BRAIN
ORIGINAL ARTICLE

A comprehensive head-to-head comparison of key plasma phosphorylated tau 217 biomarker tests

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Plasma phosphorylated-tau 217 (p-tau217) is currently the most promising biomarker for reliable detection of Alzheimer's disease pathology. Various p-tau217 assays have been developed, but their relative performance is unclear. We compared key plasma p-tau217 tests using cross-sectional and longitudinal measures of amyloid-β (Aβ)-PET, tau-PET and cognition as outcomes and benchmarked them against CSF biomarker tests.

Samples from 998 individuals [mean (range) age 68.5 (20.0–92.5) years, 53% female] from the Swedish BioFINDER-2 cohort, including both cognitively unimpaired and cognitively impaired individuals, were analysed. Plasma p-tau217 was measured with mass spectrometry assays [the ratio between phosphorylated and non-phosphorylated (%p-tau217$_{WashU}$) and p-tau217$_{WashU}$] and with immunoassays (p-tau217$_{Lilly}$, p-tau217$_{Janssen}$ and p-tau217$_{ALZpath}$). CSF biomarkers included p-tau217$_{Lilly}$, the US Food and Drug Administration-approved p-tau181/Aβ42$_{Elecsys}$, and p-tau181$_{Elecsys}$. All plasma p-tau217 tests exhibited a high ability to detect abnormal Aβ-PET [area under the curve (AUC) range: 0.91–0.96] and tau-PET (AUC range: 0.94–0.97). Plasma %p-tau217$_{WashU}$ had the highest performance, with significantly higher AUCs than all the immunoassays ($P_{diff} < 0.007$). For detecting Aβ-PET status, %p-tau217$_{WashU}$ had an accuracy of 0.93 (immunoassays: 0.83–0.88), sensitivity of 0.91 (immunoassays: 0.84–0.87) and a specificity of 0.94 (immunoassays: 0.85–0.89). Among immunoassays, p-tau217$_{Lilly}$ and plasma p-tau217$_{ALZpath}$ had higher AUCs than plasma p-tau217$_{Janssen}$ for Aβ-PET status ($P_{diff} < 0.006$), and p-tau217$_{Lilly}$ outperformed plasma p-tau217$_{ALZpath}$ for tau-PET status ($P_{diff} = 0.025$). Plasma %p-tau217$_{WashU}$ exhibited stronger associations with all PET load outcomes compared with immunoassays; baseline Aβ-PET load (R^2: 0.72; immunoassays: 0.47–0.58; $P_{diff} < 0.001$), baseline tau-PET load (R^2: 0.51; immunoassays: 0.38–0.45; $P_{diff} < 0.001$), longitudinal Aβ-PET load (R^2: 0.53; immunoassays: 0.31–0.38; $P_{diff} < 0.001$) and longitudinal tau-PET load (R^2: 0.50; immunoassays: 0.35–0.43; $P_{diff} < 0.014$). Among immunoassays, plasma p-tau217$_{Lilly}$ was more associated with Aβ-PET load than plasma p-tau217$_{Janssen}$ ($P_{diff} < 0.020$) and with tau-PET load than both plasma p-tau217$_{Janssen}$ and plasma p-tau217$_{ALZpath}$ (all $P_{diff} < 0.010$). Plasma %p-tau217 also correlated more strongly with baseline cognition (Mini-Mental State Examination) than all immunoassays (R^2: %p-tau217$_{WashU}$: 0.33; immunoassays: 0.27–0.30; $P_{diff} < 0.024$). The main results were replicated in an external cohort from Washington University in St Louis ($n = 219$). Finally, p-tau217$_{NULISA}$ showed similar performance to other immunoassays in subsets of both cohorts.

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In summary, both mass spectrometry- and immunoassay-based p-tau217 tests generally perform well in identifying Aβ-PET, tau-PET and cognitive abnormalities, but %p-tau217$_{WashU}$ performed significantly better than all the examined immunoassays. Plasma %p-tau217 may be considered as a stand-alone confirmatory test for Alzheimer's disease pathology, whereas some immunoassays might be better suited as triage tests where positive results are confirmed with a second test, which needs to be determined by future reviews incorporating results from multiple cohorts.

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Introduction

Detection of Alzheimer's disease (AD) pathology, i.e. amyloid-β (Aβ) plaques and hyperphosphorylated tau aggregates, with accurate blood tests is of great interest for both research and clinical trials, and clinical implementation is already underway.[1-3] For optimal clinical management of AD, it is crucial to ensure a timely and accurate diagnosis in a scalable and cost-effective manner. This is becoming even more important with the launch of disease-modifying therapies in several countries.[1,4,5] Tau phosphorylated at threonine 217 (p-tau217) has emerged as a leading plasma biomarker of AD pathology along the disease continuum ranging from presymptomatic to symptomatic disease stages.[1,3,6-10] Several p-tau217 assays have been developed using a variety of methods. The performance of these different assays can differ depending on analytical factors (e.g. the specific antibodies used), or the measurement techniques [e.g. mass spectrometry (MS) versus immunoassays]. A comparison of the different assays is needed to determine the extent to which different assays provide an alternative to established CSF and imaging markers. Previously, we showed that MS-based measures of p-tau217 performed the best out of a variety of p-tau217 and p-tau181 plasma assays to predict CSF Aβ status in 135 individuals with mild cognitive impairment (MCI).[8] It has also been shown that several p-tau217 immunoassays perform similarly when predicting Aβ status.[11-14] Nonetheless, it is not yet clear whether different p-tau217 assays perform similarly in relation to other key measures

of AD, such as cross-sectional and longitudinal measures of Aβ-PET load, tau-PET load and cognition, and how they compare with well-established CSF markers in these contexts. Furthermore, it is important to determine which plasma p-tau217 tests fulfil the proposed minimal requirements for use in clinical practice either as a stand-alone confirmatory test or as a triage test where positive or intermediate results are confirmed with a higher-performing test, such as CSF or PET.[15]

