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

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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 (Aβ) 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 $Aβ_{42/40}$) 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 ($p\text{-tau217}^{WashU}$) exhibited significantly better performance than all other plasma p-tau biomarkers when detecting abnormal Aβ status [area under curve (AUC) = 0.947; $P_{diff} < 0.015$] or progression to Alzheimer's dementia (AUC = 0.932; $P_{diff} < 0.027$). Among immunoassays, $p\text{-tau217}^{Lilly}$ had the highest AUCs (0.886–0.889), which was not significantly different from the AUCs of $p\text{-tau217}^{Janss}$, $p\text{-tau181}^{ADx}$ and $p\text{-tau181}^{WashU}$ ($AUC_{range}$ 0.835–0.872; $P_{diff} > 0.09$), but higher compared with AUC of $p\text{-tau231}^{UGOT}$, $p\text{-tau181}^{Lilly}$, $p\text{-tau181}^{UGOT}$, $p\text{-tau181}^{Fuji}$ and $p\text{-tau181}^{Splex}$ ($AUC_{range}$ 0.642–0.813; $P_{diff} \leq 0.029$). Correlations between plasma and CSF values were strongest for $p\text{-tau217}^{WashU}$ (R = 0.891) followed by $p\text{-tau217}^{Lilly}$ (R = 0.755; $P_{diff} = 0.003$ versus $p\text{-tau217}^{WashU}$) and weak to moderate for the rest of the p-tau biomarkers ($R_{range}$ 0.320–0.669). In conclusion, our findings suggest that among all tested plasma p-tau assays, mass spectrometry-based measures of p-tau217 perform best when identifying mild cognitive impairment patients with abnormal brain Aβ or those who will subsequently progress to Alzheimer's dementia. Several other assays ($p\text{-tau217}^{Lilly}$, $p\text{-tau217}^{Janss}$, $p\text{-tau181}^{ADx}$ and $p\text{-tau181}^{WashU}$) showed relatively high and consistent accuracy across both outcomes. The results further indicate that the highest performing assays have performance metrics that rival the gold standards of Aβ-PET and CSF. If further validated, our findings will have significant impacts in diagnosis, screening and treatment for Alzheimer's dementia in the future.

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## Introduction

Alzheimer's disease neuropathologic changes in the brain, i.e. accumulation amyloid-β (Aβ) plaques and neurofibrillary tangles containing hyperphosphorylated tau (p-tau), can be detected in living people using PET scanning or quantification of Aβ and p-tau proteins levels in CSF.[1] Although Aβ- and tau-PET as well as CSF $Aβ_{42/40}$ and p-tau are highly accurate and validated diagnostic and prognostic biomarkers of Alzheimer's disease[2–4] that have been widely used in research settings, blood-based tests are needed for implementation in clinical practice globally and to facilitate patient screening and selection in clinical trials.[3,5]

In CSF, soluble p-tau species change in different stages and progression of Alzheimer's disease.[6] A growing number of studies have demonstrated that three variants of p-tau, p-tau181, p-tau217 and p-tau231, measured in blood plasma hold great promise as biomarkers of Alzheimer's disease-related Aβ and tau pathologies.[7–11] At the same time, there are reported differences in the performance of different plasma p-tau species and assays. For example, p-tau217 [measured using either mass spectrometry (MS) or immunoassays] has consistently shown higher accuracy for detecting abnormal CSF and PET biomarker status and differentiating Alzheimer's disease from other neurodegenerative disorders (in both clinical and neuropathological cohorts) and controls than p-tau181, even though the effect sizes were in many cases relatively small.[7,10,12,13] Some data also suggest that while plasma p-tau231 and p-tau181 perform equally well as diagnostic biomarkers in later dementia phase of Alzheimer's disease, p-tau231 starts to increase earlier than p-tau181 and is more strongly associated with Aβ and tau PET measures in preclinical disease stages.[14–16] However, it is at present unclear how much varying performance of the plasma p-tau biomarkers is attributable to analytical measurement methods. Several immunoassays[17] and an MS-based method[7] have been developed for determination of different p-tau species in plasma and used across different studies making their interpretation challenging. MS is considered to be the 'gold standard' for protein identification and analysis and, although published work shows that MS-based plasma Aβ measures might more accurately reflect brain Aβ pathology in Alzheimer's disease than immunoassays,[18] a direct comparison of these methods for blood p-tau quantification is currently lacking. Some studies, on the other hand, compared several of the available plasma p-tau immunoassays. P-tau217 measured with two 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 (ADD) 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, MS 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 analysed 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 ADD.

