Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease

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12   **Abbreviations:** Aβ = amyloid-β; AUC = area under the curve; CSF = cerebrospinal fluid; MCI

13   = mild cognitive impairment; MMSE = mini-mental state examination; MS = mass spectrometry;

14   ROC = receiver operating characteristic; PET = positron emission tomography; p-tau =

15   phosphorylated tau

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Abstract

Plasma phospho-tau (p-tau) species have emerged as the most promising blood-based biomarkers of Alzheimer's disease. Here, we performed a head-to-head comparison of p-tau181, p-tau217 and p-tau231 measured using 10 assays to detect abnormal brain amyloid-β status and predict future progression to Alzheimer's dementia. The study included 135 patients with baseline diagnosis of mild cognitive impairment (mean age 72.4 years; 60.7% women) who were followed for an average of 4.9 years. Seventy-one participants had abnormal Aβ-status (i.e., abnormal CSF Aβ42/40) at baseline; and 45 of these Aβ-positive participants progressed to Alzheimer's dementia during follow-up. P-tau concentrations were determined in baseline plasma and CSF. P-tau217 and p-tau181 were both measured using immunoassays developed by Lilly Research Laboratories (Lilly) and mass spectrometry assays developed at Washington University (WashU). P-tau217 was also analysed using Simoa immunoassay developed by Janssen Research and Development (Janss). P-tau181 was measured using Simoa immunoassay from ADxNeurosciences (ADx), Lumipulse immunoassay from Fujirebio (Fuji) and Splex immunoassay from Mesoscale Discovery (Splex). Both p-tau181 and p-tau231 were quantified using Simoa immunoassay developed at the University of Gothenburg (UGOT). We found that the mass spectrometry-based p-tau217 (p-tau217^{WashU}) exhibited significantly better performance than all other plasma p-tau biomarkers when detecting abnormal Aβ status (AUC=0.947; p_{diff}<0.015) or progression to Alzheimer's dementia (AUC=0.932; p_{diff}<0.027). Among immunoassays, p-tau217^{Lilly} had the highest AUCs (0.886-0.889), which was not significantly different from the AUCs of p-tau217^{Janss}, p-tau181^{ADx} and p-tau181^{WashU} (AUC$_{range}$, 0.835-0.872; p_{diff}>0.09), but higher compared with AUC of p-tau231^{UGOT}, p-tau181^{Lilly}, p-tau181^{UGOT}, p-tau181^{Fuji}, and p-tau181^{Splex} (AUC$_{range}$, 0.642-0.813; p_{diff} ≤0.029). Correlations between plasma

1   and CSF values were strongest for p-tau217^{WashU} (R=0.891) followed by p-tau217^{Lilly} (R=0.755;

2   p$_{diff}$=0.003 vs p-tau217^{WashU}) and weak to moderate for the rest of the p-tau biomarkers (R$_{range}$,

3   0.320-0.669). In conclusion, the findings suggest that among all tested plasma p-tau assays, mass

4   spectrometry-based measures of p-tau217 perform best when identifying mild cognitive

5   impairment patients with abnormal brain Aβ or those who will subsequently progress to

6   Alzheimer's dementia. Several other assays (p-tau217^{Lilly}, p-tau217^{Janss}, p-tau181^{ADx}, and p-

7   tau181^{WashU}) showed relatively high and consistent accuracy across both outcomes. The results

8   further indicate that the highest performing assays have performance metrics that rival the gold

9   standards of Aβ-PET and CSF. If further validated, our findings will have significant impacts in

10   diagnosis, screening and treatment for Alzheimer's dementia in the future.

11

1 Introduction

2 Alzheimer's disease neuropathologic changes in the brain, i.e., accumulation amyloid-β (Aβ)

3 plaques and neurofibrillary tangles containing hyperphosphorylated tau (p-tau), can be detected

4 in living people using positron emission tomography (PET) scanning or quantification of Aβ

5 and p-tau proteins levels in CSF.[1] Although Aβ- and tau-PET as well as CSF Aβ42/40 and p-tau

6 are highly accurate and validated diagnostic and prognostic biomarkers of Alzheimer's disease[2-4]

7 that have been widely used in research settings, blood-based tests are needed for implementation

8 in clinical practice globally and to facilitate patient screening and selection in clinical trials.[3,5]

9

10 In CSF, soluble p-tau species change in different stages and progression of Alzheimer's disease.[6]

11 A growing number of studies have demonstrated that three variants of p-tau, p-tau181, p-tau217

12 and p-tau231, measured in blood plasma hold great promise as biomarkers of Alzheimer's

13 disease related Aβ and tau pathologies.[7-11] At the same time, there are reported differences in the

14 performance of different plasma p-tau species and assays. For example p-tau217 (measured

15 using either mass spectrometry [MS] or immunoassays) has consistently shown higher accuracy

16 for detecting abnormal CSF and PET biomarker status and differentiating Alzheimer's disease

17 from other neurogenerative disorders (in both clinical and neuropathological cohorts) and

18 controls than p-tau181, even though the effect sizes were in many cases relatively small.[7,10,12,13]

19 Some data also suggest that while plasma p-tau231 and p-tau181 perform equally well as

20 diagnostic biomarkers in later dementia phase of Alzheimer's disease, p-tau231 starts to increase

21 earlier than p-tau181 and is more strongly associated with Aβ and tau PET measures in

22 preclinical disease stages.[14-16] However, it is at present unclear how much varying performance

23 of the plasma p-tau biomarkers is attributable to analytical measurement methods. Several

24 immunoassays[17] and an MS-based method[7] have been developed for determination of different

25 p-tau species in plasma and used across different studies making their interpretation challenging.

26 MS is considered as "the gold standard" for protein identification and analysis and although

27 published work shows that MS-based plasma Aβ measures might more accurately reflect brain

28 Aβ pathology in Alzheimer's disease than immunoassays,[18] a direct comparison of these

29 methods for blood p-tau quantification is currently lacking. Some studies, on the other hand,

30 compared several of the available plasma p-tau immunoassays. P-tau217 measured with two

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different immunoassays developed by Lilly Research Laboratories and Janssen Research and Development have both been shown to accurately predict abnormal CSF Aβ status and future conversion to Alzheimer's disease dementia in patients with mild cognitive impairment (MCI).[19] In contrast, a certain degree of variability has been found in performance of different p-tau181 immunoassays[12,20]. Interestingly, differences in the performance between plasma p-tau217 and p-tau181 appears much smaller when both biomarkers are measured with Lilly immunoassays that only differ in phospho-specific capture antibodies compared to the differences between Lilly p-tau217 and other p-tau181 immunoassays.[10,12,13] Collectively, these findings suggest that immunoassay components (e.g., antibodies, other reagents, detection systems) may affect the performance of p-tau biomarkers and illustrate the importance of conducting head-to-head comparisons of different plasma p-tau immunoassays. On the other hand, mass spectrometry measurement of tau peptides generated by trypsinization or other enzymatic digestions may be confounded by the presence of various endogenously produced tau truncated species.[21] Expanding on previous preliminary studies, with the additional aim to compare MS-based methods and immunoassays, we analyzed p-tau181, p-tau217 and p-tau231 using 10 assays in plasma samples from a cohort of MCI patients who were followed for up to 9.5 years to monitor progression of clinical symptoms. We tested the ability of p-tau biomarkers to identify participants with abnormal CSF Aβ status and to predict future progression from MCI to Alzheimer's disease dementia.

