Comparing tau status determined via plasma pTau181, pTau231 and [18F]MK6240 tau-PET

Cécile Tissot,[a,b,c] Joseph Therriault,[a,b,c] Peter Kunach,[a,b,c] Andréa L. Benedet,[a,b,c,d] Tharick A. Pascoal,[a,b,c,e]
Nicholas J. Ashton,[f,g,h] Thomas K. Karikari,[d,e] Stijn Servaes,[a,b,c] Firoza Z. Lussier,[a,b,c] Mira Chamoun,[a,b,c] Dana L. Tudorascu,[e]
Jenna Stevenson,[a,b,c] Nesrine Rahmouni,[a,b,c] Nina Margherita Poltronetti,[a,b,c] Vanessa Pallen,[a,b,c] Gleb Bezgin,[a,b,c] Min Su Kang,[a,b,c]
Sulantha S. Mathotaarachchi,[a,b,c] Yi-Ting Wang,[a,b,c] Jaime Fernandez Arias,[a,b,c] Pamela Cristina Lukasewicz Ferreira,[e]
João Pedro Ferrari-Souza,[e,i] Eugeen Vanmechelen,[j] Kaj Blennow,[k,l] Henrik Zetterberg,[f,l,m] Serge Gauthier,[a,b,n] and
Pedro Rosa-Neto [a,b,c,*]

[a]McGill University, Montreal, QC, Canada
[b]McGill University Research Centre for Studies in Aging, Douglas Hospital, McGill University, 6875 La Salle Blvd − FBC room 3149, Verdun, QC H4H 1R3, Canada
[c]Translational Neuroimaging Laboratory, Alzheimer's Disease Research Unit, Le Centre intégré universitaire de santé et de services sociaux (CIUSSS) de l'Ouest-de-l'Île-de-Montréal, Department of Neurology and Neurosurgery, Psychiatry and Pharmacology and Therapeutics, McGill University, McGill University Research Centre for Studies in Aging, Douglas Research Institute, Montreal, Canada
[d]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Sweden
[e]University of Pittsburgh, Pittsburgh, PA, USA
[f]Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Sweden
[g]King's College London, Institute of Psychiatry, Psychology and Neuroscience, Maurice Wohl Clinical Neuroscience Institute, London, UK
[h]NIHR Biomedical Research Centre for Mental Health and Biomedical Research Unit for Dementia at South London and Maudsley NHS Foundation, London, UK
[i]Graduate program in Biological Sciences, Biochemistry, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[j]ADx NeuroSciences, Ghent, Belgium
[k]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
[l]UK Dementia Research Institute at UCL, London, United Kingdom
[m]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United Kingdom
[n]Douglas Hospital Research Centre, Verdun, QC, Canada

Summary

Background Tau in Alzheimer's disease (AD) is assessed via cerebrospinal fluid (CSF) and Positron emission tomography (PET). Novel methods to detect phosphorylated tau (pTau) in blood have been recently developed. We aim to investigate agreement of tau status as determined by [18F]MK6240 tau-PET, plasma pTau181 and pTau231.

Methods We assessed cognitively unimpaired young, cognitively unimpaired, mild cognitive impairment and AD individuals with [18F]MK6240, plasma pTau181, pTau 231, [18F]AZD4694 amyloid-PET and MRI. A subset underwent CSF assessment.

We conducted ROC curves to obtain cut-off values for plasma pTau epitopes. Individuals were categorized as positive or negative in all biomarkers. We then compared the distribution among concordant and discordant groups in relation to diagnosis, Aβ status, *APOEε4* status, [18F]AZD4694 global SUVR, hippocampal volume and CSF pTau181.

Findings The threshold for positivity was 15.085 pg/mL for plasma pTau181 and 17.652 pg/mL for plasma pTau231. Most individuals had concordant statuses, however, 18% of plasma181/PET, 26% of plasma231/PET and 25% of the pTau231/pTau181 were discordant. Positivity to at least one biomarker was often accompanied by diagnosis of

cognitive impairment, Aβ positivity, *APOEε4* carriership, higher levels of [^{18}F]AZD4694 global SUVR, hippocampal atrophy and CSF pTau181.

Interpretation Plasma pTau181, pTau231 and [^{18}F]MK6240 seem to reflect different stages of tau progression. Plasma biomarkers can be useful in the context of diagnostic information and clinical trials, to evaluate the disease stage. Moreover, they seem to confidently evaluate tau-PET positivity.

Research in context

Evidence before this study

Assessment of tau *in vivo* is done through positron emission tomography (PET) or cerebrospinal fluid (CSF) assessment. However, those methods are costly and invasive. Research is now focusing on blood-based biomarkers to have an efficient and inexpensive way to assess tau pathology rapidly. Previous work was done on the correlation between plasma phosphorylated-tau (pTau) and tau-PET. However, no study compared the concordance and discordance of tau status, depending on the tau biomarker assessed, either using plasma pTau epitopes or tau-PET. In this study, we compared tau status assessed with plasma pTau231 and pTau181 and [^{18}F]MK6240 tau-PET.

Added value of this study

The current work demonstrated that most individuals have concordant statuses. This implies assessments of blood tau seem to confidently evaluate tau presence in the brain of individuals along the Alzheimer's disease spectrum. Additionally, there was an important proportion of individuals showing discrepancy, *i.e.* they were negative to one biomarker but positive to another. This leads to the idea that plasma pTau231, pTau181 and tau-PET reflect different stages of tau progression.

Implications of all available evidence

Even though statuses using either plasma pTau231, pTau181 or tau-PET did not present perfect concordance, it corroborates a study conducted using CSF demonstrating that biofluid markers are earlier predictors of tau pathology, as compared to tau-PET. Longitudinal analyses are required to assess the disease biomarker trajectories in the plasma. However, our study emphasizes the importance of plasma assessment, and supports its use in clinical and diagnostic settings to assess tau pathology.

Introduction

The core characteristics of Alzheimer's disease (AD) are the accumulation of amyloid-*β* (A*β*) plaques and phosphorylated tau (pTau) tangles, and plaque-surrounding neurites in the brain, then leading to neurodegeneration.[1] Positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) assessments are used to detect the presence of AD pathologies *in vivo*. Due to the high cost and perceived invasiveness of these methods, recent research has been focusing on blood-based biomarkers of AD to diagnose and facilitate clinical trial recruitment.[2] It was recently demonstrated that ultra-sensitive assays for tau phosphorylated at threonine-181 (pTau181) and threonine-231 (pTau231) in plasma[3−7] provide an inexpensive way to determine the presence of brain neurofibrillary tangles *in vivo*.

However, recent studies also provided evidence of variability in the biomarker status depending on the method used,[8] which also seems to depend on the clinical stage. CSF A*β* has been suggested to precede A*β*-PET positivity.[9] Similarly, further evidence supports the idea that CSF pTau181 precedes tau-PET positivity.[10] Plasma biomarkers seem to coincide with CSF results more closely than with PET biomarkers.[11]

Plasma assessments of pTau are promising tools to aid in the diagnosis and clinical management of patients with cognitive impairment, though many questions remain.[12] An important one is the degree to which elevated concentrations of different plasma pTau epitopes deliver similar information, and predict tau positivity status as determined by PET. Here we investigate the concordance and discordance of plasma pTau181, plasma pTau231 and [^{18}F]MK6240 tau-PET biomarker statuses.

