38.

Google Scholar

50Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002; 297: 353- 356.

Google Scholar

51Gurol ME, Irizarry MC, Smith EE, et al. Plasma beta-amyloid and white matter lesions in AD, MCI, and cerebral amyloid angiopathy. Neurology. 2006: 66.

Google Scholar

52Toledo JB, Vanderstichele H, Figurski M, et al. Factors affecting Abeta plasma levels and their utility as biomarkers in ADNI. Acta neuropathologica. 2011: 122.

Google Scholar

53Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. JAMA. 2020; 324: 772- 781.

Google Scholar

54O'Connor A, Karikari TK, Poole T, et al. Plasma phospho-tau181 in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study. Mol Psychiatry. 2020.

Google Scholar

55Karikari TK, Emeršič A, Vrillon A, et al. Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2020.

Google Scholar

56Suárez-Calvet M, Karikari TK, Ashton NJ, et al. Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in Aβ pathology are detected. EMBO Mol Med. 2020; 12:e12921.

Google Scholar