Materials and methods

Participants

The study was approved by the Ethics Committee at the University of Lund and the patients and/or their relatives gave their informed consent (for research). We included 135 individuals with clinical diagnosis of MCI at baseline who were recruited at the Memory Clinic at Skåne University Hospital in Malmö, Sweden.[19,22,23] All participants underwent a thorough physical, neurological, and psychiatric examination, as well as a clinical interview focusing on cognitive symptoms and activities of daily living function by physicians with an expertise in cognitive

1 disorders. Patients with MCI at baseline had to fulfill the criteria by Petersen,[24] including (1)

2 memory complaint, preferably corroborated by an informant; (2) objective memory impairment

3 adjusted for age and education, as judged by the physician; (3) preservation of general cognitive

4 functioning, as determined by the clinician's judgment based on a structured interview with the

5 patient and a Mini Mental Status Examination (MMSE) score greater than or equal to 24; (4)

6 zero or minimal impairment of daily life activities; and (5) not fulfilling the DSM-IIIR criteria

7 for dementia. The exclusion criteria were (1) significant unstable systemic illness or organ

8 failure; (2) current significant alcohol or substance misuse; and (3) cognitive impairment that

9 could be explained by other specific non-neurodegenerative disorders such as brain tumor or

10 subdural hematoma. Study participants were followed for an average of 4.9 (SD=2.1) years. The

11 MCI-ADD group included participants who progressed to Alzheimer's disease dementia during

12 follow-up. Patients who received a diagnosis of Alzheimer's disease were required to meet the

13 DSM-IIIR criteria for dementia and the criteria of probable Alzheimer's disease defined by

14 NINCDS-ADRDA[25] and have abnormal CSF Aβ42/40 ratio.[19] The criteria for non-AD dementia

15 diagnosis in this MCI cohort have been previously described.[22,23] Stable MCI (sMCI) patients

16 and MCI who progressed to non-Alzheimer's disease dementia were classified as non-

17 progressors and further stratified into Aβ-negative (A-) and Aβ-positive (A+) groups based on

18 the CSF Aβ42/40 ratio status. The characteristics of the study participants are given in Table 1.

19 ## CSF and plasma sampling and analysis

20 CSF and blood sample were drawn in the morning while participants were not necessarily non-

21 fasting. Blood was collected in six K2-EDTA-plasma tubes and centrifuged at 2000g, +4°C for

22 10 minutes. Following centrifugation plasma was aliquoted into 1.5-ml polypropylene tubes (1

23 ml per tube) and stored at −80°C. CSF was obtained by lumbar puncture and stored at −80°C in

24 polypropylene tubes following the Alzheimer's Association flow chart for lumbar puncture and

25 CSF sample processing.[26] All samples went through one freeze–thaw cycle before the analysis

26 when 0.2-0.5ml were further aliquoted into LoBind tubes. P-tau217 was measured as

27 phosphorylation occupancy at Thr217 using MS assay developed at Washington University (p-

28 tau217[WashU]),[7] Meso Scale Discovery (MSD) immunoassay developed by Lilly Research

29 Laboratories (p-tau217[Lilly]) [10,27] and Single molecule arrays (Simoa) immunoassay developed by

30 Janssen Research and Development (p-tau217[Janss]).[19,28,29] P-tau181 was measured as

phosphorylation occupancy at Thr181 using MS-WashU assays (p-tau181[WashU]),[7] MSD immunoassay developed by Lilly Research Laboratories (p-tau181[Lilly]),[8,30] Simoa immunoassay developed at the University of Gothenburg (p-tau181[UGOT]),[9] Simoa immunoassay developed by ADx Neurosciences (p-tau181[ADx]),[20,31] Lumipulse immunoassay developed by Fujirebio (p-tau181[Fuji]) and Splex immunoassay from MSD (p-tau181[Splex]). P-tau231 was measured using in-house Simoa immunoassay developed at the University of Gothenburg (p-tau231[UGOT]).[14] We also tested p-tau231[Splex] assay from MSD. However, this assay failed to detect any measurable p-tau231 in a pilot study of eight plasma samples (four from Aβ-negative and the other four from Aβ-positive individuals) analyzed across 2 runs and therefore was not included in the present study. P-tau217[Lilly] and p-tau217[Janss] data in overlapping sample have been reported previously.[19] CSF samples (N=78) were analyzed using p-tau217[WashU], p-tau217[Lilly], p-tau217[Janss], p-tau181[WashU], p-tau181[ADx], p-tau181[UGOT], p-tau181[Fuji] and p-tau231[UGOT] assays. CSF Aβ40 and Aβ42 levels were assessed using commercially available MSD immunoassays. Amyloid positivity was defined based on CSF Aβ42/40 and a previously described threshold of 0.07.[22,23] All samples were analyzed by staff blinded to the clinical data. Further details of the p-tau analyses are described in the Supplementary Methods and data on assay performance are shown in Table 2 and Supplementary Figure 1.

Statistical analysis

SPSS (version 28, IBM, Armonk, NY, US) and R (version 4.1.2) in RStudio[32] were used for statistical analysis. Demographic and clinical data were compared with Mann-Whitney U, Kruskal-Wallis and chi-square (sex and *APOE* ε4 positivity) tests. Group differences in the log10-transformed biomarker levels were assessed with univariate general linear models adjusting for age and sex and additionally for duration of follow-up when comparing MCI participants who progressed to Alzheimer's disease dementia with those who did not. In figures, fold changes relative to the mean of the A- sMCI group are presented to aid interpretation of biomarker levels across comparisons. Correlations between CSF and plasma were examined using Spearman test and we used bootstrapping (n=2000 iterations) to test differences in the correlation coefficients. Diagnostic accuracies of CSF biomarkers were assessed using receiver operating characteristic (ROC) curve analysis. The Youden index with bootstrapping (n=2000 iterations) was used to determine sensitivity, specificity and accuracy with 95% confidence

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1 interval (CI) at optimal thresholds. Area under the curve (AUC) of two ROC curves were
2 compared with DeLong test with adjustment for multiple comparisons using Benjamini-
3 Hochberg false discovery rate method.[33] For p-tau181[UGOT] and p-tau181[Splex] assays, plasma
4 samples from 124 and 101 participants, respectively, were analyzed and included in the main
5 analysis. However, we performed a sensitivity analysis in subsamples where all plasma p-tau
6 measures were available. Two-sided $p<0.05$ was considered statistically significant.

7 Data availability

8 Anonymized data will be shared by request from a qualified academic investigator for the sole
9 purpose of replicating procedures and results presented in the article and as long as data transfer
10 is in agreement with EU legislation on the general data protection regulation and decisions by the
11 Ethical Review Board of Sweden and Region Skåne, which should be regulated in a material
12 transfer agreement.