Methods

Study participants and ethics

Data was obtained from the TRIAD cohort,[13] from October 2017 to February 2020. The project was approved

by the Douglas Institute Research Ethics Board and written consent was obtained from all participants (Protocols: IUSMD 16-60 and 16-61). 284 individuals (30 cognitively unimpaired young (CUY), 162 cognitively unimpaired (CU), 60 Mild Cognitive Impairment (MCI) and 32 AD) underwent plasma pTau181 and pTau231 assessments, [^{18}F]MK6240 tau-PET, [^{18}F]AZD4694 amyloid-PET, MRI, and a neuropsychological evaluation. Among them, 151 participants were also subjected to CSF pTau181 assessment (22 CUY, 79 CU, 34 MCI and 16 AD). Details on the information gathered from participants can be found here: https://triad.tnl-mcgill.com/. CU individuals are defined as having no cognitive impairment.[14] Consistent with the biological AD research framework from the National Institute of Aging-Alzheimer's Association,[15] participants without a diagnosis of MCI or AD with subjective memory complaints were analyzed with CU individuals. In addition to standard clinical assessments, Mini Mental State Examination (MMSE) and Clinical Dementia Rating (CDR) total scores were used to define MCI operationally as a total MMSE score of 26 or above and a global CDR of 0.5,[16] and dementia due to AD as MMSE lower than 26 and a global CDR above 0.5.[15] No participant met the criteria for another neurological or major neuropsychiatric disorder.

PET processing
PET acquisition and processing of [^{18}F]MK6240 and [^{18}F]AZD4694 can be found elsewhere.[17]

A composite mask including the entorhinal, amygdala, fusiform, inferior and middle temporal cortices was used to calculate [^{18}F]MK6240 temporal meta-ROI SUVR. Those regions are said to capture the changes associated with AD.[10,18] We used a published threshold of 1.24 temporal meta-ROI SUVR[19] to determine tau-PET positivity. In this study, the authors set the threshold by calculating the mean SUVR + 2 standard deviations from the CUY population. A global [^{18}F]AZD4694 SUVR value was estimated by averaging the SUVR from the precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior and posterior cingulate cortices.[20] The cut-off value for positivity was above a published threshold of 1.55[13] global SUVR, used to classify participants as Aβ positive (Aβ+) or Aβ negative (Aβ-). Finally, hippocampal volume was also extracted from MRI images using FreeSurfer.

Biofluid measurements
All plasma pTau biomarkers were measured using *in-house* Single Molecular Array (Simoa) methods Simoa HD-X instruments (Quanterix, Billerica, MA, USA). Methods were described in the supplementary material, and further detailed elsewhere.[3,7] CSF pTau181 was measured via Lumipulse, at the Clinical

Neurochemistry Laboratory, University of Gothenburg, Mölndal, Sweden, by scientists blinded to participants' clinical information.

Statistical analyses
Receiver Operating Characteristic (ROC) curves analyses were performed to assess the optimal cut-off value for plasma pTau181, and pTau231. CUY were considered as the healthy group, contrasted with AD (Youden Index). We used the CUY as the healthy group as it is known that tau pathology is also related to aging, thus can be observed in CU elderlies.[21,22] CUY were not used in subsequent analyses. Exploratory analyses were also conducted using CU as the healthy group, contrasted with AD. Each individual was categorized as positive or negative in all biomarkers. We obtained four groups: concordant plasma pTau negative / PET negative (Plasma-/PET-), discordant plasma pTau positive / PET negative (Plasma+/PET-), discordant plasma pTau negative / PET positive (Plasma-/PET+) and concordant plasma pTau positive / PET positive (Plasma+/PET+). In the case of plasma pTau231 and pTau181 analyses, the four groups were: concordant negative (pTau231-/pTau181-), discordant plasma pTau231 positive / pTau181 negative (pTau231+/pTau181-), discordant plasma pTau231 negative / pTau181 positive (pTau231-/pTau181+) and concordant positive (pTau231+/pTau181+).

We conducted Spearman correlation analysis between [^{18}F]MK6240 SUVR, plasma pTau181 and pTau231. Using ANOVA and chi-square tests when appropriate, we compared the demographic variables in all groups, and calculated the coefficient of variation.

Further ROC curves were conducted to see how plasma pTau epitopes predicted Aβ-PET positivity.

Role of funders: This work was supported by the Weston Brain Institute, Canadian Institute of Health Research (CIHR), and Fonds de recherche du Québec − Santé. None of the funders had a role in the study design, data collection, data analyses, interpretation or writing of the report.

Results

Demographics
There were significant differences between the diagnostic groups in terms of age, plasma pTau181 and pTau231 levels, temporal meta-ROI SUVR, hippocampal volume, *APOEε4* genotype and CSF pTau181. From CU to AD, individuals had higher levels of plasma pTau181, pTau231, CSF pTau181 and temporal meta-ROI [^{18}F]MK6240 SUVR, as well as lower hippocampal volume. Moreover, *APOEε4* carriership was more common in individuals with cognitive impairment (either MCI or AD). Similarly, there was a slightly significant difference in the years of education, being higher in

	CUY	CU	MCI	AD	P value
Number of individuals	30	162	60	32	
Age (mean ± sd)	23.0 ± 2.1	69.4 ± 10.3	70.3 ± 9.1	64.9 ± 10.4	<0.001
Sex (Female (%))	19 (63)	102 (63)	27 (45)	16 (50)	0.073
Education (mean ± sd)	17.0 ± 2.2	15.4 ± 3.7	14.8 ± 4.0	13.9 ± 3.4	0.006
Plasma pTau181 pg/mL (mean ± sd)	8.0 ± 3.6	11.3 ± 6.9	16.1 ± 8.6	26.8 ± 12.9	<0.001
Plasma pTau231 pg/mL (mean ± sd)	9.2 ± 5.9	15.4 ± 8.6	18.1 ± 9.5	27.6 ± 11.0	<0.001
Temporal meta-ROI SUVR (mean ± sd)	1.0 ± 0.1	1.1 ± 0.2	1.6 ± 0.8	2.6 ± 0.9	<0.001
Hippocampal Volume (mean±sd)	4.1 ± 0.3	3.5 ± 0.4	3.5 ± 0.4	2.9 ± 0.4	<0.001
APOEε4 (data available)	30	158	58	29	0.002
0 (N (%))	22 (73)	116 (73)	30 (52)	14 (48)	
1 (N (%))	8 (27)	40 (25)	22 (38)	12 (41)	
2 (N (%))	0 (0)	2 (1)	6 (10)	3 (10)	
CSF pTau181 pg/mL (data available)	22	79	34	16	
(mean ± sd)	22.4 ± 7.5	43.2 ± 25.2	76.8 ± 50.2	110.6 ± 63.4	<0.001

Table 1: Demographics from the TRIAD cohort.

CUY as compared to the other diagnostic groups. However, there was no statistically significant difference in terms of sex (Table 1). Similar results were observed in the subgroup that underwent CSF assessment (Supplementary Table 1). Moreover, the coefficient of variation (Supplementary Table 2) showed high variation biofluid measures (CSF and plasma), as compared to low variation in imaging (MRI and PET).

Discrepancies between statuses of plasma pTau231, pTau181 and tau-PET

CUY were only used for the calculation of cut-off values. Using ROC curves (contrasting CUY versus AD — supplementary Fig. 1), we determined that the cut-off value

for positivity for plasma pTau181 was 15.085 pg/mL, and the value for plasma pTau231 was 17.652 pg/mL, using *in vitro* phosphorylated full-length recombinant tau 441 in both cases.[3,7] When using CU as the healthy group, the cut-off value did not differ for plasma pTau181. Even though the cut-off for plasma pTau231 was higher, it did not impact the results of this study. In the exploratory analyses, we calculated the area under the curve for sensitivity and specificity of plasma pTau231 and pTau181 to evaluate Aβ positivity as assessed via [^{18}F]AZD4694. Analyses revealed that plasma pTau epitopes have acceptable AUC to discriminate between amyloid statuses.