To this end, we conducted a head-to-head study comparing the performance and accuracy of five different plasma p-tau217 assays, using both high-performing MS- and immunoassay-based tests, in a large Swedish sample covering the full spectrum of AD ($n = 998$), with sensitivity analyses performed in cognitively unimpaired and cognitively impaired individuals separately. The blood tests were compared in their abilities to discriminate between a normal versus abnormal Aβ- and tau-PET status. We also compared their continuous associations with baseline levels and the rate of change in Aβ- and tau-PET values, and cognitive test scores. Plasma biomarker performances were also compared with relevant CSF tests, i.e. the US Food and Drug Administration (FDA)-approved p-tau181/Aβ42 ratio and p-tau181 tests, and p-tau217. Finally, the results were replicated using plasma biomarkers in an external American cohort ($n = 219$). We hypothesized that the best plasma p-tau217 tests would be on par with the CSF tests, supporting a possible substitution of CSF tests with plasma biomarkers for AD.

Materials and methods

Participants

BioFINDER-2 cohort

The study population consisted of participants from the prospective Swedish BioFINDER-2 study (NCT03174938), recruited between 2017 and 2022, from the Skåne University Hospital and Ängelholm Hospital. Participants were cognitively normal ($n = 375$) or had subjective cognitive decline (SCD; $n = 139$), mild cognitive impairment (MCI; $n = 256$) or dementia attributable to AD ($n = 131$) or to other causes (frontotemporal dementia, dementia with Lewy bodies, Parkinson's disease dementia, vascular dementia or unspecified dementia; $n = 97$). Non-AD dementia patients were not excluded, in order to represent the range of patients from a memory clinic population. Overall exclusion criteria included: (i) presence of systemic illness preventing study participation; (ii) significant non-dementia neurological or psychiatric diseases; (iii) current alcohol or substance abuse; and (iv) unwilling to undergo imaging or lumbar puncture. All participants were required to be proficient in Swedish. Cognitively normal and SCD participants formed the group of cognitively unimpaired individuals ($n = 514$). The inclusion criteria here included not meeting MCI or dementia criteria (for further details, see Salvadó *et al.*[16]). Participants with MCI exhibited significant cognitive decline on at least one domain from a neuropsychological test battery, defined as performing below 1.5 standard deviations below the normative score, and a Mini-Mental State Examination (MMSE) score between 24 and 30. If participants scored between 24 and 30 on the MMSE but performed normally on the neuropsychological test battery, they were considered as SCD.[17] AD dementia was diagnosed based on Diagnostic and Statistical Manual of Mental Disorders 5 criteria and confirmed with Aβ biomarkers based on the National Institute on Aging and Alzheimer's Association criteria for AD.[18] Patients with MCI or dementia formed the group of cognitively impaired individuals ($n = 484$). For this study, participants were included only if they had all plasma and CSF biomarkers available at baseline ($n = 998$).[19,20] The BioFINDER-2 study was approved by the Swedish Ethical Review Authority. Each participant and/or their relatives provided informed consent. The study adhered to guidelines outlines in the World Medical Association Declaration of Helsinki and the Department of Health and Human Services Belmont Report.

Knight ADRC cohort

The Knight ADRC cohort is composed of community-dwelling volunteers enrolled in ageing studies in Washington University in St. Louis. All participants underwent comprehensive clinical assessments, including neurological examinations and cognitive testing, and were included if they had all fluid biomarkers available ($n = 219$).[21] Participants with a clinical dementia rating of zero were classified as cognitively unimpaired ($n = 195$) and those with a clinical dementia rating higher than zero as cognitively impaired ($n = 27$).[22] All participants gave written informed consent, and ethical approval was granted by the Washington University Human Research Protection Office (protocol: 201109100).

Plasma biomarkers

Blood was collected in K_2-EDTA-plasma tubes and centrifuged at 2000*g*, +4°C for 10 min. Plasma was aliquoted into 1.5 ml polypropylene tubes (1 ml per tube) and stored at −80°C. Plasma % p-tau217$_{WashU}$ and plasma p-tau217$_{WashU}$ were derived from non-phosphorylated tau217 and p-tau217 concentrations measured with a liquid chromatography tandem high-resolution MS method developed at Washington University (WashU).[7,23] The plasma % p-tau217$_{WashU}$ was determined by dividing the level of tau phosphorylated at residue 217 by the concentration of the non-phosphorylated version of the same tau peptide. Plasma p-tau217$_{Lilly}$ concentrations were measured with the Meso Scale Discovery (MSD) immunoassay developed by Lilly Research Laboratories.[17,24,25] Plasma p-tau217$_{Janssen}$ concentrations were measured using a single molecule array (Simoa) immunoassay developed by Johnson & Johnson Innovative Medicine, formerly Janssen R&D,[12,26,27] which is commercially available at Quanterix as LucentAD p217 assay. Plasma p-tau217$_{ALZpath}$ was measured with the ALZpath pTau217 assay and is commercially available.[28] More detailed descriptions regarding the assays can be found in the Supplementary material. In the Knight ADRC cohort, plasma biomarkers were measured with the same assays as in BioFINDER-2, except that the Janssen and ALZpath assays were not available. For a subset of participants, measurements with the multiplex NULISA pTau-217 assay (p-tau217$_{NULISA}$)[29] were available in BioFINDER-2 ($n = 463$) and the Knight ADRC cohort ($n = 97$). In the BioFINDER-2 cohort, the NULISA multiplex assay was also compared with their prototype singleplex assay ($n = 617$).