13 Results

14 Participants

15 The study included 45 MCI patients who progressed to AD dementia (MCI-ADD), 64 non-
16 progressors with normal Aβ-status (A-) and 26 A+ non-progressors (Table 1). There were
17 differences in age (H(2)=19.0, $p<0.001$), sex ($\chi^2(2)$=8.1, p=0.018), MMSE (H(2)=30.1, $p<0.001$),
18 *APOE* ε4 carriership ($\chi^2(2)$=33.0, $p<0.001$) and follow-up duration (H(2)=23.3, $p<0.001$)
19 between the groups. The MCI-ADD group was on average older, had lower MMSE and shorter
20 follow-up time than both non-progressor groups ($p<0.001$). There were more women among
21 MCI-ADD compared with A+ non-progressors (p=0.005) and A- non-progressors (p=0.056),
22 whereas *APOE* ε4 positivity rate was lower in A- non-progressors than both A+ non-progressors
23 and MCI-ADD ($p<0.001$)

Associations with Aβ pathology

We first assessed how well plasma p-tau species measured with different assays identified individuals with abnormal baseline Aβ status among all study participants with baseline diagnosis of MCI (Figure 1A, Table 3). In ROC curve analysis, the mass spectrometry-based p-tau217 assay (p-tau217^{WashU}) performed significantly better than all other p-tau biomarkers with AUC of 0.947 (95% CI, 0.907-0.987; p_{diff}<0.015). Among immunoassays, p-tau217^{Lilly} had the highest AUC (AUC=0.886; CI, 0.827-0.944), which was not significantly different from the AUCs of p-tau217^{Janss} (AUC=0.858; 95% CI, 0.795-0.920; $p_{diff=}$0.38), p-tau181^{ADx} (AUC=0.841; 95% CI, 0.768-0.913; $p_{diff=}$0.24) and p-tau181^{WashU} (AUC=0.835; 95% CI, 0.765-0.906; $p_{diff=}$0.20), but higher compared with AUC of p-tau231^{UGOT}, p-tau181^{Lilly}, p-tau181^{UGOT}, p-tau181^{Fuji}, and p-tau181^{Splex} (AUC$_{range}$, 0.642-0.784; p_{diff} ≤0.029). For comparison, the AUCs of the best performing CSF p-tau assays in a subsample of 78 participants with CSF measures available ranged between 0.948 and 0.975 (p-tau217^{WashU}, AUC=0.975; p-tau181^{ADx}, AUC=0.961; p-tau181^{WashU}, AUC=0.954; p-tau217^{Lilly}, AUC=0.952; p-tau217^{Janss}, AUC=0.948). CSF p-tau showed significantly higher AUCs than corresponding plasma p-tau for most assays (Supplementary Table 1).

When testing differences in plasma p-tau levels between A+ and A- groups, we found that all 10 p-tau biomarkers were significantly higher in A+ MCI than A- MCI (Figure 2). However, the fold increase in the A+ group compared with the A- group was largest for the p-tau217^{WashU} (mean=3.6, SD=1.9), followed by p-tau217^{Janss} (mean=2.7, SD=1.8), P-tau217^{Lilly} (mean=2.0, SD=1.0), and p-tau181^{ADx} (mean=1.8, SD=0.8) and ranging between 1.2 and 1.4 for the rest of the biomarkers.

Prediction of future progression to Alzheimer's disease dementia

We next studied the performance of the plasma p-tau biomarkers to predict future clinical progression to Alzheimer's disease dementia (Figure 1B, Table 4). When distinguishing MCI patients who progressed to Alzheimer's disease dementia during follow-up from those who did not, p-tau217^{WashU} again showed significantly higher AUC than all other p-tau biomarkers (AUC=0.932; 95% CI, 0.891-0.974; p_{diff}<0.027) followed by p-tau217^{Lilly} (AUC=0.889; 95% CI,

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1   0.833-0.946). P-tau217^{Janss} (AUC=0.872; 95% CI, 0.814-0.931; $p_{diff=}$0.53), p-tau181^{ADx}

2   (AUC=0.846; 95% CI, 0.777-0.916; $p_{diff=}$0.16) and p-tau181^{WashU} (AUC=0.835; 95% CI, 0.764-

3   0.906; $p_{diff=}$0.09) were non-inferior to p-tau217^{Lilly}, whereas p-tau231^{UGOT}, p-tau181^{Lilly}, p-

4   tau181^{UGOT}, p-tau181^{Fuji}, and p-tau181^{Splex} all had significantly lower AUCs (AUC$_{range}$, 0.688-

5   0.813; p_{diff} ≤0.013). For comparison, the AUCs of the best performing CSF p-tau assays in a

6   subsample of 78 participants with CSF measures available ranged between 0.907 and 0.943 (p-

7   tau217^{WashU}, AUC=0.943; p-tau217^{Janss}, AUC=0.928; p-tau217^{Lilly}, AUC=0.926; p-tau181^{ADx},

8   AUC=0.924; p-tau181^{Fuji}, AUC=0.907). The differences in AUCs between CSF and

9   corresponding plasma p-tau assays were not significant (Supplementary Table 1).

10

11   We also found differences in plasma concentrations of all p-tau biomarkers except p-tau181^{Fuji}

12   between the A- non-progressor, A+ non-progressor and MCI-ADD groups (Figure 3). Post-hoc

13   analysis revealed that plasma levels of p-tau217 (when measured with three different assays), but

14   not p-tau181 or ptau231, were higher in MCI-ADD than A+ non-progressors (p<0.002). At the

15   same time, the three p-tau217 biomarkers as well as the best performing p-tau181 biomarkers (p-

16   tau181^{WashU} and p-tau181^{ADx}) were increased in both A+ non-progressors and MCI-ADD

17   compared with A- non-progressors (p≤0.001). P-tau217^{WashU} showed the largest fold increase in

18   both MCI-ADD (mean$_=$4.3, SD=1.7) and A+ non-progressors (mean$_=$2.5, SD=1.4) compared

19   with A- non-progressors. Fold increase was also larger in MCI-ADD (mean$_{range}$, 2.0-3.2) than in

20   A+ non-progressors (mean$_{range}$, 1.4-1.9) for p-tau217^{Lilly}, p-tau217^{Janss} and p-tau181^{ADx}.

21 Correlations between plasma and CSF p-tau

22   Finally, we examined associations between plasma and CSF p-tau biomarkers (Figure 4). CSF p-

23   tau concentrations are presented in Supplementary Table 2. In line with other results of this

24   study, the strongest correlations between CSF and plasma were seen for p-tau217^{WashU} (R=0.891;

25   95% CI, 0.832-0.930), followed by p-tau217^{Lilly} (R=0.755; 95% CI, 0.635-0.839) with significant

26   difference in correlation coefficients between the two biomarkers (p=0.003). The correlations

27   were weak to moderate for the rest of the biomarkers (R$_{range}$, 0.320-0.669).

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1 Plasma p-tau217^{WashU} correlated strongly with plasma p-tau217^{Lilly}, p-tau217^{Janss}, p-tau181^{ADx}

2 and p-tau181^{WashU} (R$_{range}$, 0.712-0.862; Supplementary Figure 2) while correlations with other

3 plasma p-tau biomarkers were weak to moderate (R$_{range}$, 0.376-0.619; Supplementary Figure 2).

Sensitivity analysis

5 The results were similar when statistical analysis was performed in smaller sub-samples where p-

6 tau181^{UGOT} and p-tau181^{Splex} data were available (Supplementary Tables 3-6). Briefly, plasma p-

7 tau217^{WashU} showed the best performance when detecting both abnormal Aβ status and

8 progression to Alzheimer's disease dementia (AUC$_{range}$, 0.927-0.955), followed by p-tau217^{Lilly}

9 (AUC$_{range}$, 0.878-0.900), p-tau217^{Janss} (AUC$_{range}$, 0.860-0.870), p-tau181^{ADx} (AUC$_{range}$, 0.832-

10 0.860) and p-tau181^{WashU} (AUC$_{range}$, 0.809-0.0.827). None of the AUCs of P-tau231^{UGOT}, p-

11 tau181^{Lilly}, p-tau181^{UGOT}, p-tau181^{Fuji}, p-tau181^{Splex} were consistently above 0.800.