Plasma181/PET demographics can be observed in Table 2, plasma231/PET in Table 3 and finally pTau231/

	Plasma-/PET-	Plasma+/PET-	Plasma-/PET+	Plasma+/PET+	P value
Number of individuals	156	26	20	52	
Diagnosis					<0.001
CU	130	20	7	5	
MCI	25	4	10	21	
AD	1	2	3	26	
Age (mean ± sd)	68.9 ± 10.3	73.3 ± 8.6	71.5 ± 5.9	66.4 ± 10.9	0.021
Sex (Female (%))	90 (58)	14 (54)	14 (70)	27 (52)	0.560
Education (mean ± sd)	15.1 ± 4.0	15.6 ± 2.6	14.2 ± 3.1	14.9 ± 3.7	0.615
Plasma pTau181 pg/mL (mean ± sd)	9.3 ± 2.8	24.2 ± 13.7	11.1 ± 2.1	25.9 ± 9.1	<0.001
Plasma pTau231 pg/mL (mean ± sd)	13.6 ± 6.7	20.3 ± 12.8	18.4 ± 8.4	27.9 ± 9.4	<0.001
Temporal meta-ROI SUVR (mean ± sd)	1.1 ± 0.1	1.1 ± 0.8	1.8 ± 0.6	2.4 ± 0.9	<0.001
Hippocampal Volume (mean±sd)	3.5 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	3.0 ± 0.4	<0.001
APOEε4 (data available)	151	24	20	50	<0.001
0 (N (%))	113 (75)	18 (75)	9 (45)	20 (40)	
1 (N (%))	37 (25)	5 (21)	10 (50)	22 (44)	
2 (N (%))	1 (1)	1 (4)	1 (5)	8 (16)	
CSF pTau181 pg/mL (data available)	78	12	14	25	<0.001
(mean ± sd)	40.0 ± 16.3	44.6 ± 14.5	86.4 ± 44.1	117.1 ± 62.1	

Table 2: Demographics of groups based on Plasma pTau181 and temporal meta-ROI SUVR.

	Plasma-/PET-	Plasma+/PET-	Plasma-/PET+	Plasma+/PET+	P value
Number of individuals	132	50	16	56	
Diagnosis					<0.001
CU	109	41	4	8	
MCI	21	8	8	23	
AD	2	1	4	25	
Age (mean ± sd)	68.6 ± 10.6	72.2 ± 8.3	68.9 ± 7.4	67.5 ± 10.7	0.094
Sex (Female (%))	77 (58)	27 (54)	12 (75)	29 (52)	0.389
Education (mean ± sd)	15.5 ± 4.0	14.6 ± 3.4	13.9 ± 3.4	14.9 ± 3.5	0.276
Plasma pTau231 pg/mL (mean ± sd)	11.0 ± 4.1	23.9 ± 8.8	13.4 ± 3.7	28.6 ± 8.7	<0.001
Plasma pTau181 pg/mL (mean ± sd)	10.1 ± 6.6	15.0 ± 9.3	16.6 ± 8.4	23.3 ± 10.3	<0.001
Temporal meta-ROI SUVR	1.1 ± 0.1	1.1 ± 0.1	1.8 ± 0.6	2.3 ± 0.9	<0.001
Hippocampal Volume (mean±sd)	3.5 ± 0.4	3.4 ± 0.4	3.2 ± 0.4	3.0 ± 0.5	<0.001
APOEε4 (data available)	127	48	15	55	<0.001
0	98 (77)	33 (69)	6 (40)	23 (42)	
1	28 (22)	14 (29)	5 (33)	27 (49)	
2	1 (1)	1 (2)	4 (27)	5 (9)	
CSF pTau181 pg/mL (data available)	59	31	9	30	<0.001
(mean ± sd)	35.3 ± 11.4	50.7 ± 18.6	88.6 ± 45.2	111.3 ± 60.5	<0.001

Table 3: Demographics of groups based on Plasma pTau231 and temporal meta-ROI SUVR.

	Plasma231-/181-	Plasma231+/181-	Plasma231-/181+	Plasma231+/181+	P value
Number of individuals	130	46	18	60	
Diagnosis					<0.001
CU	103	34	10	15	
MCI	25	10	4	21	
AD	2	2	4	24	
Age (mean ± sd)	68.7 ± 10.3	70.7 ± 8.6	67.8 ± 10.5	68.9 ± 10.8	0.657
Sex (Female (%))	78 (60)	26 (57)	11 (61)	30 (50)	0.614
Education (mean ± sd)	15.3 ± 4.1	14.3 ± 3.4	15.3 ± 3.0	15.1 ± 3.5	0.509
Plasma pTau181 pg/mL (mean ± sd)	9.0 ± 2.8	11.0 ± 2.2	23.7 ± 13.1	25.8 ± 10.1	<0.001
Plasma pTau231 pg/mL (mean ± sd)	11.0 ± 4.1	22.9 ± 6.2	12.9 ± 4.2	29.1 ± 9.9	<0.001
Temporal meta-ROI SUVR	1.1 ± 0.2	1.3 ± 0.5	1.4 ± 0.6	2.1 ± 1.0	<0.001
Hippocampal Volume (mean±sd)	3.5 ± 0.4	3.4 ± 0.4	3.3 ± 0.4	3.0 ± 0.4	<0.001
APOEε4 (data available)	126	45	16	58	<0.001
0	93 (74)	29 (64)	11 (69)	27 (47)	
1	31 (25)	16 (36)	2 (1)	25 (43)	
2	2 (2)	0 (0)	3 (2)	5 (10)	
CSF pTau181 pg/mL (data available)	59	33	9	28	<0.001
(mean ± sd)	39.0 ± 18.4	61.6 ± 35.6	64.8 ± 45.2	102.9 ± 62.7	

Table 4: Demographics of groups based on Plasma pTau231 and Plasma pTau181.

181 in Table 4. In all analyses, we observed significant differences in diagnostic groups regarding plasma pTau181 and pTau231 levels, temporal meta-ROI SUVR, hippocampal volume, *APOEε4* presence, and CSF pTau181, while no significant differences in age, sex or years of education.

Significant correlations were observed between [18F] MK6240 SUVR in the temporal meta-ROI and plasma pTau181 ($R = 0.48$, $p < 0.001$ [Spearman correlation]) (Figure 1a), and pTau231 ($R = 0.49$, $p < 0.001$ [Spearman correlation]) (Figure 1b), as well as between plasma pTau231 and pTau181 ($R = 0.60$, $p < 0.001$ [Spearman correlation]) (Figure 1c).