CSF biomarkers

CSF was obtained through lumbar puncture during the same session as blood collection and stored at −80°C in polypropylene tubes. Samples were handled in agreement with the suggested protocol.[30] CSF p-tau217$_{Lilly}$ concentrations were measured with the MSD immunoassay developed by Lilly Research Laboratories. CSF p-tau181, Aβ40 and Aβ42 concentrations were measured with the Roche Elecsys CSF immunoassays on a fully automated Cobas instrument (Roche Diagnostics International).[31] The ratio between CSF p-tau181 and Aβ42 (CSFp-tau181/Aβ42) is approved by the

FDA to detect Alzheimer's pathology in cognitively impaired individuals. Participants were categorized as being CSF Aβ-positive based on a CSF Aβ42/Aβ40 threshold of <0.075, determined with Gaussian mixture modelling.[17,32] In the Knight ADRC cohort, CSF was collected at the same time as blood sampling. CSF Aβ42, Aβ40 and p-tau181 were measured with the Lumipulse G1200 automated immunoassay platform (Fujirebio). The Aβ42/40 ratio from Lumipulse is also FDA approved.

PET

PET scans were performed at Skåne University Hospital in Lund, Sweden. Aβ-PET images were acquired 90–110 min after the injection of ~185 MBq of ^{18}F-flutemetamol in participants without dementia ($n = 698$), who did not undergo Aβ-PET by study design. In patients with dementia, Aβ-positivity was determined by CSF Aβ42/40 instead. The standardized uptake value ratios (SUVRs) were created with the whole cerebellum as reference region. Aβ-PET positivity was defined as having an SUVR in the neocortical meta region of interest (ROI) of ≥1.033, defined through Gaussian mixture modelling.[33] A subset of participants ($n = 448$) underwent Aβ-PET longitudinally for an average follow-up of 3.63 ± 2.30 years. Almost all participants ($n = 961$) had tau-PET data available. Images were acquired 70–90 min after injection of ~370 MBq of ^{18}F-RO948. Tau-PET positivity was characterized as ≥1.362 SUVR in the temporal-meta ROI with the inferior cerebellar cortex as reference region.[34,35] A subset of participants ($n = 524$) had follow-up tau-PET data available over the course of 3.46 ± 2.09 years. Further details have been described elsewhere.[17,36] The Swedish Medical Products Agency and the local Radiation Safety Committee at Skåne University Hospital, Sweden, approved the PET imaging. In the Knight ADRC cohort, PET-scans were obtained within 1 year of plasma and CSF collection. Aβ-PET was performed with either tracer ^{18}F-florbetapir (AV45) or ^{11}C-Pittsburgh Compound B (PiB).[21] The mean cortical SUVR was calculated based on the averaged signal of neocortical ROIs using cerebellar grey matter as the reference regions. Centiloids were derived from SUVR values and a cut-off of ≥37 centiloids was defined for Aβ-PET positivity.[37]

Cognition

We focused on two cognitive measures. First, we included the MMSE,[38] which measured global cognitive function, with a range of 0–30, in which 0 is the worst possible score. Second, we included a modified version of the Preclinical Alzheimer Cognitive Composite (mPACC), which measures different aspects of cognition associated with early stages of cognitive decline, in which lower scores indicate more cognitive impairment. The mPACC included z-scores on animal fluency, MMSE, Symbol Digit Modalities Test and Alzheimer's Disease Assessment Scale delayed recall scores (counted twice).[39] Dementia patients were excluded from mPACC analyses ($n = 228$). In the Knight ADRC cohort, we used a global cognitive composite, which is similar to the mPACC used in the main cohort. This composite was created based on the Free and Cued Selective Reminding Test (FCSRT) free recall, animal fluency and Trail-Making Test (TMT)-A and -B.[16]

Statistical analyses

Statistical analyses were carried out in R (version 4.3.2). Fluid biomarkers were $\log_{10}$-transformed (except p-tau217$_{NULISA}$, because these values are already log-transformed in the multiplex assay) and z-scored using cognitively unimpaired Aβ− individuals as the reference group. Correlations between biomarkers were assessed with scaled linear regression models. The z-scores were scaled to facilitate comparisons between the biomarkers. Abilities to discriminate between Aβ- or tau-PET positive or negative participants were assessed with receiver operating characteristic (ROC) curve analysis ('pROC' package). The area under the curve (AUC) and accuracy (proportion of correctly predicted outcomes) of two ROC curves were compared with bootstrapping (1000 iterations). In brief, significant differences in test metrics (e.g. AUC) were assessed by analysing the generated distribution of the bootstrapped differences. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated using the threshold derived from the optimization of Youden's index ('pROC' package). In a complementary analysis, for each biomarker, we grouped participants into either PET-negative or PET-positive groups, with the PET-positive group further split into quartiles. Wilcoxon signed-rank tests were used to compare differences between neighbouring quartiles. We also looked at the associations with continuous measures of PET SUVR and cognitive performance at baseline, in addition to their rates of change. For the linear regression models, we included Aβ-PET, tau-PET, MMSE score and mPACC as outcomes, with the plasma or CSF biomarkers as predictors (one model per biomarker and outcome). For each outcome, the adjusted R^2 of each biomarker model was compared using the same bootstrapping method (same sample size, 1000 iterations). Linear mixed models were used to obtain the individual rate of change ('lme4 package') from longitudinal Aβ-PET, tau-PET, MMSE score and mPACC, using random intercepts and random time slopes. These slopes were then used as the outcome in a linear regression model with the fluid biomarkers as predictors. Again, we compared the adjusted R^2 of each biomarker model with bootstrapping (1000 iterations). The same analyses were performed in the Knight ADRC cohort, with the available outcomes: Aβ-PET status or SUVR and a global cognitive composite. The AUC models and linear regression models were all adjusted for age and sex. Cognition models were also adjusted for years of education. The *P*-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method, applied across all comparisons per model, for each outcome. Hence, all reported *P*-values are FDR corrected. Two-sided $P < 0.05$ was considered as statistically significant. All main analyses were performed including all participants. Analyses were then repeated only in cognitively unimpaired and only in cognitively impaired groups as sensitivity analyses.