Discussion

13 Recently developed blood tests for Aβ and p-tau are anticipated to transform Alzheimer's disease

14 research and care. Here we sought to directly compare currently available methods for

15 determinations of p-tau in blood in order to establish which of these methods are accurate

16 enough to be useful for implementation in clinical practice or drug trials. In this study including

17 patients with MCI, plasma p-tau217 quantified using MS-based assay showed very high

18 accuracy when both identifying participants with abnormal Aβ status and those who progress to

19 Alzheimer's disease dementia during follow-up with AUCs>0.93 which was higher than for the

20 other p-tau biomarkers. Further, this assay exhibited significantly higher correlations with p-tau

21 levels in CSF than the other p-tau assays. However, p-tau217^{Lilly}, p-tau217^{Janss}, p-tau181^{ADx} and

22 p-tau181^{WashU} all displayed relatively high and consistent accuracy across both outcomes

23 (AUC$_{range}$, 0.835-0.889), whereas the performance of other biomarkers (p-tau231^{UGOT}, p-

24 tau181^{Lilly}, p-tau181^{UGOT}, p-tau181^{Fuji}, p-tau181^{Splex}) was significantly inferior (AUC$_{range}$, 0.642-

25 0.813). Of note, there was no added value of combining different plasma p-tau species (p-

26 tau217^{WashU}, ptau181^{ADx} and p-tau231^{UGOT}) when either distinguishing normal from abnormal

27 Aβ status or predicting future progression to Alzheimer's disease dementia (data not shown).

28

MS-based measure of plasma p-tau217 has previously shown very good accuracy to detect Aβ pathology in 2 mixed cohorts of cognitively healthy controls, MCI participants and patients at different stages of Alzheimer's disease .[7] Using an improved version of the same MS assay (now requiring lower volume of plasma) we demonstrate that p-tau217[WashU] accurately predicted abnormal Aβ status as well as future progression to Alzheimer's disease dementia in a sample of MCI patients. One novel finding of the present study is that MS p-tau217[WashU] performed significantly better than p-tau217 quantified with immunoassays. A possible explanation for this may be that MS-based detection methods are highly accurate and potentially more so than immunoassays, and therefore could more reliably quantify low abundance proteins in protein-rich matrices such as blood as was seen for plasma Aβ.[18]

We also found that p-tau217[WashU] performed better than p-tau181[WashU] corroborating the results of an earlier MS-based study.[7] The higher performance of p-tau217 over p-tau181 has been shown for immunoassays-based p-tau measures[10,12,13] as well as for CSF p-tau217 and p-tau181[34,35] and could be due to the specificity of p-tau217 for Alzheimer's disease (this biomarker is found at considerably lower levels in people without Alzheimer's disease compared to p-tau181) and to a greater dynamic range of p-tau217, i.e. larger fold increase in relation to developing Aβ and tau pathologies. Among eight immunoassays tested in the present study, p-tau217[Lilly] displayed numerically highest AUCs which were significantly different from the AUCs of several p-tau181 biomarkers. However, p-tau217[Lilly], p-tau217[Janss] and p-tau181[ADx] all exhibited comparable accuracies for both abnormal Aβ status and progression to Alzheimer's disease dementia indicating substantial variability in the performance of p-tau181 that is most likely caused by the differences in antibodies and analytical procedures used across the assays.

Our study has several limitations. The overall sample size was moderate with a relatively small number of A+ non-progressor and participant with CSF data which might have affected the analysis. The cohort was restricted to MCI participants and it is possible that the performance of the plasma p-tau assays varies across disease stages warranting future investigations in individuals with preclinical Alzheimer's disease. Nevertheless, our findings in MCI patients are very relevant given that this patient group represent the most likely target population to receive disease-modifying therapies in the clinical settings in the coming years. Replication in more

heterogeneous and ethnically diverse population-based cohorts is also needed. Finally, future larger studies should establish if combining individual plasma p-tau biomarkers with other accessible demographic and clinical measures could further improve their diagnostic and prognostic accuracy as has previously been shown for plasma p-tau217.[36]

In conclusion, we show that there are significant and meaningful differences in the performance of plasma p-tau assays that have to be taken into account when interpreting results from published work. Our data support superior performance of MS p-tau217 to detect abnormal Aβ status and progression to Alzheimer's disease dementia in MCI patients. In addition, we report relatively high and consistent accuracy for several p-tau immunoassays for both outcomes. Overall, our findings indicate that certain MS-based methods and immunoassays might be suitable for implementation in drug trials and clinical practice whereas others require substantial improvement. An important consideration is that compared with immunoassays, currently available research-based MS analytical technologies are more labor intensive and time consuming with less throughput. However, with the development of commercial fully automated MS platforms which have already increased capacity and speed with automated systems, MS platforms can provide reasonable clinical access.

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Competing interests

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, reMYND, Passage Bio, Roche, Samumed,

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Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, 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, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, 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 (outside submitted work).

RJB has received research funding from Avid Radiopharmaceuticals, Janssen, Roche/Genentech, Eli Lilly, Eisai, Biogen, AbbVie, Bristol Myers Squibb, and Novartis. Washington University and RJB have equity ownership interest in C2N Diagnostics. RJB and NRB receive income based on technology (blood plasma assay, and methods of diagnosing AD with phosphorylation changes) licensed by Washington University to C2N Diagnostics. RJB receives income from C2N Diagnostics for serving on the scientific advisory board. RJB serves on the Roche Gantenerumab Steering Committee as an unpaid member.

MJP is an employee of Avid radiopharmaceuticals, a wholly owned subsidiary of Eli Lilly and Company, and is a minor stockholder in Eli Lilly

OH has acquired research support (for the institution) from ADx, AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, Fujirebio, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Amylyx, Alzpath, BioArctic, Biogen, Cerveau, Fujirebio, Genentech, Novartis, Roche, and Siemens. The rest of authors do not report any disclosures.

GTB and HK are employees of Janssen Research and Development.

JV and ES are employees of ADx NeuroSciences.

EVM is a co-founder of ADx NeuroSciences.

MV is an employee of Fujirebio Europe N.V.

Supplementary material

Supplementary material is available at *Brain* online.

References

1.   Scheltens P, Blennow K, Breteler MM, *et al*. Alzheimer's disease. *Lancet*. Jul 30 2016;388(10043):505-17. doi:10.1016/S0140-6736(15)01124-1

2.   Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol Sci*. May 2015;36(5):297-309. doi:10.1016/j.tips.2015.03.002

3.   Hansson O. Biomarkers for neurodegenerative diseases. *Nat Med*. Jun 2021;27(6):954-963. doi:10.1038/s41591-021-01382-x

4.   Ossenkoppele R, van der Kant R, Hansson O. Tau biomarkers in Alzheimer's disease: towards implementation in clinical practice and trials. Rapid Review. *The Lancet Neurology*. Published online May 25, 2022;

5.   Blennow K. Phenotyping Alzheimer's disease with blood tests. *Science*. Aug 6 2021;373(6555):626-628. doi:10.1126/science.abi5208

6.   Barthelemy 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*. Mar 2020;26(3):398-407. doi:10.1038/s41591-020-0781-z

7.   Barthelemy NR, Horie K, Sato C, Bateman RJ. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *J Exp Med*. Nov 2 2020;217(11)doi:10.1084/jem.20200861

8.   Janelidze 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 Medicine*. 2020/03/01 2020;26(3):379-386. doi:10.1038/s41591-020-0755-1

9.   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*. May 2020;19(5):422-433. doi:10.1016/S1474-4422(20)30071-5