# 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 disorders. Patients with MCI at baseline had to fulfill the criteria by Petersen,[24] including (i) memory complaint, preferably corroborated by an informant; (ii) objective memory impairment adjusted for age and education, as judged by the physician; (iii) preservation of general cognitive functioning, as determined by the clinician's judgement based on a structured interview with the patient and a Mini-Mental Status Examination (MMSE) score ≥24; (iv) zero or minimal impairment of daily life activities; and (v) not fulfilling the DSM-IIIR criteria for dementia. The exclusion criteria were (i) significant unstable systemic illness or organ failure; (ii) current significant alcohol or substance misuse; and (iii) cognitive impairment that could be explained by other specific non-neurodegenerative disorders such as brain tumour or subdural haematoma. Study participants were followed for an average of 4.9 (SD = 2.1) years. The MCI-ADD group included participants who progressed to ADD during follow-up. Patients who received a diagnosis of Alzheimer's disease were required to meet the DSM-IIIR criteria for dementia and the criteria of probable Alzheimer's disease defined by NINCDS-ADRDA[25] and have abnormal CSF $A\beta_{42/40}$ ratio.[19] The criteria for non-ADD diagnosis in this MCI cohort have been previously described.[22,23] Stable MCI patients and MCI who progressed to non-ADD were classified as non-progressors and further stratified into Aβ-negative (A–) and Aβ-positive (A+) groups based on the CSF $A\beta_{42/40}$ ratio status. The characteristics of the study participants are given in Table 1.