Results

Demographics and correlations between biomarkers

Plasma and CSF concentrations were assessed in 998 individuals in the BioFINDER-2 study, with a mean age of 68.5 (12.1) years and 53% female. In total, 52% ($n = 514$) were considered as cognitively unimpaired, and 48% ($n = 484$) as cognitively impaired (see Table 1 for demographic information). When correlating the z-scores of different plasma tests with each other, the two MS plasma methods, as expected, had the highest correlation, with an R^2 of 0.92, whereas other correlations ranged between 0.61 and 0.80 (Fig. 1). A correlation matrix and correlations between raw values can be found in Supplementary Figs 1 and 2. It must be noted that p-tau217 is the numerator for %p-tau217, hence these measures are not independent. All plasma p-tau217 biomarkers were associated with estimated glomerular filtration rate (eGFR) (standard β ranging from −0.17 to −0.10, where lower

Table 1 Demographics

Parameter	Total sample $n = 998$	Cognitively unimpaired $n = 514$	Cognitively impaired $n = 484$
Characteristics			
Age, years	68.5 ± 12.1 (20.0–92.5)	64.7 ± 14.1 (20.0–92.5)	72.6 ± 7.6 (44.3–89.1)
Female, *n* (%)	524 (53)	299 (58)	225 (46)
Education, years	12.8 ± 3.9 (3–36)	13.1 ± 3.6 (6–36)	12.5 ± 4.3 (3–33)
MMSE score	26.9 ± 3.8 (6–30)	28.9 ± 1.2 (24–30)	24.6 ± 4.3 (6–30)
mPACC score[a]	−0.97 ± 1.58 (−6.07–2.30)	0.11 ± 0.81 (−2.10–2.30)	−1.75 ± 0.86 (−4.51–0.59)
APOE-ε4 carriers, *n* (%)	471 (47)	214 (42)	247 (53)
Aβ+ participants, *n* (%)	441 (44)	126 (25)	315 (65)
Cognitive status	–	375 controls/139 SCD	256 MCI/228 dementia
Chronic kidney disease, *n* (%)	186 (19)	80 (16)	106 (22)
eGFR	71.3 ± 13.6 (21.6–122.6)	73.5 ± 13.8 (26.4–122.6)	69.1 ± 12.9 (21.6–102.0)
PET			
^{18}F-flutemetamol global Aβ-PET SUVR[b]	1.11 ± 0.30 (0.81–2.24)	1.03 ± 0.23 (0.81–1.91)	1.30 ± 0.36 (0.81–2.24)
^{18}F-RO948 temporal-meta ROI tau-PET SUVR[b]	1.34 ± 0.43 (0.85–4.29)	1.17 ± 0.15 (0.86–2.54)	1.52 ± 0.54 (0.85–4.29)
Plasma biomarkers			
%p-tau217$_{WashU}$, %	1.76 ± 1.73 (0.21–12.81)	1.07 ± 0.88 (0.21–7.47)	2.50 ± 2.07 (0.24–12.81)
p-tau217$_{WashU}$, pg/ml	0.00 ± 0.01 (0.00–0.04)	0.00 ± 0.00 (0.00–0.02)	0.01 ± 0.01 (0.00–0.04)
p-tau217$_{Lilly}$, pg/ml	0.31 ± 0.29 (0.03–2.01)	0.20 ± 0.13 (0.03–1.30)	0.43 ± 0.36 (0.03–2.01)
p-tau217$_{Janssen}$, pg/ml	0.07 ± 0.07 (0.00–0.47)	0.05 ± 0.04 (0.00–0.29)	0.10 ± 0.08 (0.01–0.47)
p-tau217$_{ALZpath}$, pg/ml	0.53 ± 0.43 (0.04–3.30)	0.36 ± 0.24 (0.04–1.61)	0.72 ± 0.50 (0.08–3.30)
CSF biomarkers			
p-tau217$_{Lilly}$, pg/ml	18.70 ± 24.67 (0.57–164.50)	8.96 ± 10.55 (0.57–97.81)	29.04 ± 30.49 (0.73–164.50)
p-tau181/Aβ42$_{Elecsys}$	0.02 ± 0.02 (0.00–0.14)	0.01 ± 0.01 (0.00–0.09)	0.03 ± 0.02 (0.00–0.14)
p-tau181$_{Elecsys}$, pg/ml	22.25 ± 12.73 (8.00–100.50)	18.00 ± 8.15 (8.00–77.26)	26.78 ± 14.97 (8.00–100.50)

Data are presented as the mean ± standard deviation (range), unless otherwise specified. Aβ positivity was defined with the CSF Aβ42/40 ratio. CKD positivity was determined as an eGFR <60 ml/min/1.73 m^2. Aβ = amyloid-β; *APOE-ε4* = apolipoprotein E carriership (carrying at least one ε4 allele); CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; mPACC = modified Preclinical Alzheimer Cognitive Composite; ROI = region of interest; SCD = subjective cognitive decline; SUVR = standardized uptake value ratio.

[a]Participants diagnosed with dementia were not included in the mPACC analyses, hence the data here are reported only for the MCI individuals with mPACC data available ($n = 236$).

[b]Participants diagnosed with dementia do not undergo Aβ-PET by study design (missing $n = 304$; in $n = 37$ cognitively unimpaired; in $n = 267$ cognitively impaired). Tau-PET is missing for $n = 23$ in the total sample ($n = 5$ cognitively unimpaired; $n = 18$ cognitively impaired).

kidney function was associated with higher p-tau217 levels), except %p-tau217$_{WashU}$, where levels were not affected by eGFR (Supplementary Fig. 3).