For 82% of individuals, the plasma181 and tau-PET assessment methods were in agreement with respect to their tau status. Among the cases where there was a discordance, plasma181+/PET- was observed more frequently. Looking more closely at the plasma181-/PET+ individuals, we observed the majority were cognitively impaired (CI), *i.e.,* MCI or AD. In the plasma231/PET plot, 76% of the individuals were also concordant in terms of their tau status. Additionally, 20% were
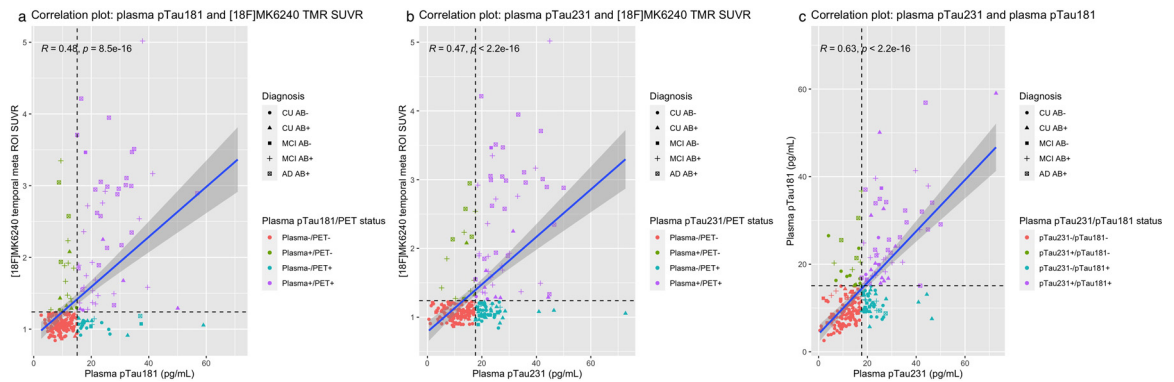
Figure 1. Correlation plots (*n* = 254). a. Correlation between plasma pTau181 and [^{18}F]MK6240 temporal meta-ROI SUVR ($R = 0.48$, $p < 0.001$), stratified by status and diagnosis. b. Correlation between plasma pTau231 and [^{18}F]MK6240 temporal meta-ROI SUVR ($R = 0.49$, $p < 0.001$) stratified by status and diagnosis. c. Correlation between plasma pTau231 and pTau181 ($R = 0.60$, $p < 0.001$) stratified by status and diagnosis.

considered plasma231+/PET-; with a high proportion of CU individuals. The plasma231-/PET+ group was in turn comprised of a high number of CI participants. In 75% of cases, both plasma pTau231 and pTau181 produced concordant estimates of tau status. Among the discordant results, the proportion of pTau231+/pTau181- was larger than the proportion of pTau231-/pTau181+; the latter was mainly composed of CI individuals.

Demographics in relation to tau statuses

We first investigated the distribution of diagnostic groups in relation to tau statuses. Plasma181/PET analyses (Figure 2a) revealed that 83% of plasma181-/PET- were CU individuals, while 90% of plasma181+/PET+ were CI. However, we observed that some MCI individuals were considered negative to both tau biomarkers (42% of MCI), and some CU were positive to plasma pTau181 and tau-PET (3% of CU). Among the individuals with tau status discordance, 77% of plasma181+/PET- were CU, while 65% of plasma181-/PET+ were CI. Plasma231/PET analyses showed a similar pattern (Figure 2b) in which 83% of plasma231-/PET- were CU and 86% of plasma231+/PET+ were CI. Nonetheless, in the plasma231-/PET- group, 2% of individuals were AD and 16% MCI, while in the plasma231+/PET+, 14% were CU. Among discordant tau status groups, 82% of plasma231+/PET- individuals were CU with the remaining 16% being MCI and 2% being AD. Finally, 75% of the plasma231-/PET+ group was CI (50% MCI and 25% AD). Plasma pTau231/pTau181 analyses also showed a pattern (Figure 2c) in which 75% obtained concordance in their tau status, and the highest proportion of discrepant individuals was in the pTau231+/pTau181-. In this group, 74% were CU; while they were 56% in pTau231-/pTau181+.

When combining diagnosis and Aβ status, we observed that majority of plasma181-/PET- individuals were CU-Aβ- (68%), the remaining being CU-Aβ+

(15%), MCI (Aβ- (11%), Aβ+ (5%)) and AD-Aβ+ (1%) (Figure 2d). Plasma181+/PET+ individuals were mainly composed of CI individuals showing Aβ positivity (2% MCI-Aβ-, 41% MCI-Aβ+, 47% AD-Aβ+). Among the plasma181/PET groups (Figure 2d), the one with the biggest proportion of CU-Aβ+ individuals was plasma181+/PET-; it is also important to note that the CU-Aβ+ group was often positive for at least one tau biomarker. Finally, cognitive impairment was usually accompanied by tau-PET positivity (plasma181-/PET+). Regarding plasma231/PET statuses, 76% of plasma231-/PET- individuals were CU-Aβ- (Figure 2e). One AD-Aβ+ individual was considered plasma231-/PET-. We observed a high proportion of CI-Aβ+ individuals in the plasma231+/PET+ analyses (42% MCI-Aβ+ and 42% AD-Aβ+). Among the individuals with discrepant tau results, CU-Aβ+ were often part of the plasma231+/PET- group. Additionally, CI individuals categorized as plasma231+/PET- were 16% MCI (12% Aβ-, 4% Aβ+) and 2% AD-Aβ+. 75% of the plasma231-/PET+ group were CI individuals (50% MCI-Aβ+, 25% AD-Aβ+). In terms of plasma comparisons, we observed that plasma pTau231-/pTau181- individuals were mainly CU-Aβ- (71%), with some CU-Aβ+ (8%), and a small proportion of MCI (10% Aβ-, 9% Aβ+) and AD-Aβ+ (2%). Conversely, 74% of the pTau231+/pTau181+ participants were categorized as CI (5% MCI-Aβ-, 32% MCI-Aβ+, 38% AD-Aβ+) (Figure 2f). In the pTau231+/pTau181- group, we mainly observed Aβ+ individuals (37% CU-Aβ+, 13% MCI-Aβ+). Lastly, the pTau231-/pTau181+ group had a high proportion of CI individuals, showing Aβ positivity (24% MCI-Aβ+ and 18% AD-Aβ+). In all three analyses, when presenting cognitive impairment and/or Aβ positivity, individuals had a tendency to be positive to at least one tau-biomarker. A table summarizing all the percentage of diagnosis and diagnosis combined with Aβ status can be found in the supplementary material (Supplementary Table 3).
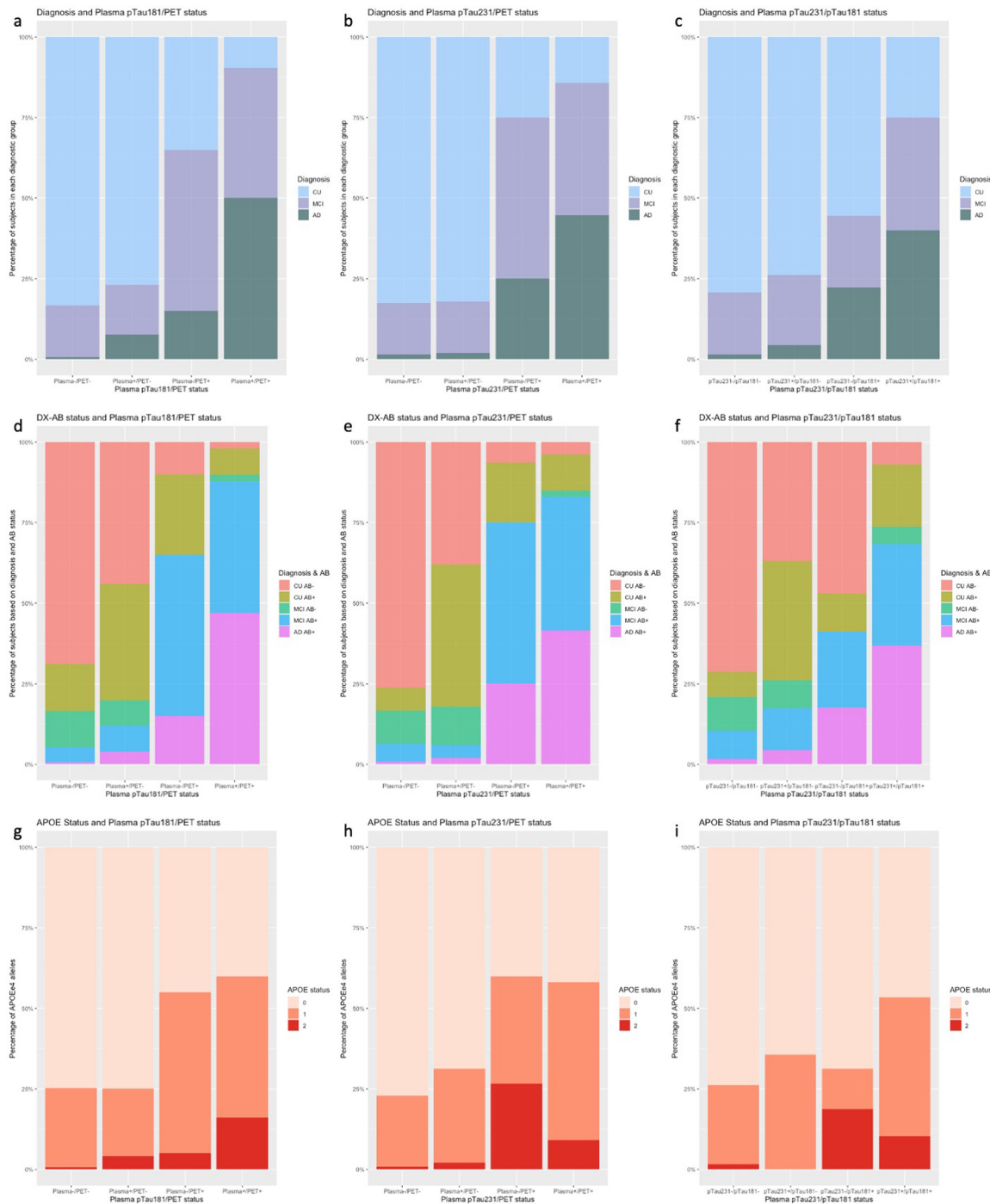
Figure 2. Demographic information in relation to plasma/PET statuses (*n* = 254). a. Diagnosis in plasma181/PET b. Diagnosis in plasma231/PET c. Diagnosis in plasma pTau231/pTau181 d. Diagnosis and Aβ status in plasma181/PET e. Diagnosis and Aβ status in plasma231/PET f. Diagnosis and Aβ status in plasma pTau231/pTau181 g. *APOEε4* status in plasma181/PET and h. *APOEε4* status in plasma231/PET. i. *APOEε4* status in plasma pTau231/pTau181.