## CSF and plasma sampling and analysis

CSF and blood sample were drawn in the morning while participants were not necessarily non-fasting. Blood was collected in six K2-EDTA-plasma tubes and centrifuged at 2000*g*, +4°C for 10 min. Following centrifugation plasma was aliquoted into 1.5-ml polypropylene tubes (1 ml per tube) and stored at −80°C. CSF was obtained by lumbar puncture and stored at −80°C in polypropylene tubes following the Alzheimer's Association flow chart for lumbar puncture and CSF sample processing.[26] All samples went through one freeze–thaw cycle before the analysis when 0.2–0.5ml were further aliquoted into LoBind tubes. P-tau217 was measured as phosphorylation occupancy at Thr217 using MS assay developed at Washington University ($p\text{-tau217}^{WashU}$),[7] Meso Scale Discovery (MSD) immunoassay developed by Lilly Research Laboratories ($p\text{-tau217}^{Lilly}$) [10,27] and Single molecule arrays (Simoa) immunoassay developed by Janssen Research and Development ($p\text{-tau217}^{Janss}$).[19,28,29] P-tau181 was measured as phosphorylation occupancy at Thr181 using MS-WashU assays ($p\text{-tau181}^{WashU}$),[7] MSD immunoassay developed by Lilly Research Laboratories ($p\text{-tau181}^{Lilly}$),[8,30] Simoa immunoassay developed at the University of Gothenburg ($p\text{-tau181}^{UGOT}$),[9] Simoa immunoassay developed by ADx Neurosciences ($p\text{-tau181}^{ADx}$),[20,31] Lumipulse immunoassay developed by Fujirebio ($p\text{-tau181}^{Fuji}$) and Splex immunoassay from MSD ($p\text{-tau181}^{Splex}$). P-tau231 was measured using in-house Simoa immunoassay developed at the University of Gothenburg ($p\text{-tau231}^{UGOT}$).[14] We also tested a $p\text{-tau231}^{Splex}$ 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) analysed across two runs, and therefore was not included in the present study. $p\text{-tau217}^{Lilly}$ and $p\text{-tau217}^{Janss}$ data in overlapping sample have been reported previously.[19] CSF samples ($n = 78$) were analysed using $p\text{-tau217}^{WashU}$, $p\text{-tau217}^{Lilly}$, $p\text{-tau217}^{Janss}$, $p\text{-tau181}^{WashU}$, $p\text{-tau181}^{ADx}$, $p\text{-tau181}^{UGOT}$, $p\text{-tau181}^{Fuji}$ and $p\text{-tau231}^{UGOT}$ assays. CSF $A\beta_{40}$ and $A\beta_{42}$ levels were assessed using commercially available MSD immunoassays. Amyloid positivity was defined based on CSF $A\beta_{42/40}$ and a previously described threshold of 0.07.[22,23] All samples were analysed by staff blinded to the clinical data. Further details of the p-tau analyses are described in the Supplementary material and data on assay performance are shown in Table 2 and Supplementary Fig. 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 $\chi^2$ (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 ADD with those who did not. In figures, fold changes relative to the mean of the A– stable MCI group are presented to aid interpretation of biomarker levels across comparisons. Correlations between CSF and plasma were examined using the 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 interval (CI) at optimal thresholds. Area under the curve (AUC) of two ROC curves were compared with a DeLong test with adjustment for multiple comparisons using the Benjamini–Hochberg false discovery rate method.[33] For $p\text{-tau181}^{UGOT}$ and $p\text{-tau181}^{Splex}$ assays, plasma samples from 124 and 101 participants, respectively, were analysed and included in the main analysis. However, we performed a sensitivity analysis in subsamples where all plasma p-tau measures were available. Two-sided $P < 0.05$ was considered statistically significant.

## Data availability

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

# Results

## Participants

The study included 45 MCI patients who progressed to ADD (MCI-ADD), 64 non-progressors with normal Aβ– status (A–) and 26 A+ non-progressors (Table 1). There were differences in age [$H(2) = 19.0$, $P < 0.001$], sex [$\chi^2(2) = 8.1$, $P = 0.018$], MMSE [$H(2) = 30.1$, $P < 0.001$], *APOE* ε4 carriership [$\chi^2(2) = 33.0$, $P < 0.001$] and follow-up

**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[b] | 1.07 (0.859–1.55) | 0.999 (0.792–1.22) | 0.927 (0.754–1.73) | 1.28 (1.05–2.16) |

Data are shown as median (interquartile range) unless otherwise specified.
[a]Aβ status was defined using the CSF $Aβ_{42/40}$ cutoff (0.07) as described in the 'Materials and methods' section
[b]p-tau217$^{Lilly}$ and p-tau217$^{Janss}$ data in overlapping sample have been reported previously.[19]
[c]p-tau181-UGOT and p-tau181-Splex data were available for 124 and 101 participants, respectively.

**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$^{UGOT}$ | 0.08 | 7.6 | 8.5 | 0 | 1 |
| p-tau181$^{Lilly}$ | 0.07 | 6.0 | 11.2 | 0 | 0.864 |
| p-tau181$^{UGOT}$ | 0.08 | 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 |

CV = coefficient of variation; LLOD = lower limit of detection.
[a]One millilitre was required for the entire multiplex assay.
[b]CVs were estimated using quality control samples; study samples were tested in singlicate.
[c]Not applicable for phosphorylation occupancy measures.
[d]Not applicable, samples in this study were tested in singlicate in one run.

duration [H(2) = 23.3, $P < 0.001$] between the groups. The MCI-ADD group was on average older, had lower MMSE and shorter follow-up time than both non-progressor groups ($P < 0.001$). There were more women among MCI-ADD compared with A+ non-progressors ($P = 0.005$) and A– non-progressors ($P = 0.056$), whereas *APOE* ϵ4 positivity rate was lower in A– non-progressors than both A+ non-progressors 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 (Fig. 1A and Table 3). In the ROC curve analysis, the MS-based p-tau217 assay (p-tau217$^{WashU}$) performed significantly better than all other p-tau biomarkers with an 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; $P_{diff} \leq 0.029$). 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 (Fig. 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$^{Janss}$