Classification of Aβ-PET status

Initially, we assessed how well the different assays could identify individuals with an abnormal Aβ-PET status using ROC analyses (Fig. 2A). Plasma %p-tau217$_{WashU}$ performed significantly better than the other plasma biomarkers, with an AUC of 0.96 (all $P_{diff} < 0.001$). Among the immunoassays, plasma p-tau217$_{Lilly}$ (AUC: 0.94) and plasma p-tau217$_{ALZpath}$ (AUC: 0.93) both had significantly higher AUCs than plasma p-tau217$_{Janssen}$ (AUC: 0.91, all $P_{diff} < 0.016$) (Supplementary Table 1). When comparing with CSF tests, no significant difference was observed between plasma %-ptau217$_{WashU}$ (AUC: 0.96) and CSF p-tau217$_{Lilly}$ (AUC: 0.95, $P_{diff} = 0.074$; Fig. 2A). Plasma %p-tau217$_{WashU}$ (AUC: 0.96) performed significantly better than CSF p-tau181/Aβ42$_{Elecsys}$ (AUC: 0.94, $P_{diff} = 0.007$), whereas plasma p-tau217$_{WashU}$, plasma p-tau217$_{Lilly}$ and plasma p-tau217$_{ALZpath}$ performed similarly to CSF p-tau181/Aβ42$_{Elecsys}$ (all $P_{diff} > 0.377$). Plasma p-tau217$_{Janssen}$ (AUC: 0.91) was inferior to CSF p-tau181/Aβ42$_{Elecsys}$ (AUC: 0.94, $P_{diff} = 0.012$). CSF p-tau181$_{Elecsys}$ had the lowest AUCs of all biomarker tests (either plasma or CSF) (Fig. 2A and Supplementary Table 1).

Next, we studied the agreement between fluid biomarker tests and Aβ-PET status (Fig. 2C and Table 2). Plasma %p-tau217$_{WashU}$ exhibited significantly higher accuracy than all other plasma biomarker tests (%p-tau217, 0.93; immunoassays, 0.83–0.88, all $P_{diff} < 0.025$; Fig. 2C). No significant differences between the accuracies of the plasma immunoassays were observed (all $P_{diff} > 0.183$). Furthermore, plasma %p-tau217$_{WashU}$ had a sensitivity of 91% (immunoassays: 84%–87%) and a specificity of 94% (immunoassays: 85%–89%) for detecting elevated Aβ-PET status (Table 2).

Classification of tau-PET status

Next, we examined how well the different diagnostic tests identified an abnormal tau-PET status using ROC analyses (Fig. 2B and Supplementary Table 1). Plasma %p-tau217$_{WashU}$ (AUC: 0.97) performed significantly better than all the plasma p-tau217 immunoassays (AUC: 0.94–0.96, all $P_{diff} < 0.007$). Among the immunoassays, plasma p-tau217$_{Lilly}$ (AUC: 0.96) and plasma p-tau217$_{Janssen}$ (AUC: 0.95) did not perform differently from each other ($P_{diff} = 0.162$). However, plasma p-tau217$_{Lilly}$ (AUC: 0.96) had a significantly higher AUC than plasma p-tau217$_{ALZpath}$ (AUC: 0.94, $P_{diff} = 0.025$). In comparison to CSF tests, plasma %p-tau217$_{WashU}$, plasma p-tau217$_{WashU}$ and CSF p-tau217$_{Lilly}$ had significantly higher AUCs (0.96–0.97) than CSF p-tau181/Aβ42$_{Elecsys}$ (AUC: 0.94, all $P_{diff} < 0.009$), but plasma p-tau217$_{Lilly}$, plasma p-tau217$_{Janssen}$ and plasma p-tau217$_{ALZpath}$ (AUC: 0.94–0.96) performed comparably to CSF p-tau181/Aβ42$_{Elecsys}$ (all $P_{diff} > 0.404$). CSF p-tau181$_{Elecsys}$ had the lowest AUC (0.87), and all other assays (from either plasma or CSF) were superior.

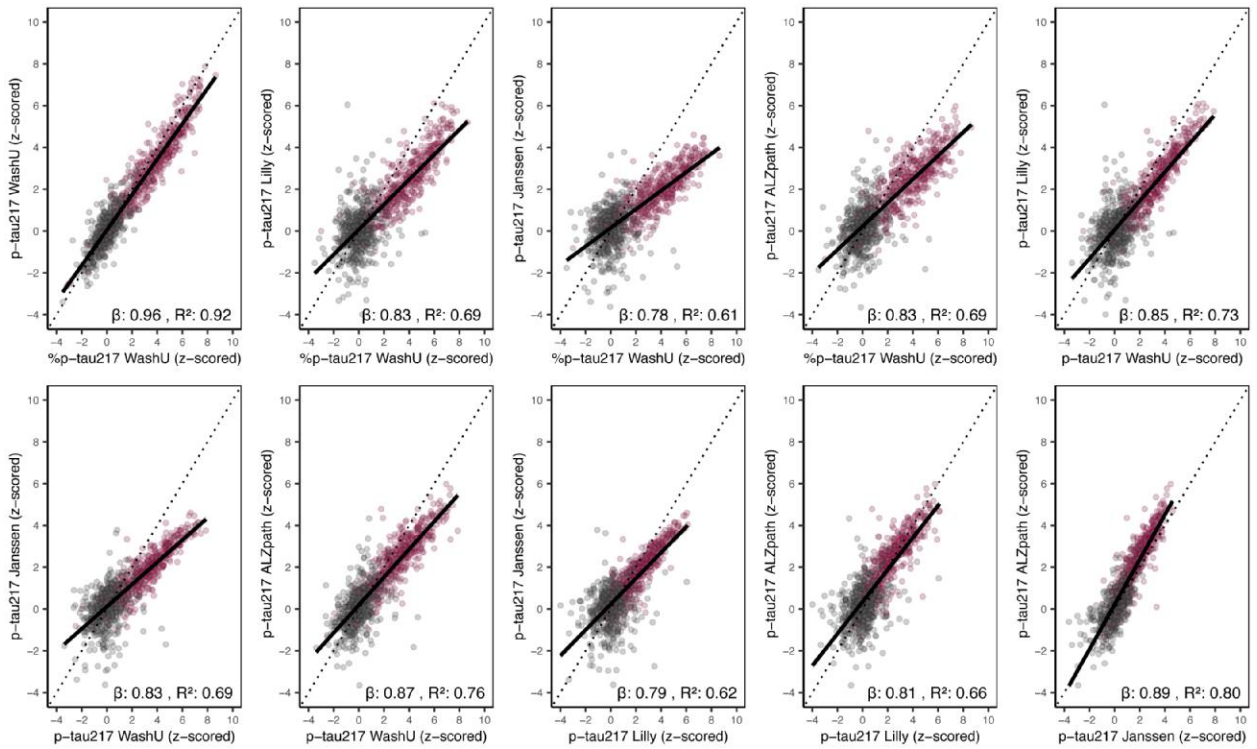

Figure 1 Correlations between plasma p-tau217 biomarkers. The reported betas are standardized and across the full sample. Z-scores were based on cognitively unimpaired, CSF Aβ– participants ($n = 364$) as the reference group. The grey dots represent CSF Aβ– participants, the red dots represent CSF Aβ+ participants. Aβ = amyloid-β.