10.   Palmqvist S, Janelidze S, Quiroz YT*, et al*. Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA*. Jul 28 2020;doi:10.1001/jama.2020.12134

11. Thijssen EH, La Joie R, Wolf A, *et al*. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nature Medicine*. 2020/03/01 2020;26(3):387-397. doi:10.1038/s41591-020-0762-2

12. Mielke MM, Frank RD, Dage JL, *et al*. Comparison of Plasma Phosphorylated Tau Species With Amyloid and Tau Positron Emission Tomography, Neurodegeneration, Vascular Pathology, and Cognitive Outcomes. *JAMA Neurol*. Sep 1 2021;78(9):1108-1117. doi:10.1001/jamaneurol.2021.2293

13. Thijssen EH, La Joie R, Strom A, *et al*. Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. *Lancet Neurol*. Sep 2021;20(9):739-752. doi:10.1016/S1474-4422(21)00214-3

14. Ashton NJ, Pascoal TA, Karikari TK, *et al*. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol*. May 2021;141(5):709-724. doi:10.1007/s00401-021-02275-6

15. Meyer PF, Ashton NJ, Karikari TK, *et al*. Plasma p-tau231, p-tau181, PET Biomarkers, and Cognitive Change in Older Adults. *Ann Neurol*. Apr 2022;91(4):548-560. doi:10.1002/ana.26308

16. Smirnov DS, Ashton NJ, Blennow K, *et al*. Plasma biomarkers for Alzheimer's Disease in relation to neuropathology and cognitive change. *Acta Neuropathol*. Feb 23 2022;doi:10.1007/s00401-022-02408-5

17. Leuzy A, Mattsson-Carlgren N, Palmqvist S, Janelidze S, Dage JL, Hansson O. Blood-based biomarkers for Alzheimer's disease. *EMBO Mol Med*. Jan 11 2022;14(1):e14408. doi:10.15252/emmm.202114408

18. Janelidze S, Teunissen CE, Zetterberg H, *et al*. Head-to-Head Comparison of 8 Plasma Amyloid-beta 42/40 Assays in Alzheimer Disease. *JAMA Neurol*. Nov 1 2021;78(11):1375-1382. doi:10.1001/jamaneurol.2021.3180

19. Groot C, Cicognola C, Bali D, *et al*. Diagnostic and prognostic performance to detect Alzheimer's disease and clinical progression of a novel assay for plasma p-tau217. *Alzheimers Res Ther*. 2022;In press

20. Bayoumy S, Verberk IMW, den Dulk B, *et al*. Clinical and analytical comparison of six Simoa assays for plasma P-tau isoforms P-tau181, P-tau217, and P-tau231. *Alzheimers Res Ther*. Dec 4 2021;13(1):198. doi:10.1186/s13195-021-00939-9

21. Hansson K, Dahlen R, Hansson O*, et al*. Use of the tau protein-to-peptide ratio in CSF to improve diagnostic classification of Alzheimer's disease. *Clin Mass Spectrom*. Nov 2019;14 Pt B:74-82. doi:10.1016/j.clinms.2019.07.002

22. Cicognola C, Janelidze S, Hertze J, *et al*. Plasma glial fibrillary acidic protein detects Alzheimer pathology and predicts future conversion to Alzheimer dementia in patients with mild cognitive impairment. *Alzheimers Res Ther*. Mar 27 2021;13(1):68. doi:10.1186/s13195-021-00804-9

23. Hertze J, Minthon L, Zetterberg H, Vanmechelen E, Blennow K, Hansson O. Evaluation of CSF biomarkers as predictors of Alzheimer's disease: a clinical follow-up study of 4.7 years. *J Alzheimers Dis*. 2010;21(4):1119-28. doi:10.3233/jad-2010-100207

24. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. Sep 2004;256(3):183-94. doi:10.1111/j.1365-2796.2004.01388.x

25. 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*. May 2011;7(3):263-9. doi:10.1016/j.jalz.2011.03.005

26. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol*. Mar 2010;6(3):131-44. doi:10.1038/nrneurol.2010.4

27. Janelidze S, Palmqvist S, Leuzy A, *et al*. Detecting amyloid positivity in early Alzheimer's disease using combinations of plasma Aβ42/Aβ40 and P-tau. *Alzheimer's & Dementia*. 2021;In press

28. Dore V, Doecke JD, Saad ZS, *et al*. Plasma p217+tau versus NAV4694 amyloid and MK6240 tau PET across the Alzheimer's continuum. *Alzheimers Dement (Amst)*. 2022;14(1):e12307. doi:10.1002/dad2.12307

29. Triana-Baltzer G, Moughadam S, Slemmon R, *et al*. Development and validation of a high-sensitivity assay for measuring p217+tau in plasma. *Alzheimers Dement (Amst)*. 2021;13(1):e12204. doi:10.1002/dad2.12204

30. 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*. Aug 2018;14(8):989-997. doi:10.1016/j.jalz.2018.02.013

1   31.   De Meyer S, Vanbrabant J, Schaeverbeke JM, *et al*. Phospho-specific plasma p-tau181
2   assay detects clinical as well as asymptomatic Alzheimer's disease. *Ann Clin Transl Neurol*. May
3   2022;9(5):734-746. doi:10.1002/acn3.51553

4   32.   Team RC. R: A Language and Environment for Statistical Computing. *R Foundation for*
5   *Statistical Computing, Vienna, Austria*. 2014;URL http://www.R-project.org/

6   33.   Robin X, Turck N, Hainard A, *et al*. pROC: an open-source package for R and S+ to
7   analyze and compare ROC curves. *BMC Bioinformatics*. Mar 17 2011;12:77. doi:10.1186/1471-
8   2105-12-77

9   34.   Barthelemy NR, Bateman RJ, Hirtz C, *et al*. Cerebrospinal fluid phospho-tau T217
10   outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET
11   amyloid-positive patient identification. *Alzheimers Res Ther*. Mar 17 2020;12(1):26.
12   doi:10.1186/s13195-020-00596-4

13   35.   Janelidze S, Stomrud E, Smith R, *et al*. Cerebrospinal fluid p-tau217 performs better than
14   p-tau181 as a biomarker of Alzheimer's disease. *Nat Commun*. Apr 3 2020;11(1):1683.
15   doi:10.1038/s41467-020-15436-0

16   36.   Palmqvist S, Tideman P, Cullen N, *et al*. Prediction of future Alzheimer's disease
17   dementia using plasma phospho-tau combined with other accessible measures. *Nat Med*. Jun
18   2021;27(6):1034-1042. doi:10.1038/s41591-021-01348-z
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Figure legends

Figure 1 ROC curve analysis for abnormal CSF Aβ42/40 status and progression to Alzheimer's disease dementia. Receiver operating characteristic (ROC) curve analysis for differentiating (**A**) mild cognitive impairment (MCI) participants with abnormal CSF amyloid-β (Aβ)42/40 from those with normal CSF Aβ42/40 and (**B**) MCI patients who progressed to Alzheimer's disease dementia during follow-up from those who did not (stable MCI patients and MCI patients who progressed to other types of dementia).