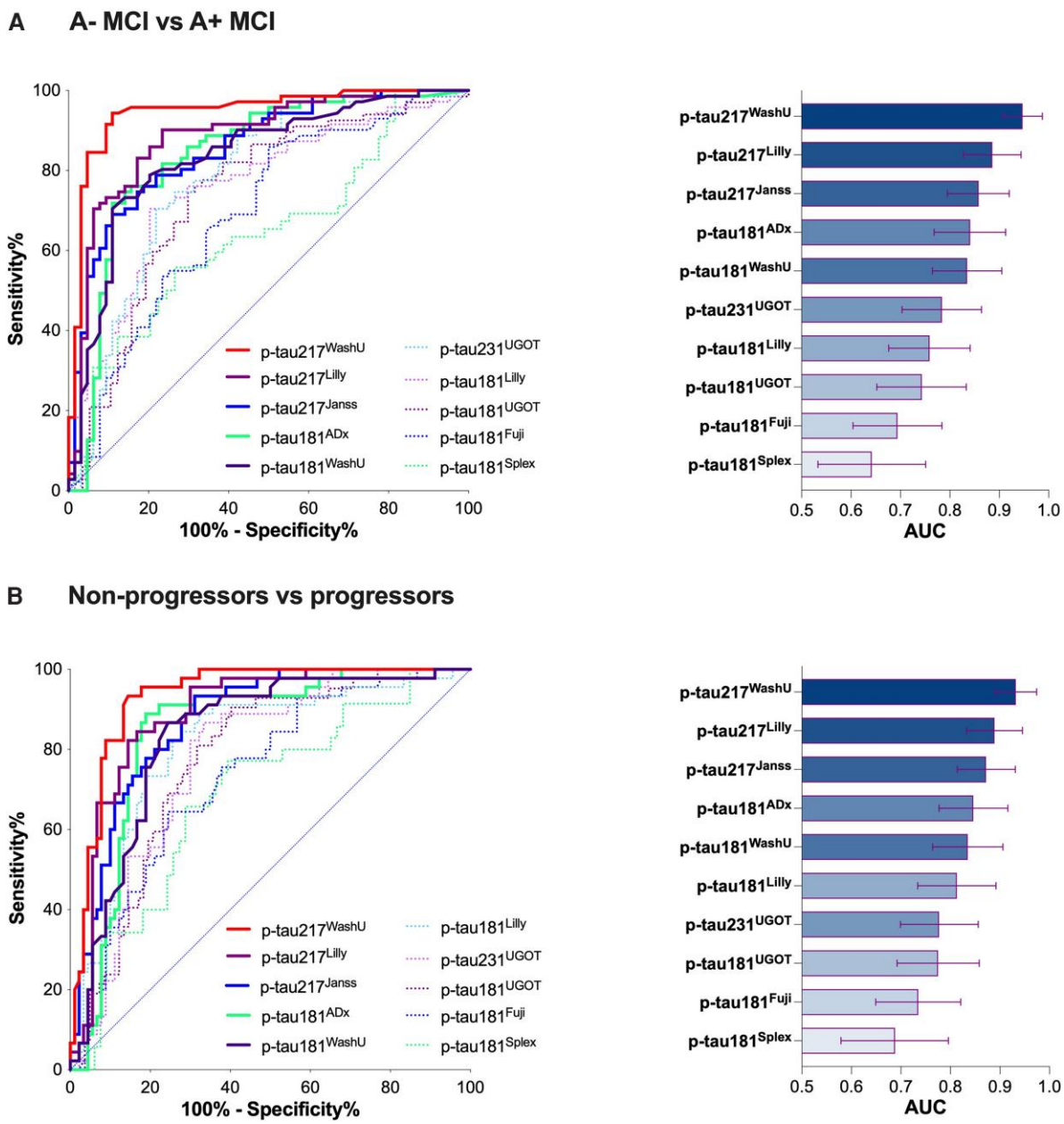


**Figure 1 ROC curve analysis for abnormal CSF $A\beta_{42/40}$ status and progression to ADD.** ROC curve analysis for differentiating (**A**) MCI participants with abnormal CSF $A\beta_{42/40}$ from those with normal CSF $A\beta_{42/40}$ and (**B**) MCI patients who progressed to ADD during follow-up from those who did not (stable MCI patients and MCI patients who progressed to other types of dementia).

(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 ranged 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 ADD (Fig. 1B and Table 4). When distinguishing MCI patients who progressed to ADD 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, 0.833–0.946). P-tau217$^{Janss}$ (AUC = 0.872; 95% CI, 0.814–0.931; $P_{diff} = 0.53$), p-tau181$^{ADx}$ (AUC = 0.846; 95% CI, 0.777–0.916; $P_{diff} = 0.16$) and p-tau181$^{WashU}$ (AUC = 0.835; 95% CI, 0.764–0.906; $P_{diff} = 0.09$) were non-inferior to p-tau217$^{Lilly}$, whereas p-tau231$^{UGOT}$, p-tau181$^{Lilly}$, p-tau181$^{UGOT}$, p-tau181$^{Fuji}$ and p-tau181$^{Splex}$ all had significantly lower AUCs ($AUC_{range}$ 0.688–0.813; $P_{diff} \leq 0.013$). For comparison, the AUCs of the best performing CSF p-tau assays in a subsample of 78 participants with CSF measures available ranged between 0.907 and 0.943 (p-tau217$^{WashU}$, AUC = 0.943; p-tau217$^{Janss}$, AUC = 0.928; p-tau217$^{Lilly}$, AUC = 0.926; p-tau181$^{ADx}$, AUC = 0.924; p-tau181$^{Fuji}$, AUC = 0.907). The differences in AUCs between CSF and corresponding plasma p-tau assays were not significant (Supplementary Table 1).

We also found differences in plasma concentrations of all p-tau biomarkers except p-tau181$^{Fuji}$ between the A– non-progressor, A+

**Table 3 Associations of plasma p-tau with CSF $A\beta_{42/40}$**

| 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) |

Data are from ROC curve analysis. MCI participants were classified as amyloid-negative ($n=64$) or as amyloid-positive ($n=71$) using CSF $A\beta_{42/40}$ as described in the methods.
[a]p-tau181-UGOT and p-tau181-Splex data were available for 124 (57 amyloid-negative, 67 amyloid-positive) and 101 (49 amyloid-negative, 52 amyloid-positive) participants, respectively.