When the accuracies were compared, no significant differences were observed between any of the fluid biomarkers, which ranged between 0.80 and 0.89 (Fig. 2D). More information regarding sensitivity and specificity can be found in Table 2.

Distinguishing between PET quantiles

To gain a better understanding of how the different fluid biomarkers tracked changes in the levels of the AD proteinopathies, we examined the levels of each fluid biomarker test between different PET stages [PET-negative participants in the 'Negative' group and PET-positive participants further split into quartiles (Q1–Q4)]. When grouping individuals by Aβ-PET SUVR, the CSF measures plateaued in individuals with the highest levels of Aβ pathology (between Q3 and Q4), but the plasma biomarkers continued to increase with higher PET load (Fig. 3A). Plasma p-tau217$_{Janssen}$ did not show a significant difference when comparing the Q2 and Q3 stages but did for the other stages. For tau-PET, more differences were observed. Plasma %p-tau217$_{WashU}$, plasma p-tau217$_{WashU}$, plasma p-tau217$_{Lilly}$ and plasma p-tau217$_{Janssen}$ did not plateau at later stages of tau-PET positivity and continued to increase with higher PET loads. Plasma p-tau217$_{ALZpath}$ showed a significant difference only when comparing the tau-PET negative group with Q1, and Q1 with Q2, but was not different for the other quantiles. The CSF Elecsys measures (p-tau181/Aβ42 and p-tau181) plateaued after distinguishing between the negative group and Q1, but CSF p-tau217$_{Lilly}$ did not (Fig. 3B).

Associations with Aβ-PET load

Next, we examined the associations between fluid biomarker test outcomes at baseline and (i) Aβ-PET load at baseline; and (ii) the rate of change of Aβ-PET load during follow-up. The results are given in Fig. 4A (cross-sectional analyses) and Fig. 4C (longitudinal analyses), with additional information in Supplementary Tables 2, and 3 and Supplementary Figs 4 and 5. Results across the different assays were highly consistent between the two types of analyses. Overall, plasma %p-tau217$_{WashU}$ showed the strongest association with Aβ-PET values, performing significantly better than all the plasma p-tau217 immunoassays, with an R^2 of 0.72 in the cross-sectional analysis (immunoassays: 0.47–0.58; all $P_{diff} < 0.001$), and with an R^2 of 0.53 in the longitudinal analysis (immunoassays: 0.31–0.38; all $P_{diff} < 0.005$). Among the immunoassays, plasma p-tau217$_{Lilly}$ ($R^2_{cross\text{-}sectional}$: 0.58; $R^2_{longitudinal}$: 0.38) and p-tau217$_{ALZpath}$ ($R^2_{cross\text{-}sectional}$: 0.56; $R^2_{longitudinal}$: 0.37) had a significantly higher R^2 than plasma p-tau217$_{Janssen}$ ($R^2_{cross\text{-}sectional}$: 0.47; $R^2_{longitudinal}$: 0.31) in both the cross-sectional and longitudinal analyses (all $P_{diff} < 0.020$) and did not differ from each other. When comparing plasma p-tau217 tests with CSF tests, plasma %p-tau217$_{WashU}$ ($R^2_{cross\text{-}sectional}$: 0.72; $R^2_{longitudinal}$: 0.53) performed significantly better than CSF p-tau217$_{Lilly}$ ($R^2_{cross\text{-}sectional}$: 0.65; $R^2_{longitudinal}$: 0.46) in both the cross-sectional and longitudinal analyses (all $P_{diff} < 0.034$) and better than CSF p-tau181/Aβ42$_{Elecsys}$ ($R^2_{cross\text{-}sectional}$: 0.62; $R^2_{longitudinal}$: 0.48) at the cross-sectional level ($P_{diff} = 0.001$). CSF p-tau217$_{Lilly}$, however, performed significantly better than plasma p-tau217$_{Lilly}$, plasma p-tau217$_{Janssen}$ and p-tau217$_{ALZpath}$ in both the cross-sectional and longitudinal analyses (all $P_{diff} < 0.021$). CSF p-tau181/Aβ42$_{Elecsys}$ performed better than plasma p-tau217$_{Janssen}$ in cross-sectional and longitudinal analyses ($P_{diff} < 0.012$), and better than plasma p-tau217$_{Lilly}$ and plasma p-tau217$_{ALZpath}$ (R^2: 0.37) in the longitudinal analyses (all $P_{diff} < 0.013$). In the cross-sectional analyses, all plasma and CSF assays had stronger associations with Aβ-PET values than CSF