Figure 2 Plasma p-tau biomarkers in amyloid-negative and -positive MCI patients. Plasma levels of phosphorylated tau (p-tau)217 (**A-C**), p-tau181 (**D-E, G-J**) and p-tau231 (**F**) measured using different assays in the amyloid-β (Aβ) negative (A-) and A+ mild cognitive impairment (MCI) groups. Aβ status was defined based on the CSF Aβ42/40 ratio. Data are presented as a fold change from the mean of the A- MCI group. Two p-tau217^{WashU} and p-tau217^{Janss} outliers in the A+ group and one p-tau181^{ADx} outlier in the A- group are not shown in (**A**), (**C**) and (**D**) but these data were included in the statistical analysis. F-values and p-values are from univariate general linear models adjusted for age and sex. Boxes show interquartile range, the horizontal lines are medians and the whiskers and outliers were plotted using the Tukey method.

Figure 3 Plasma p-tau biomarkers in MCI participants who progressed to Alzheimer's disease dementia during follow-up and amyloid-negative and -positive non-progressors. Plasma levels of phosphorylated tau (p-tau)217 (**A-C**), p-tau181 (**D-E, G-J**) and p-tau231 (**F**) measured using different assays in patients with mild cognitive impairment (MCI) who progressed to Alzheimer's disease dementia during follow-up (MCI-ADD), amyloid-β negative (A-) and A+ non-progressor MCI patients. Aβ status was defined based on the CSF Aβ42/40 ratio. Data are presented as a fold change from the mean of the A- MCI group. Two p-tau217^{WashU} and p-tau217^{Janss} outliers in the MCI-ADD group and one p-tau181^{ADx} outlier in the A- group are not shown in (**A**), (**C**) and (**D**) but these data were included in the statistical analysis. F-values and p-values are from univariate general linear models adjusted for age, sex

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1 and follow-up time. Boxes show interquartile range, the horizontal lines are medians and the
2 whiskers and outliers were plotted using the Tukey method.

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4 **Figure 4 Correlations between CSF and plasma p-tau.** Heatmap showing Spearman
5 coefficients for correlations between plasma CSF and plasma p-tau measured using different
6 assays (p-tau181^{UGOT}, N=72; p-tau181^{Splex}, N=52; all other biomarker N=78). Correlations
7 between plasma and CSF p-tau measured with the same assay are highlighted in orange except
8 plasma p-tau181^{Lilly} and p-tau181^{Splex} for which corresponding CSF assay data were not
9 available.

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1 **Table 1 Demographic and clinical characteristics**

	Overall	Non-progressors A−[a]	Non-progressors A+[a]	MCI-ADD
N	135	64	26	45
Age, years	74.0 (66.0–79.0)	70.5 (63.0–76.8)	72.0 (65.0–76.0)	78.0 (73.5–81.0)
Female, n (%)	82 (60.7)	37 (57.8)	11 (42.3)	34 (75.6)
MMSE	28.0 (26.0–29.0)	28.0 (27.0–29.0)	28.0 (27.0–29.3)	26.0 (25.0–27.0)
APOE ε4 positivity, n (%)	75 (55.6)	19 (29.7)	20 (76.9)	36 (80.0)
Follow-up time, years	4.6 (3.3–6.6)	6.21 (4.02–7.21)	5.16 (3.90–6.64)	3.64 (2.68–4.65)
Plasma p-tau				
p-tau217[WashU], %	1.36 (0.742–3.25)	0.753 (0.614–0.951)	1.88 (1.27–2.73)	3.49 (2.91–4.73)
p-tau217[Lilly], pg/ml[b]	0.247 (0.170–0.404)	0.177 (0.146–0.201)	0.275 (0.200–0.359)	0.442 (0.330–0.532)
p-tau217[Janss], pg/ml[b]	0.055 (0.030–0.105)	0.034 (0.020–0.049)	0.066 (0.036–0.104)	0.109 (0.077–0.173)
p-tau181[ADx], pg/ml	29.7 (19.3–46.3)	19.5 (10.4–27.3)	30.0 (22.8–45.0)	46.3 (38.8–63.7)
p-tau181[WashU], %	23.5 (19.8–28.7)	20.3 (18.2–22.7)	24.5 (20.7–29.0)	28.4 (25.7–32.1)
p-tau231[UGOT], pg/ml	20.9 (15.7–27.3)	16.8 (12.7–21.4)	22.0 (17.6–27.2)	26.9 (22.6–33.1)
p-tau181[Lilly], pg/ml	1.90 (1.42–2.59)	1.57 (1.20–1.90)	1.77 (1.49–2.26)	2.59 (2.04–3.30)
p-tau181[UGOT], pg/ml[c]	2.46 (1.72–3.55)	1.88 (1.49–2.58)	2.43 (1.89–3.45)	3.38 (2.58–4.07)
p-tau181[Fuji], pg/ml	4.80 (3.64–5.75)	3.83 (3.01–5.14)	4.73 (3.74–5.57)	5.61 (4.77–6.25)
p-tau181[Splex], pg/ml[c]	1.07 (0.859–1.55)	0.999 (0.792–1.22)	0.927 (0.754–1.73)	1.28 (1.05–2.16)

2 A=amyloid-β; ADD=Alzheimer's disease dementia; MCI=mild cognitive impairment; MMSE=Mini Mental State Examination; p-
3 tau=phosphorylated tau.

4 [a]Aβ status was defined using the CSF Aβ42/40 cutoff (0.07) as described in the methods. Data are shown as median (IQR) unless otherwise
5 specified.

6 [b]p-tau217[Lilly] and p-tau217[Janss] data in overlapping sample have been reported previously.[19]

7 [c]p-tau181-UGOT and p-tau181-Splex data were available for 124 and 101 participants, respectively.

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1   **Table 2 Analytical performance of plasma p-tau assays**

Plasma biomarkers	Required plasma volume, ml	Intra-assay CV, %	Inter-assay CV, %	Samples below LLOD, %	LLOD, pg/ml
p-tau217[WashU]	1[a]	3.3[b]	3.5[b]	0	NA[c]
p-tau217[Lilly]	0.07	6.8	10.1	15.6	0.150
p-tau217[Janss]	0.2	23.7	12.4	0	0.013
p-tau181[ADx]	0.1	11.1	3.8	16.3	2.312
p-tau181[WashU]	1[a]	3.7[b]	0.4[b]	0	NA[c]
p-tau231[UGOT]	0.8	7.6	8.5	0	1
p-tau181[Lilly]	0.07	6.0	11.2	0	0.864
p-tau181[UGOT]	0.8	8.2	10.9	0	0.5
p-tau181[Fuji]	0.13	NA[d]	NA[d]	0	0.052
p-tau181[Splex]	0.06	4.8	13.5	0	0.190

2   CV=coefficient of variation; LLOD=lower limit of detection; p-tau=phosphorylated tau.

3   [a]1ml was required for the entire multiplex assay.

4   [b]CVs were estimated using quality control samples; study samples were tested in singlicate.

5   [c]Not applicable for phosphorylation occupancy measures.

6   [d]Not applicable, samples in this study were tested in singlicate in one run.