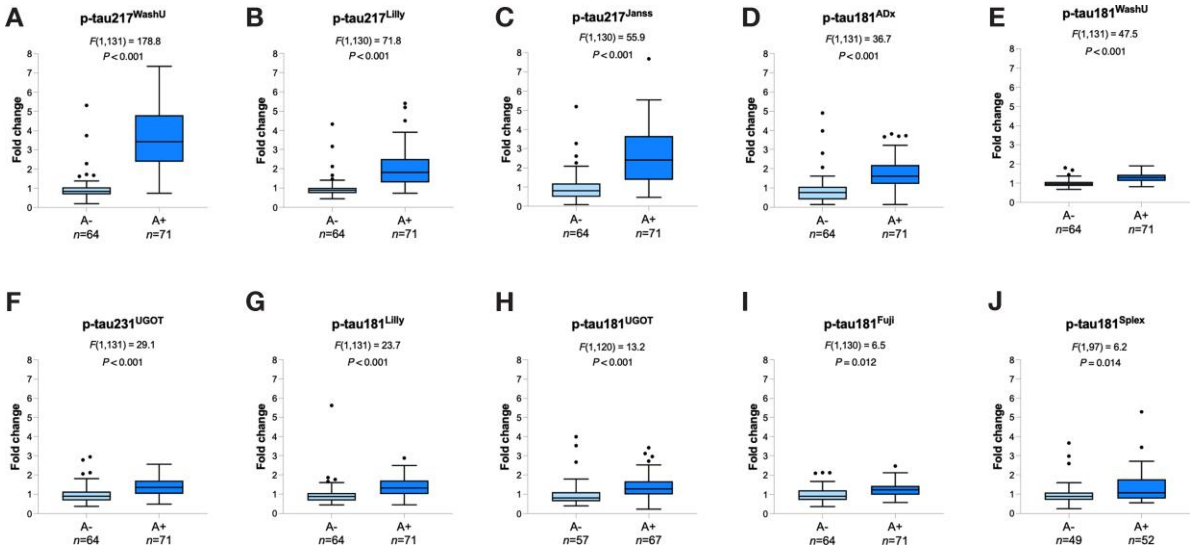


**Figure 2 Plasma p-tau biomarkers in amyloid-negative and -positive MCI patients.** Plasma levels of p-tau217 (**A–C**), p-tau181 (**D–E** and **G–J**) and p-tau231 (**F**) measured using different assays in the A– and A+ MCI groups. Aβ status was defined based on the CSF $A\beta_{42/40}$ ratio. Data 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.

non-progressor and MCI-ADD groups (Fig. 3). *Post hoc* analysis revealed that plasma levels of p-tau217 (when measured with three different assays), but not p-tau181 or p-tau231, were higher in MCI-ADD than A+ non-progressors ($P<0.002$). At the same time, the three p-tau217 biomarkers as well as the best performing p-tau181 biomarkers (p-tau181$^{WashU}$ and p-tau181$^{ADx}$) were increased in both A+ non-progressors and MCI-ADD compared with A– non-progressors ($P\leq0.001$). P-tau217$^{WashU}$ showed the largest fold increase in both MCI-ADD (mean = 4.3, SD = 1.7) and A+ non-progressors (mean = 2.5, SD = 1.4) compared with A– non-progressors. Fold increase was also larger in MCI-ADD ($mean_{range}$ 2.0–3.2) than in A+ non-progressors ($mean_{range}$ 1.4–1.9) for p-tau217$^{Lilly}$, p-tau217$^{Janss}$ and p-tau181$^{ADx}$.

## Correlations between plasma and CSF p-tau

Finally, we examined associations between plasma and CSF p-tau biomarkers (Fig. 4). CSF p-tau concentrations are presented in Supplementary Table 2. In line with other results of this study, the strongest correlations between CSF and plasma were seen for p-tau217$^{WashU}$ (R = 0.891; 95% CI, 0.832–0.930), followed by p-tau217$^{Lilly}$ (R = 0.755; 95% CI, 0.635–0.839) with significant difference in correlation coefficients between the two biomarkers ($P=0.003$). The correlations were weak to moderate for the rest of the biomarkers ($R_{range}$ 0.320–0.669).

Plasma p-tau217$^{WashU}$ correlated strongly with plasma p-tau217$^{Lilly}$, p-tau217$^{Janss}$, p-tau181$^{ADx}$ and p-tau181$^{WashU}$ ($R_{range}$ 0.712–0.862; Supplementary Fig. 2), while correlations with other plasma p-tau biomarkers were weak to moderate ($R_{range}$ 0.376–0.619; Supplementary Fig. 2).

## Sensitivity analysis

The results were similar when statistical analysis was performed in smaller sub-samples where p-tau181$^{UGOT}$ and p-tau181$^{Splex}$ data were available (Supplementary Tables 3–6). Briefly, plasma

**Table 4 Associations of plasma p-tau with future progression to ADD**

| 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) |

Data are from a ROC curve analysis. Forty-five MCI participants progressed to ADD during follow-up and 90 remained stable or progressed to non-ADD.
[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-ADD) participants, respectively.