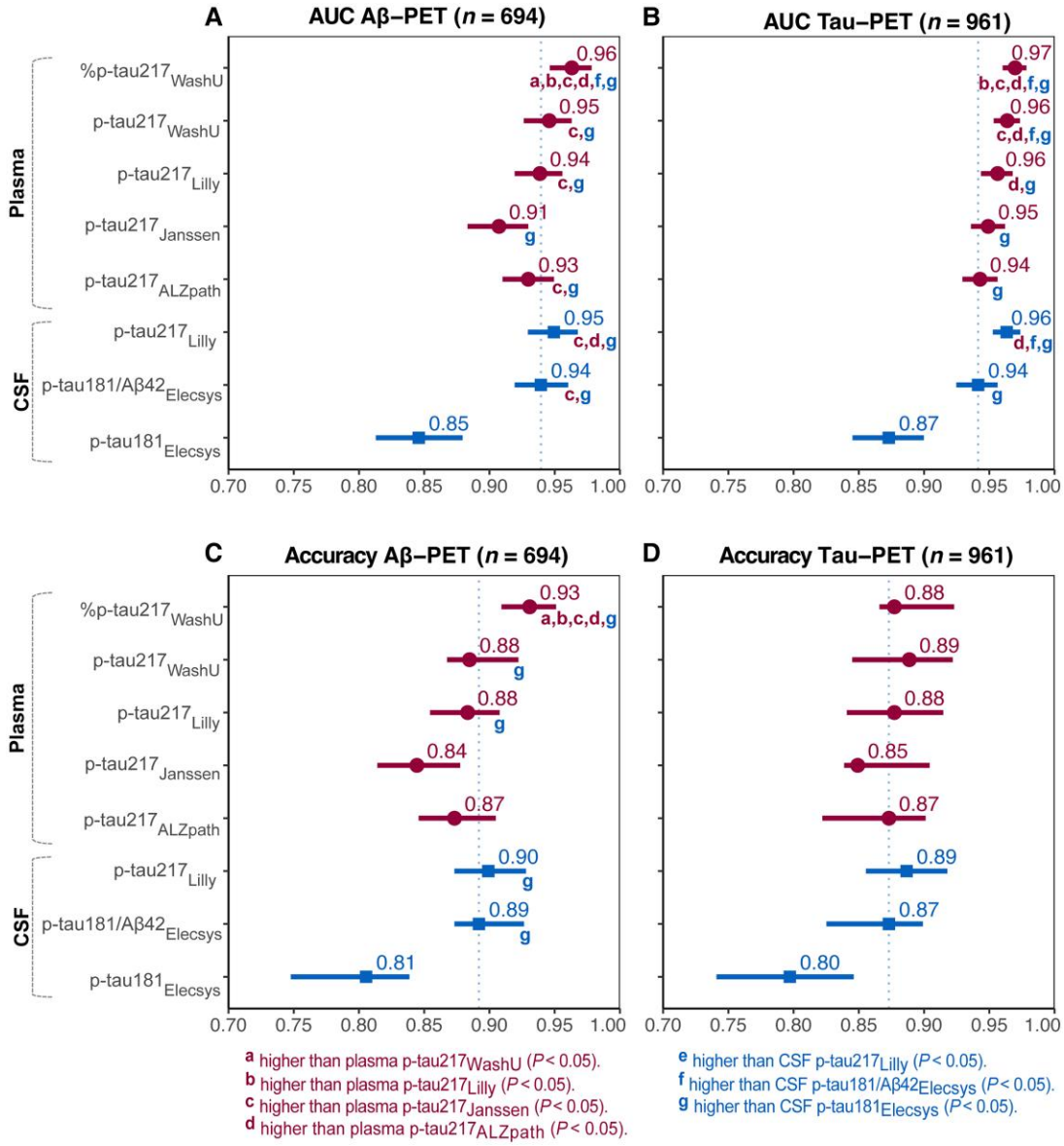

Figure 2 Comparisons of area under the curve and accuracy between plasma p-tau217 biomarkers for Aβ-PET and tau-PET status. (**A**) AUC comparisons for Aβ-PET status; (**B**) AUC comparisons for tau-PET status; (**C**) accuracy comparisons for Aβ-PET status; and (**D**) accuracy comparisons for tau-PET status. Dots and squares represent the AUC or accuracy, and bars represent the 95% confidence interval. The dashed line is drawn at CSF p-tau181/Aβ42$_{Elecsys}$ to facilitate comparison of the other tests with the current US Food and Drug Administration-approved test. Significant differences between assays were assessed through bootstrapping, and all *P*-values were false discovery rate corrected. Models were corrected for age and sex. Aβ = amyloid-β.

p-tau181$_{Elecsys}$ ($R^2_{cross\text{-}sectional}$: 0.38; $R^2_{longitudinal}$: 0.25). This was replicated in the longitudinal analyses, except for plasma p-tau217$_{Janssen}$.

Associations with tau-PET load

We then examined the associations between biomarker performance and tau-PET load. The results are given in Fig. 4B (cross-sectional analyses) and Fig. 4D (longitudinal analyses), with additional information in Supplementary Tables 2, and 3 and Supplementary Figs 6 and 7. Results were similar across both the cross-sectional and longitudinal analyses. Plasma % p-tau217$_{WashU}$ performed significantly better than all the plasma p-tau217 immunoassays, with an R^2 of 0.51 in the cross-sectional analysis (immunoassays: 0.38–0.45; all $P_{diff} < 0.001$) and with an R^2 of 0.50 in the longitudinal analysis (immunoassays: 0.35–0.43; all $P_{diff} < 0.014$). Among the plasma immunoassays, plasma p-tau217$_{Lilly}$ ($R^2_{cross\text{-}sectional}$: 0.45; $R^2_{longitudinal}$: 0.43) performed significantly better than p-tau217$_{Janssen}$ ($R^2_{cross\text{-}sectional}$: 0.39; $R^2_{longitudinal}$: 0.36) and p-tau217$_{ALZpath}$ ($R^2_{cross\text{-}sectional}$: 0.38; $R^2_{longitudinal}$: 0.35) in both cross-sectional (all $P_{diff} < 0.001$) and longitudinal analyses (all $P_{diff} < 0.019$). Plasma p-tau217$_{Janssen}$ and plasma p-tau217$_{ALZpath}$ did not differ from each other in either analysis.