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1 **Table 3 Associations of plasma p-tau with CSF Aβ42/40**

Plasma p-tau	AUC (95% CI)		P-value versus p-tau217[WashU]	P-value versus p-tau217[Lilly]	Specificity (95% CI)		Sensitivity (95% CI)		Accuracy (95% CI)
p-tau217[WashU]	0.947	(0.907–0.987)	NA	0.015	90.6	(82.8–98.4)	94.4	(84.5–98.6)	92.6 (88.1–96.3)
p-tau217[Lilly]	0.886	(0.827–0.944)	0.015	NA	84.4	(71.9–96.9)	85.9	(67.6–95.8)	84.4 (78.5–90.4)
p-tau217[Janss]	0.858	(0.795–0.920)	0.004	0.38	87.5	(65.6–95.3)	74.6	(60.6–91.5)	80.0 (73.3–86.7)
p-tau181[ADx]	0.841	(0.768–0.913)	<0.001	0.24	85.9	(68.8–95.3)	77.5	(66.2–93.0)	81.5 (74.8–87.4)
p-tau181[WashU]	0.835	(0.765–0.906)	<0.001	0.20	87.5	(73.4–95.3)	76.1	(64.8–88.7)	81.5 (74.8–87.4)
p-tau231[UGOT]	0.784	(0.703–0.864)	<0.001	0.029	73.4	(46.9–87.5)	78.9	(64.8–98.6)	76.3 (69.6–82.2)
p-tau181[Lilly]	0.759	(0.676–0.841)	<0.001	<0.001	78.1	(65.6–89.1)	71.8	(60.6–84.5)	75.6 (68.1–82.2)
p-tau181[UGOT a]	0.743	(0.652–0.833)	<0.001	0.005	70.2	(50.9–86.0)	79.1	(59.7–92.5)	74.2 (66.9–81.5)
p-tau181[Fuji]	0.694	(0.604–0.784)	<0.001	<0.001	56.3	(40.6–85.9)	84.5	(50.7–93.0)	69.6 (62.2–76.3)
p-tau181[Splex a]	0.642	(0.533–0.751)	<0.001	<0.001	79.6	(22.4–98.0)	53.8	(26.9–100.0)	65.3 (58.4–73.3)

2 Data are from ROC curve analysis. MCI participants were classified as amyloid-negative (n=64) or as amyloid-positive (n=71) using CSF
3 Aβ42/40 as described in the methods. AUC=area under the curve; CSF = cerebrospinal fluid; CI=confidence interval; MCI=mild cognitive
4 impairment; ROC=Receiver Operating Characteristic; p-tau=phosphorylated tau.

5 ªp-tau181-UGOT and p-tau181-Splex data were available for 124 (57 amyloid-negative, 67 amyloid-positive) and 101 (49 amyloid-negative, 52
6 amyloid-positive) participants, respectively.

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1 **Table 4 Associations of plasma p-tau with future progression to Alzheimer's disease dementia**

Plasma p-tau	AUC (95% CI)	P-value versus p-tau217^WashU	P-value versus p-tau217^Lilly	Specificity (95% CI)	Sensitivity (95% CI)	Accuracy (95% CI)
p-tau217^WashU	0.932 (0.891–0.974)	NA	0.027	86.7 (77.8–93.3)	95.6 (84.4–100.0)	88.9 (83.7–94.1)
p-tau217^Lilly	0.889 (0.833–0.946)	0.027	NA	83.3 (65.6–93.3)	88.9 (73.3–100.0)	84.4 (75.6–90.4)
p-tau217^Janss	0.872 (0.814–0.931)	0.027	0.53	74.4 (61.1–91.1)	91.1 (71.1–100.0)	80.0 (71.9–87.4)
p-tau181^ADx	0.846 (0.777–0.916)	0.007	0.16	81.1 (72.2–88.9)	91.1 (80.0–97.8)	84.4 (77.8–90.4)
p-tau181^WashU	0.835 (0.764–0.906)	0.001	0.09	76.7 (64.4–86.7)	88.9 (77.8–97.8)	80.7 (72.6–86.7)
p-tau181^Lilly	0.813 (0.734–0.892)	0.002	0.013	74.4 (60.0–86.7)	86.7 (71.1–97.8)	77.8 (70.4–85.2)
p-tau231^UGOT	0.777 (0.699–0.856)	<0.001	0.009	68.9 (57.8–81.1)	86.7 (73.3–95.6)	74.8 (67.4–81.5)
p-tau181^UGOT a	0.775 (0.692–0.858)	<0.001	0.014	65.9 (52.4–82.9)	88.1 (69.0–97.6)	73.4 (64.5–81.5)
p-tau181^Fuji	0.735 (0.649–0.821)	<0.001	0.002	70.0 (40.0–86.7)	75.6 (53.3–97.8)	71.1 (57.8–79.3)
p-tau181^Splex a	0.688 (0.579–0.796)	<0.001	<0.001	66.7 (50.0–90.9)	74.3 (42.9–91.4)	69.3 (59.4–78.2)

2 Data are from ROC curve analysis. 45 MCI participants progressed to Alzheimer's disease dementia during follow-up and 90 remained stable or
3 progressed to non-Alzheimer's disease dementia. AUC=area under the curve; CI=confidence interval; MCI=mild cognitive impairment;
4 ROC=Receiver Operating Characteristic; p-tau=phosphorylated tau

5 ^a p-tau181-UGOT and p-tau181-Splex data were available for 124 (82 non-progressors, 42 MCI-ADD) and 101 (66 non-progressors, 35 MCI-
6 ADD) participants, respectively.

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A **A- MCI vs A+ MCI**
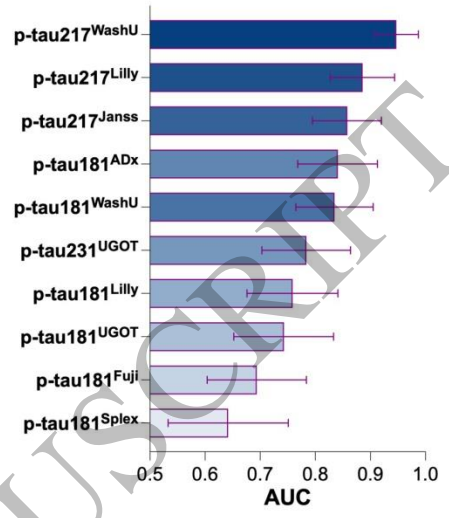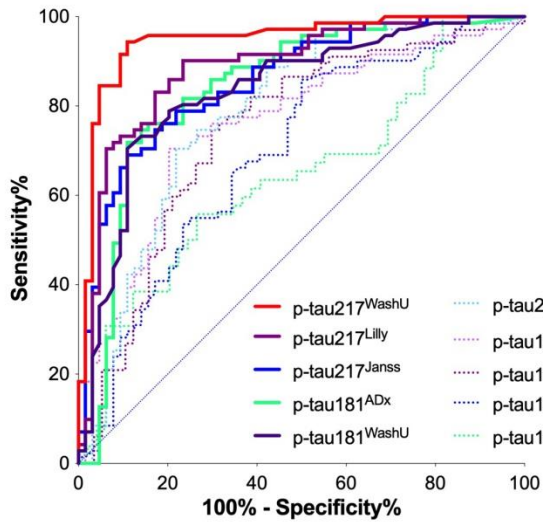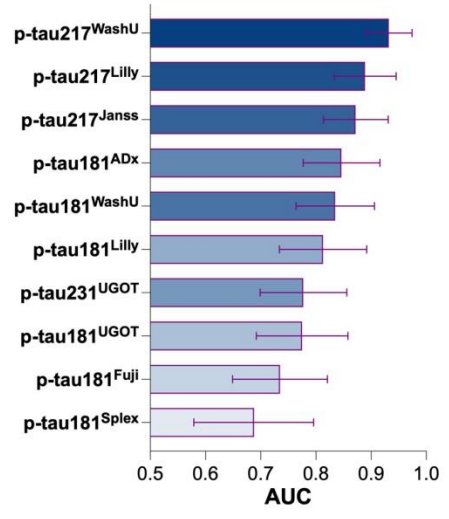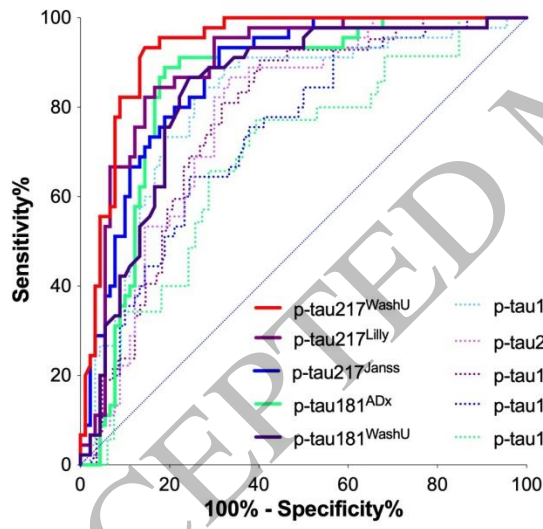