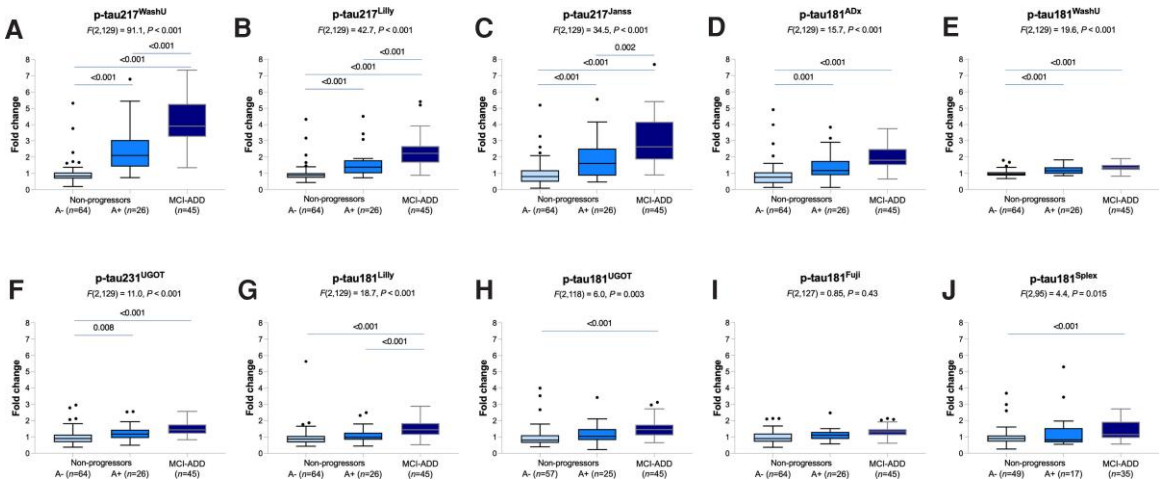


**Figure 3 Plasma p-tau biomarkers in MCI participants who progressed to ADD during follow-up and amyloid-negative and -positive non-progressors.** Plasma levels of p-tau217 (**A**–**C**), p-tau181 (**D**–**E** and **G**–**J**) and p-tau231 (**F**) measured using different assays in patients with MCI who progressed to ADD during follow-up (MCI-ADD), A– and A+ non-progressor MCI patients. Aβ status was defined based on the CSF $Aβ_{42/40}$ 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 and follow-up time. Boxes show interquartile range, the horizontal lines are medians and the whiskers and outliers were plotted using the Tukey method.

p-tau217$^{WashU}$ showed the best performance when detecting both abnormal Aβ status and progression to ADD ($AUC_{range}$ 0.927–0.955), followed by p-tau217$^{Lilly}$ ($AUC_{range}$ 0.878–0.900), p-tau217$^{Janss}$ ($AUC_{range}$ 0.860–0.870), p-tau181$^{ADx}$ ($AUC_{range}$ 0.832–0.860) and p-tau181$^{WashU}$ ($AUC_{range}$ 0.809–0.827). None of the AUCs of P-tau231$^{UGOT}$, p-tau181$^{Lilly}$, p-tau181$^{UGOT}$, p-tau181$^{Fuji}$ or p-tau181$^{Splex}$ were consistently above 0.800.