APOE genotype was assessed in a subgroup of 245 individuals. The plasma181/PET analyses showed an incremental relationship in the proportion of *APOEε4*

carriers, heterozygous or homozygous (Figure 2g). Indeed, 25% of plasma181-/PET- carried at least one *APOEε4* allele, as compared to 60% of plasma181

+/PET+. *APOEε4* status followed tau-PET positivity more closely than plasma positivity, with 55% of plasma181-/PET+ having at least one *APOEε4* allele, and only 25% in the plasma181+/PET- group. Plasma231/PET analyses revealed that plasma231-/PET- had a low proportion (77%) and plasma231+/PET+ had a high proportion (58%) of *APOEε4* carriers (Figure 2h). *APOEε4* status, in this case too, seemed to correlate with tau-PET positivity closely, with 60% of plasma231-/PET+ and 58% of plasma231+/PET+ being *APOEε4* carriers. Finally, in the plasma pTau231/pTau181 analyses, concordant negative individuals were mainly not *APOEε4* carriers (74%), while concordant negative were mainly *APOEε4* carriers (53%) (Figure 2i). The discordant groups had a slightly high proportion of *APOEε4* carriers: 36% in pTau231+/pTau181- and 31% in pTau231-/pTau181+.

AD biomarkers in relation to tau statuses

We first examined the Aβ status distribution in the different tau-assessment groups, based on [^{18}F]AZD4694 SUVR.[13] We observed that 80% of plasma181-/PET- were Aβ-, while 96% of plasma181+/PET+ were Aβ+ (Figure 3a). Among the cases with a single positive tau biomarker (plasma181+/PET- and plasma181-/PET+), we observed a high percentage of Aβ+ individuals (48% for plasma181+/PET- and 90% for plasma181-/PET+), as compared to the plasma181-/PET- group. Similarly, in the plasma231/PET analyses, we observed a high proportion of Aβ- individuals in the plasma231-/PET- (87%), and Aβ+ individuals in the plasma231+/PET+ (94%) (Figure 3b). Individuals with discrepant tau statuses had a 50% risk of being Aβ+ for plasma231+/PET-, and 94% in the plasma231-/PET+. In both plasma/PET analyses, PET+ individuals had a significantly higher risk of being Aβ+, independently of the plasma status. Finally, 82% of the pTau231-/pTau181- were also categorized as Aβ- (Figure 3c). Comparatively, 88% of the pTau231+/pTau181+ were Aβ+. The groups showing discrepancy in terms of tau statuses had similar results, meaning 54% of the pTau231+/pTau181- and 53% of the pTau231-/pTau181+ had a positive Aβ status.

We compared the [^{18}F]AZD4694 global SUVR levels in each group. Plasma181/PET (Figure 3d) and plasma231/PET (Figure 3e) analyses revealed significant differences in [^{18}F]AZD4694 SUVR among all the groups, except between the plasma-/PET+ and plasma+/PET+ groups. The pTau231/pTau181 analyses revealed that there were significant differences among all groups, except when individuals had discrepant tau results (Figure 3f).

We then investigated hippocampal volume results in each group. Plasma181/PET analyses revealed significant differences among all groups, except between the discrepant groups (plasma181+/PET- and plasma181-/PET+) (Figure 3g). Similarly, plasma231/PET showed

significant differences between groups, except for plasma231+/PET- and plasma231-/PET+ as well as plasma231-/PET+ and plasma231+/PET+ (Figure 3h). In the case of pTau231/pTau181, the groups not presenting a statistically significant difference were pTau231-/pTau181- and pTau231+/pTau181- as well as pTau231+/pTau181- and pTau231-/pTau181+ (Figure 3i).

Finally, a subgroup of 129 individuals underwent CSF pTau181 assessment, among which 79 CU, 34 MCI and 16 AD. Among the plasma181/PET analyses, we did not obtain significant differences between plasma181-/PET- and plasma181+/PET- as well as plasma181-/PET+ and plasma181+/PET+ (Figure 3j). The remaining group comparisons had significant differences. Plasma231/PET revealed significant differences among all groups except between plasma231-/PET+ and plasma231+/PET+ (Figure 3k). Lastly, in the pTau231/pTau181 analyses, we discovered statistically significant difference between pTau231-/pTau181- and pTau231-/pTau181+ as well as pTau231+/pTau181- and pTau231-/pTau181+ (Figure 3l). In the remaining group comparisons, we obtained statically significant differences.

Discussion

The current study sought to compare the concordance and discordance of plasma pTau181, pTau231 and [^{18}F]MK6240 SUVR positivity in a well-characterized cohort study of aging and AD. In all cases, the rates of concordance were higher than the rates of discordance. The highest rate of concordance was between plasma pTau181 and tau-PET. Discrepant groups differed between the plasma181/PET, plasma231/PET and pTau231/pTau181 statuses, suggesting plasma pTau231, plasma pTau181 and tau-PET abnormality reflect different stages of tau pathology progression. Positivity for one tau biomarker was often accompanied by cognitive impairment, Aβ-PET positivity status, and elevated hippocampal atrophy and CSF pTau181 levels, as well as higher risk of carrying at least one *APOEε4* allele.