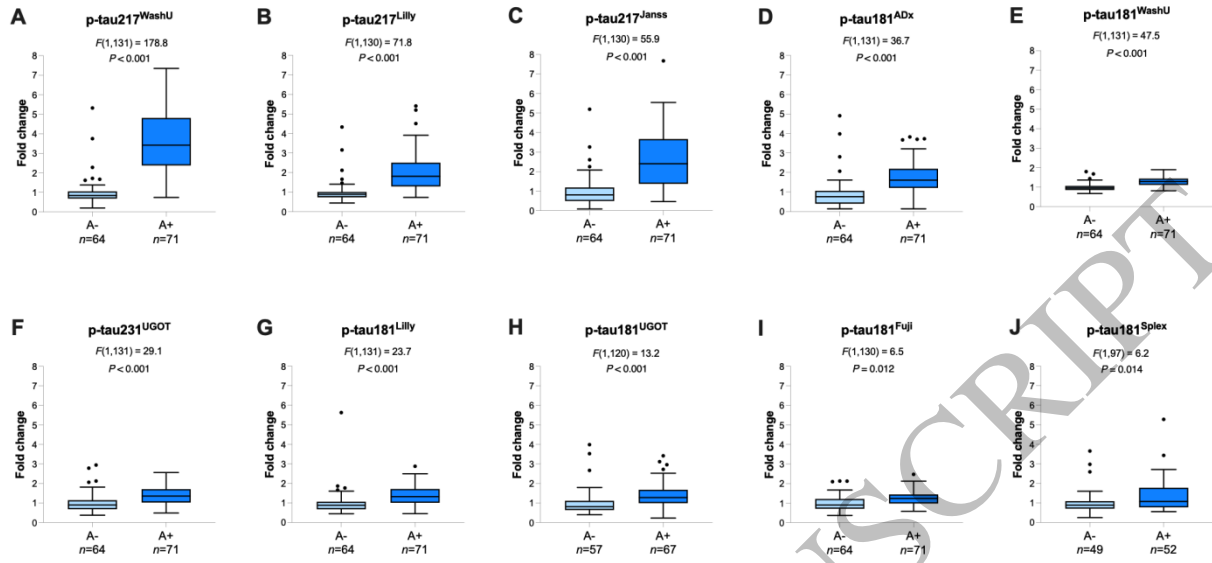**B** **Non-progressors vs progressors**

Figure 1
199x204 mm (.33 x DPI)

Figure 2
185x85 mm (.33 x DPI)

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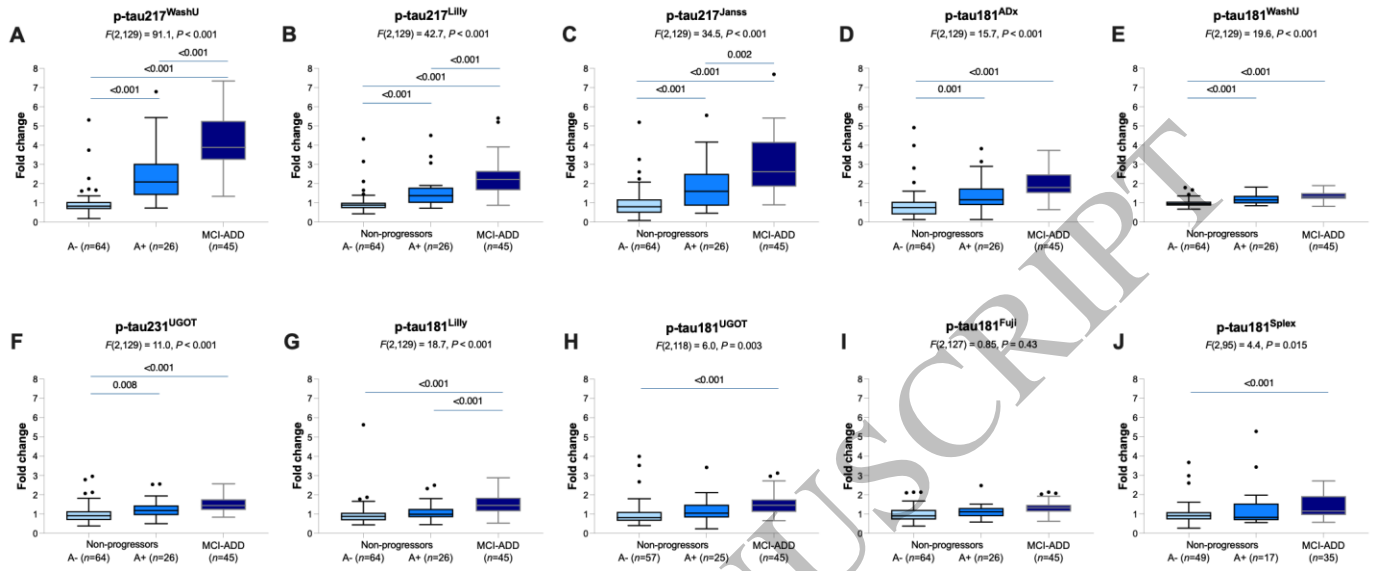
Figure 3
185x76 mm (.33 x DPI)

5

CSF

	p-tau217^{WashU}	p-tau217^{Lilly}	p-tau217^{Janss}	p-tau181^{ADx}	p-tau181^{WashU}	p-tau231^{UGOT}	p-tau181^{UGOT}	p-tau181^{Fuji}
p-tau217^{WashU}	0.891	0.858	0.837	0.836	0.789	0.682	0.696	0.766
p-tau217^{Lilly}	0.805	0.755	0.747	0.722	0.754	0.630	0.643	0.654
p-tau217^{Janss}	0.700	0.682	0.659	0.619	0.645	0.487	0.513	0.589
p-tau181^{ADx}	0.685	0.665	0.667	0.669	0.633	0.597	0.592	0.571
p-tau181^{WashU}	0.716	0.681	0.659	0.648	0.580	0.521	0.544	0.621
p-tau231^{UGOT}	0.427	0.421	0.406	0.376	0.366	0.320	0.320	0.368
p-tau181^{UGOT}	0.436	0.449	0.469	0.420	0.369	0.414	0.410	0.441
p-tau181^{Fuji}	0.421	0.384	0.368	0.362	0.329	0.257	0.288	0.372
p-tau181^{Lilly}	0.535	0.537	0.512	0.490	0.547	0.427	0.423	0.445
p-tau181^{Splex}	0.279	0.327	0.291	0.308	0.255	0.316	0.340	0.321

(Row axis labeled "Plasma")

1

2 **Figure 4**

3 **90x87 mm (.33 x DPI)**

ACCEPTED MANUSCRIPT