## Discussion

Recently developed blood tests for Aβ and p-tau are anticipated to transform Alzheimer's disease research and care. Here we sought to directly compare currently available methods for determinations of p-tau in blood in order to establish which of these methods are accurate enough to be useful for implementation in clinical practice or drug trials. In this study including patients with MCI, plasma p-tau217 quantified using MS-based assay showed very high accuracy when both identifying participants with abnormal Aβ status and those who progress to ADD during follow-up with AUCs > 0.93, which was higher than for the other p-tau biomarkers. Furthermore, this assay exhibited significantly higher correlations with p-tau levels in CSF than the other p-tau assays. However, p-tau217$^{Lilly}$, p-tau217$^{Janss}$, p-tau181$^{ADx}$ and p-tau181$^{WashU}$ all displayed relatively high and consistent accuracy across both outcomes ($AUC_{range}$ 0.835–0.889), whereas the performance of other biomarkers (p-tau231$^{UGOT}$, p-tau181$^{Lilly}$, p-tau181$^{UGOT}$, p-tau181$^{Fuji}$, p-tau181$^{Splex}$) was significantly inferior ($AUC_{range}$ 0.642–0.813). Of note, there was no added value of combining different plasma p-tau species (p-tau217$^{WashU}$, ptau181$^{ADx}$ and p-tau231$^{UGOT}$) when either distinguishing normal from abnormal Aβ status or predicting future progression to ADD (data not shown).

MS-based measure of plasma p-tau217 has previously shown very good accuracy to detect Aβ pathology in two 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 ADD in a sample of MCI patients. One novel finding of the present study is that MS

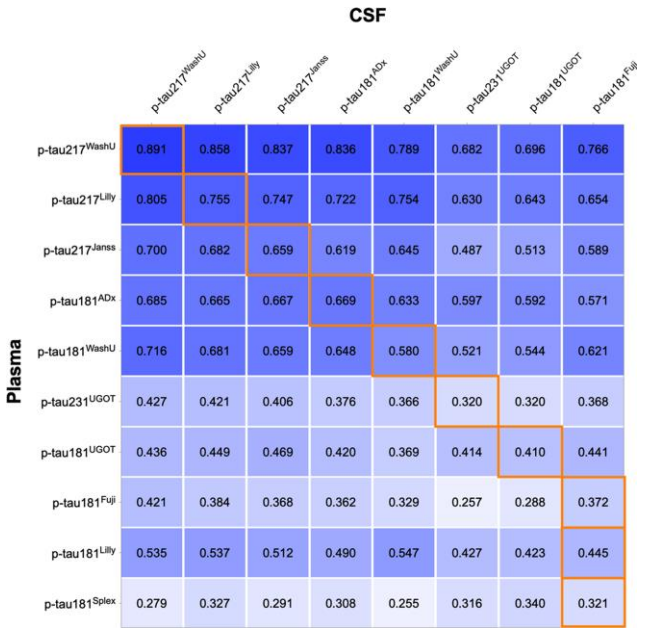


**Figure 4 Correlations between CSF and plasma p-tau.** Heat map showing Spearman coefficients for correlations between CSF and plasma p-tau measured using different assays (p-tau181$^{UGOT}$, $n = 72$; p-tau181$^{Splex}$, $n = 52$; all other biomarkers $n = 78$). Correlations between plasma and CSF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

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 ADD 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-progressors and participants 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 represents the most likely target population to receive disease-modifying therapies in the clinical setting 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 ADD 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 labour 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

H.Z. 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, 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). K.B. 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). R.J.B. has received research funding from Avid Radiopharmaceuticals, Janssen, Roche/Genentech, Eli Lilly, Eisai, Biogen, AbbVie, Bristol Myers Squibb and Novartis. Washington University and R.J.B. have equity ownership interest in C2N Diagnostics. R.J.B. and N.R.B. receive income based on technology (blood plasma assay, and methods of diagnosing Alzheimer's disease with phosphorylation changes) licensed by Washington University to C2N Diagnostics. R.J.B. receives income from C2N Diagnostics for serving on the scientific advisory board. R.J.B. serves on the Roche Gantenerumab Steering Committee as an unpaid member. M.J.P. is an employee of Avid radiopharmaceuticals, a wholly owned subsidiary of Eli Lilly and Company, and is a minor stockholder in Eli Lilly. O.H. 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. G.T.B. and H.K. are employees of Janssen Research and Development. J.V. and E.S. are employees of ADx NeuroSciences. E.V.M. is a co-founder of ADx NeuroSciences. M.V. is an employee of Fujirebio Europe N.V.

## Supplementary material

Supplementary material is available at *Brain* online.

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