Previous work on CSF revealed that, among the groups presenting discrepant tau results, CSF pTau abnormality was more common than tau-PET abnormality.[10] Moreover, other studies reported that CSF pTau epitopes seemed to appear at different stages of the disease.[23,24] We found a similar pattern for plasma pTau181 and pTau231. Differences observed in the statuses of plasma pTau231, pTau181 and tau-PET suggest distinct stages of tau continuum. In both plasma/PET analyses, the plasma-/PET+ group was the smallest, and individuals often had cognitive impairment. Plasma pTau231 and pTau181 are known to be specific to AD,[3,7] while tau-PET can also be observed in other tauopathies.[25] However, most CI individuals in the plasma-/PET+ groups were categorized as Aβ+, one of the core characteristics of AD,[1] suggesting they are also on the AD spectrum. Additionally, plasma+/PET- individuals
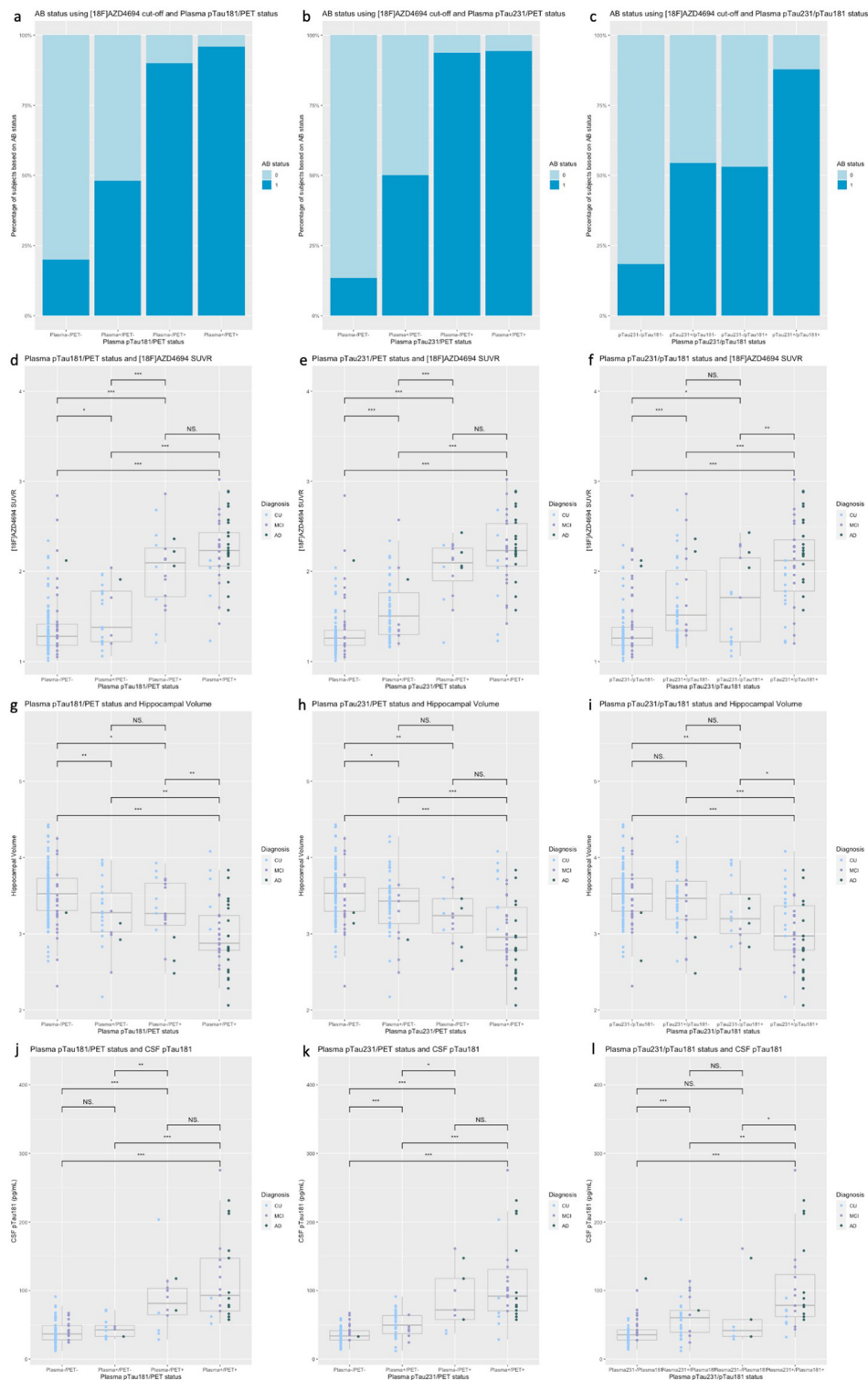
Figure 3. Alzheimer's disease biomarkers in relation to plasma/PET statuses ($n = 254$). a. Aβ status in plasma181/PET b. Aβ status in plasma231/PET c. Aβ status in plasma pTau231/pTau181. d. [^{18}F]AZD4694 global SUVR in plasma181/PET e. [^{18}F]AZD4694 global SUVR in plasma231/PET f. [^{18}F]AZD4694 global SUVR in plasma pTau231/pTau181 g. Hippocampal volume in plasma181/PET h. Hippocampal volume in plasma231/PET i. Hippocampal volume in plasma pTau231/pTau181 j. CSF pTau181 levels in plasma181/PET k. CSF pTau181 levels in plasma231/PET l. CSF pTau181 levels in plasma pTau231/pTau181.

were mostly cognitively unimpaired individuals, some presenting a positive Aβ status. We observed a higher proportion of discordant individuals in the plasma231/PET analyses, as compared to plasma181/PET. Specifically, there were more plasma231+/PET- individuals, as compared to plasma181+/PET- individuals. Finally, there was some discordance among the plasma epitopes. Plasma pTau231+/pTau181- group was more common as compared to pTau231-/pTau181+. The first group showed Aβ positivity, when the latter individuals usually presented cognitive impairment accompanied by Aβ positivity. When combining both plasma biomarkers, we observed a high rate of pathological as well as cognitive signs of AD. This suggests that even plasma pTau181 and pTau231 reflect different stages of tau continuum, potentially extending species-specific phosphorylation differences in CSF.[24] Using plasma pTau, our study extends recent CSF biomarker modeling studies which provide evidence that CSF pTau231 abnormality precedes CSF pTau181 abnormality.[23,26] This follows the framework in which plasma biomarkers are early detectors of AD pathology.[11] Tau-PET has been proven effective in providing information regarding the risk of clinical deterioration in the following months.[27] Having a blood-based assessment giving a strong predictive value of tau-PET status would be critical for both clinical trials and diagnostic settings.[28]

Individuals negative to all tau assessment methods, plasma pTau181, pTau231 and tau-PET were mainly CU-Aβ-, not APOEε4 carriers, with low levels of [18F]AZD4694 global SUVR, hippocampal atrophy and CSF pTau181. Conversely, when individuals were positively concordant in all tau assessment methods, they were often MCI-Aβ+ or AD-Aβ+, with at least one APOEε4 allele, and high levels of [18F]AZD4694 global SUVR, hippocampal atrophy and CSF pTau181. The majority of plasma (231 or 181)+/PET- individuals are either CU-Aβ+ and CI-Aβ+ individuals, which might reflect early stages of the disease. In these cases, tau levels assessed via [18F]MK6240 PET may be below the threshold for positivity. Interestingly, plasma pTau231 positivity, more common than plasma pTau181 positivity, was often observed in individuals categorized as CU-Aβ+.

It has been proposed that plasma pTau181[3] and pTau231[7] are predictors of AD dementia, and differentiate it from other types of dementia. Conversely, tau-PET is thought to bind to neurofibrillary tangles in the brain. The discordancy may thus result from the difference between the methods, rather than being truly discordant.

Presence of at least one APOEε4 allele is a known risk factor for developing AD.[29] Even though research mostly linked it to the presence of Aβ, recent studies focused on its relationship with tau. It has been demonstrated that APOEε4 acts on several mechanisms, including decreasing the clearance of Aβ in the brain, thus leading to higher brain levels of Aβ as well as

tau.[30] In both plasma/PET analyses, we observed that APOEε4 presence closely correlated with tau-PET positivity, as having at least one APOEε4 was associated with more than a 50% chance of being tau-PET positive. Concerning the pTau231/pTau181 analyses, the concordant negative and discordant groups had similar results, revealing they had around a 25% risk of having at least one APOEε4. However, more than 50% of the individuals in the pTau231+/pTau181+ group had at least one APOEε4.

We also investigated the relationship between the plasma231 and plasma181/PET and pTau231/pTau181 groups with established AD biomarkers. Plasma biomarkers, in combination with clinical and demographic information, have been proposed to help in the detection of Aβ positivity.[31] Our study corroborates this idea, demonstrating that positivity to one tau biomarker correlates with a higher risk of being Aβ positive. Other studies already presented the strong relationship between Aβ and the three biomarkers independently.[3,7] Individuals that obtained a concordant positive tau status for all assessment methods were almost exclusively Aβ positive, while individuals with concordant negative tau were almost exclusively Aβ negative. Accepted biomarker models of AD propose that Aβ accumulation arises before the presence of tau aggregates,[32] and is thus considered an early marker of the disease. The results of both plasma/PET analyses were similar; when individuals were positive to plasma pTau, there was a 50% risk of Aβ positivity, even when obtaining a negative tau-PET. However, when they were tau-PET positive, the risk of being Aβ+ increased dramatically, irrespective of the plasma (231 or 181) status. PTau231/pTau181 analyses revealed that participants had a 50% risk of being Aβ+ when positive to either plasma pTau biomarker. However, combining both results led to an almost certain positive Aβ status. This leads to the hypothesis that tau-PET or the combination of two plasma epitopes, rather than one of pTau231 or pTau181, are great predictors of Aβ status.

When conducting analyses using [18F]AZD4694 global SUVR, we observed that there were no significant differences between the plasma-/PET+ and plasma+/PET+ groups, either using plasma181/PET or plasma231/PET. All other groups had a statistically significant different [18F]AZD4694 SUVR. We noticed a strong variability in the discrepant groups, emphasizing the idea that some individuals might not be on the AD spectrum, while others could be at early disease stages, with a certain build-up of pathology without cognitive impairment.[33,34] Regarding pTau231/181, we observed no significant difference between the discrepant groups. This might be due to the high variability of the pTau levels. We can further hypothesize that combining both biomarkers could be critical in predicting the levels of cortical Aβ, hence be used to predict the advancement of AD pathology.[35]

We observed that PET status was the best predictor of hippocampal atrophy as all tau-PET+ individuals had low levels of hippocampal volume. It is also important to note that positivity to at least one tau biomarker was related to higher rates of hippocampal atrophy, however, we seemed to obtain similar results when using either pTau231 or 181 combined with tau-PET. Analyses conducted on the comparison between plasma pTau epitopes yet revealed that pTau181 positivity was more closely related to hippocampal atrophy than pTau231. This corroborates the framework in which pTau181 appears at later stages of the disease, when hippocampal atrophy is more prominent.[22]

For the established AD biomarker CSF pTau181, rates of concordance and discordance differed widely between analyses. It is important to note that not all participants of the TRIAD cohort underwent a lumbar puncture, lowering the number of individuals in the above results. Plasma biomarkers are thought to closely follow CSF biomarkers in the progress of the disease.[11] Our study adds to the research framework in which CSF levels of pTau181 are accompanied by abnormal levels of plasma pTau, either 181 or 231, tau-PET, or both, and might reach a plateau at a later disease stage. Again, it seems that the combination of both plasma biomarkers, or tau-PET, was a better predictor of high CSF pTau181 levels.

Importantly, biomarkers assessed in the plasma have crossed the blood-brain barrier (BBB), they are thus at low concentrations as compared to measures in the brain.[36] It has been suggested that the BBB is compromised in aging and disease progression,[37] leading to an increasing concentration of brain proteins in the plasma as the disease advances. Plasma biomarkers are also known to have a broad coefficient of variation, and may present higher false positive rates, as compared to the more direct assessment of cerebral tau pathology using PET. Plasma assays are a proxy of cortical tau, and do not represent exactly the same components of tau accumulation process as assessed with tau-PET. Moreover, we also focused here on specific phosphorylated sites (i.e. pTau181 and pTau231). Those phosphorylated sites are already thought to appear at different stages of Alzheimer's disease.[24] Conversely tau-PET assesses neurofibrillary tangles load in the brain,[17] leading to a more direct measure of cortical tau. Because there is this inherent difference, and because similar results were observed when studying the differences between of CSF and tau-PET statuses,[10] we do not expect a perfect concordance between tau statuses. Additionally, in our study, ROC curves were conducted based on clinical diagnosis defined through clinical testing. Clinical diagnosis does not perfectly reflect Aβ and tau pathologies at the individual-level. Indeed, recent research showed there is not always a full accordance between the biologically-defined and the clinically-defined AD diagnosis.[19]

As new AD therapeutic methods are focusing on Aβ aggregates, we wondered to which degree plasma pTau markers could predict Aβ-PET status. AUC were considered acceptable in discriminating individuals based on Aβ status. As Aβ is known to accumulate years before the onset of clinical symptoms, and appears before tau accumulation,[1] we expected a strong correlation.

We decided to use here CUY as the reference group to calculate the cut-off values for plasma biomarkers. Brain accumulation of the AD hallmarks is known to be continuous, and CU elderlies tend to show pathology even without cognitive impairment.[21] When using the CU elderlies as the control group, the cut-off was the same for plasma pTau181. For plasma pTau231, we obtained a higher cut-off, however, it did not impact the results observed in this study.

We compared the relationship between tau phospho-forms and as well as their relation to tau-PET status. Even though most individuals had concordant statuses in tau assessment methods, discordant cases were also observed. Analyses comparing plasma231/PET, plasma181/PET and pTau231/pTau181 led to the idea that plasma pTau231, pTau181, and [^{18}F]MK6240 tau-PET reflect distinct aspects of tau accumulation. Our results corroborate a study conducted using CSF pTau epitopes; in autosomal dominant AD, hyperphosphorylation of tau occurred early and exhibited a pattern of site-specific changes at different stages of the disease.[23,24,38] Longitudinal studies are needed to confirm the ordering of plasma pTau231, pTau181 and tau-PET abnormality. This is potentially useful in clinical trials, in which a plasma test could provide information on the tau pathology stage, rather than using CSF or PET, which are costly and invasive.

The principal strength of the study is the use of a well-characterized cohort of individuals, that underwent gold standard procedures of PET assessment for amyloid-β and tau. Plasma assessments for pTau epitopes also used the most advanced methodologies.[3,7] However, a limitation of our study is the lack of longitudinal measures, which would assess the disease biomarker trajectory. We could also investigate whether individuals that are positive to one biomarker are more prone to be positive to another one later on, as well as convert to dementia. This could be observed either in individuals that obtained discrepant the plasma/PET results and even between the plasma biomarkers. Moreover, it is important to note that the TRIAD cohort is comprised of a sample of individuals willing to participate in dementia research, thus involving recruitment and sampling biases. Nonetheless, the results show that plasma/PET and pTau231/181 groups correlate well with demographic and clinical information, as well as established biomarkers of AD.

Novel plasma biomarkers and tau-PET measures reflect different stages of tau pathological progression. Even though most measures have concordant statuses, it is thought that plasma biomarkers come at earlier stages of the disease. Positivity to one biomarker is often accompanied by cognitive impairment, presence of Aβ, higher levels of CSF pTau181, as well as higher risk of having at least one APOEε4.

Declaration of interests
Nothing to disclose.

Contributors
CT: conceptualization, formal analysis, methodology, investigation, writing; JT: conceptualization, methodology, writing, verification of underlying data; PK: conceptualization, validation; ALB: conceptualization, methodology, validation; TAP: conceptualization, methodology, writing; NJA: validation, investigation, data curation; TKK: validation, investigation, data curation; SS: investigation, data curation; FZL: conceptualization, data curation, verification of underlying data; MC: data curation, project administration; DLT: methodology; JS: project administration; NR: project administration; NMP: data curation, methodology; VP: data curation, methodology; GB: data curation, software; MSK: software, resources; SSM: software, resources; YTW: data curation; JFA: data curation; PCLF: investigation; JPFS: investigation; EV: methodology; KB: methodology; HZ: methodology; SG: supervision; PRN: writing, supervision. All authors read and approved the manuscript.

Data sharing statement
All data presented in this study is available upon request to the corresponding author. ☐Data is not publicly available as it contains information that could compromise the privacy of research participants.

Supplementary materials
Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2022.103837.

Reference
1 Jack CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 2018;14(4):535–562.
2 Hampel H, Bryant SEO, Molinuevo JL, et al. Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. *Nat Rev Neurol.* 2018. https://doi.org/10.1038/s41582-018-0079-7.
3 Karikari 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(5):422–433.
4 Mielke 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. *Alzheimers Dement.* 2018;14(8):989–997.
5 Tatebe 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. *Mol Neurodegener.* 2017;12(1):1–11.
6 Yang SY, Chiu MJ, Chen TF, Lin CH, Chen WP, Yang CC. P3-255: assay of plasma phosphorylated tau protein (Threonine 181) and total tau protein in vascular dementia, Parkinson's disease, frontotemporal dementia, and early-stage alzheimer's disease. *Alzheimers Dement.* 2018;14(7S_Part_22):P1172–P1172.
7 Ashton NJ, Pascoal TA, Karikari TK, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol.* 2021(0123456789). Available from: http://www.ncbi.nlm.nih.gov/pubmed/33585983.
8 Mattsson N, Leuzy A, Janelidze S. The implications of different approaches to define AT(N) in Alzheimer disease. *Neurology.* 2020:94.
9 Palmqvist S, Mattsson N, Hansson O. Cerebrospinal fluid analysis detects cerebral amyloid-β accumulation earlier than positron emission tomography. *Brain.* 2016;139(4):1226–1236.
10 Meyer PF, Binette AP, Gonneaud J, Breitner JCS, Villeneuve S. Characterization of alzheimer disease biomarker discrepancies using cerebrospinal fluid phosphorylated tau and AV1451 positron emission tomography. *JAMA Neurol.* 2019;77(4):508–516.
11 Palmqvist S, Insel PS, Stomrud E, et al. Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease. *EMBO Mol Med.* 2019;11(12):1–13.
12 Thijssen EH, Rabinovici GD. Rapid progress toward reliable blood tests for Alzheimer's disease. *JAMA Neurol.* 2021;78(2):143–145.
13 Therriault J, Benedet AL, Pascoal TA, et al. Determining amyloid-beta positivity using [18F]AZD4694 PET imaging. *J Nucl Med.* 2020.
14 Jack CR, Wiste HJ, Schwarz CG, et al. Longitudinal tau PET in ageing and Alzheimer's disease. *Brain.* 2018;141(5):1517–1528.
15 McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011;7(3):263–269.
16 Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med.* 2004;256(3):183–194.
17 Pascoal TA, Therriault J, Benedet AL, et al. 18F-MK-6240 PET for early and late detection of neurofibrillary tangles. *Brain.* 2020;143 (9):2818–2830.
18 Ossenkoppele R, Rabinovici GD, Smith R, et al. Discriminative accuracy of [18F]flortaucipir positron emission tomography for Alzheimer disease vs other neurodegenerative disorders. *JAMA J Am Med Assoc.* 2018;320(11):1151–1162.
19 Therriault J, Pascoal TA, Benedet AL, et al. Frequency of biologically defined alzheimer disease in relation to age, sex, APOE ε4, and cognitive impairment. *Neurology.* 2021;96(7):e975–e985.
20 Jack CR, Wiste HJ, Weigand SD, et al. Defining imaging biomarker cut points for brain aging and Alzheimer's disease. *Alzheimers Dement.* 2017;13(3):205–216.
21 Jagust W. Imaging the evolution and pathophysiology of Alzheimer disease. *Nat Rev Neurosci.* 2018;19(11):687–700. https://doi.org/10.1038/s41583-018-0067-3.
22 Tissot C, Benedet AL, Therriault J, et al. Plasma pTau181 predicts cortical brain atrophy in aging and Alzheimer's disease. *Alzheimers Res Ther.* 2021;13(1):69–80.
23 Suárez-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 (12):1–19.

24 Barthélemy NR, Li Y, Joseph-Mathurin N, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat Med*. 2020;26(3):398–407.

25 Leuzy A, Chiotis K, Lemoine L, et al. Tau PET imaging in neurodegenerative tauopathies—still a challenge. *Mol Psychiatry*. 2019;24 (8):1112–1134.

26 Ashton NJ, Benedet AL, Pascoal TA, et al. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. *EBioMedicine*. 2022;76:103836. https://doi.org/10.1016/j.ebiom.2022.103836.

27 Lu M, Pontecorvo MJ, Devous MD, et al. Aggregated tau measured by visual interpretation of flortaucipir positron emission tomography and the associated risk of clinical progression of mild cognitive impairment and alzheimer disease: results from 2 phase III clinical trials. *JAMA Neurol*. 2021;78(4):445–453.

28 Gauthier S, Therriault J, Pascoal T, Rosa-Neto P. Impact of p-tau181 and p-tau217 levels on enrollment for randomized clinical trials and future use of anti-amyloid and anti-tau drugs. *Expert Rev Neurother*. 2020;20(12):1211–1213.

29 Zhao N, Liu C, Qiao W, Bu G. Review apolipoprotein E, receptors, and modulation of Alzheimer's disease. *Biol Psychiatry*. 2018;83 (4):347–357. https://doi.org/10.1016/j.biopsych.2017.03.003.

30 Therriault J, Benedet AL, Pascoal TA, et al. APOEε4 potentiates the relationship between amyloid-β and tau pathologies. *Mol Psychiatry*. 2020. https://doi.org/10.1038/s41380-020-0688-6.

31 Tosun D, Veitch D, Aisen P, et al. Detection of β-amyloid positivity in Alzheimer's disease neuroimaging initiative participants with demographics, cognition, MRI, and plasma biomarkers. *Brain Commun*. 2021.

32 Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297(5580):353–356.

33 Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol*. 1999;45(3):358–368.

34 Chételat G, La Joie R, Villain N, et al. Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. *NeuroImage Clin*. 2013;2(1):356–365. https://doi.org/10.1016/j.nicl.2013.02.006.

35 Jack CR, Wiste HJ, Vemuri P, et al. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. *Brain*. 2010;133(11):3336–3348.

36 Snyder HM, Carrillo MC, Grodstein F, et al. Developing novel blood-based biomarkers for Alzheimer's disease. *Alzheimers Dement*. 2014;10:109–114.

37 Zipser BD, Johanson CE, Gonzalez L, et al. Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. *Neurobiol Aging*. 2007;28(7):977–986.

38 Karikari 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. *Alzheimers Dement*. 2020:(August):1–13.