When comparing plasma p-tau217 tests with CSF tests, we found that plasma %p-tau217$_{WashU}$ ($R^2_{cross\text{-}sectional}$: 0.51; $R^2_{longitudinal}$: 0.50) had a significantly higher R^2 than both CSF

Table 2 Identification of abnormal Aβ- or tau-PET status in the full sample ($n = 998$)

PET	Biomarkers	Sensitivity	Specificity	PPV	NPV
Aβ-PET[a]					
Plasma	%p-tau217$_{WashU}$	0.914	0.940	0.892	0.953
	p-tau217$_{WashU}$	0.922	0.864	0.787	0.953
	p-tau217$_{Lilly}$	0.865	0.893	0.815	0.924
	p-tau217$_{Janssen}$	0.836	0.849	0.750	0.905
	p-tau217$_{ALZpath}$	0.840	0.891	0.807	0.911
CSF	p-tau217$_{Lilly}$	0.889	0.904	0.835	0.938
	p-tau181/Aβ42$_{Elecsys}$	0.848	0.916	0.845	0.918
	p-tau181$_{Elecsys}$	0.693	0.867	0.738	0.839
Tau-PET[b]					
Plasma	%p-tau217$_{WashU}$	0.980	0.850	0.633	0.994
	p-tau217$_{WashU}$	0.930	0.878	0.668	0.979
	p-tau217$_{Lilly}$	0.915	0.867	0.646	0.975
	p-tau217$_{Janssen}$	0.960	0.820	0.585	0.987
	p-tau217$_{ALZpath}$	0.891	0.868	0.642	0.968
CSF	p-tau217$_{Lilly}$	0.955	0.868	0.658	0.987
	p-tau181/Aβ42$_{Elecsys}$	0.910	0.863	0.638	0.973
	p-tau181$_{Elecsys}$	0.826	0.789	0.509	0.945

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated using the threshold derived from the optimization of the Youden index ('pROC' package in R).
[a]Aβ-PET cut-off: ≥1.033. Participants with dementia do not undergo Aβ-PET by study design (missing $n = 304$).
[b]Tau-PET cut-off: ≥1.362. Tau-PET is missing for $n = 23$.

p-tau217$_{Lilly}$ ($R^2_{cross\text{-}sectional}$: 0.47; $R^2_{longitudinal}$: 0.44) and CSF p-tau181/Aβ42$_{Elecsys}$ ($R^2_{cross\text{-}sectional}$: 0.44; $R^2_{longitudinal}$: 0.41) in both the cross-sectional (all $P_{diff} < 0.015$) and longitudinal analyses (all $P_{diff} < 0.043$). However, CSF p-tau217$_{Lilly}$ ($R^2_{cross\text{-}sectional}$: 0.47; $R^2_{longitudinal}$: 0.44) performed better than both p-tau217$_{Janssen}$ ($R^2_{cross\text{-}sectional}$: 0.39; $R^2_{longitudinal}$: 0.36) and plasma p-tau217$_{ALZpath}$ in both the cross-sectional (all $P_{diff} < 0.001$) and longitudinal analyses ($R^2_{cross\text{-}sectional}$: 0.38; $R^2_{longitudinal}$: 0.35, all $P_{diff} < 0.017$). Furthermore, CSF p-tau181/Aβ42$_{Elecsys}$ performed better than plasma p-tau217$_{ALZpath}$ in the cross-sectional analysis ($P_{diff} = 0.037$). CSF p-tau181$_{Elecsys}$ performed worse than all other tests.

Associations with cognition

We next examined the biomarker associations with cognition cross-sectionally. The results were generally similar for both cross-sectional analyses of MMSE (Fig. 5A) and mPACC (Fig. 5B), and further information is given in Supplementary Table 2 and Supplementary Figs 8 and 9. Plasma %p-tau217$_{WashU}$ had the largest cross-sectional associations with cognitive measures, with an R^2 of 0.33 for MMSE and 0.37 for the mPACC when controlling for age, sex and years of education. Plasma %p-tau217$_{WashU}$ showed significantly larger associations with MMSE compared with all plasma p-tau217 immunoassays (R^2: 0.27–0.30, all $P_{diff} < 0.024$), and larger associations with mPACC than plasma p-tau217$_{Lilly}$ (R^2: 0.33) and plasma p-tau217$_{Janssen}$ (R^2: 0.33, all $P_{diff} < 0.024$). When comparing plasma and CSF tests, plasma %-tau217$_{WashU}$ was equal or better compared with the CSF tests. CSF p-tau217$_{Lilly}$ (R^2: 0.34) exhibited stronger associations with MMSE than all plasma immunoassays (all $P_{diff} < 0.042$). Furthermore, CSF p-tau181/Aβ42$_{Elecsys}$ (R^2: 0.32) exhibited stronger associations with MMSE than plasma p-tau217$_{Janssen}$ (R^2: 0.27) and p-tau217$_{ALZpath}$ (R^2: 0.27, all $P_{diff} < 0.030$). With the mPACC as the outcome, both CSF p-tau217$_{Lilly}$ (R^2: 0.37) and CSF p-tau181/Aβ42$_{Elecsys}$ (R^2: 0.38) exhibited stronger associations than plasma p-tau217$_{Lilly}$ (R^2: 0.33) and p-tau217$_{Janssen}$ (R^2: 0.33, all $P_{diff} < 0.043$).

We next examined the biomarker associations with cognition longitudinally. The results were generally similar for longitudinal analyses of both MMSE (Fig. 5C) and mPACC (Fig. 5D). Further information is given in Supplementary Table 3 and Supplementary Figs 10 and 11. Plasma %p-tau217$_{WashU}$ had the strongest association with change in MMSE (R^2: 0.48), which was significantly higher than all CSF tests (R^2: 0.26–0.42), in addition to plasma p-tau217$_{Janssen}$ (R^2: 0.38) and p-tau217$_{ALZpath}$ (R^2: 0.37, all $P_{diff} < 0.025$). Plasma p-tau217$_{Lilly}$ (R^2: 0.45) showed a stronger association with change in MMSE than plasma p-tau217$_{Janssen}$ (R^2: 0.38), plasma p-tau217$_{ALZpath}$ (R^2: 0.37), CSF p-tau181/Aβ42$_{Elecsys}$ (R^2: 0.39) and CSF p-tau181$_{Elecsys}$ (R^2: 0.26, all $P_{diff} < 0.030$). No differences between plasma p-tau217$_{Janssen}$ and plasma p-tau217$_{ALZpath}$ were observed here. For the longitudinal of change in mPACC, fewer differences between assays were observed. Plasma %p-tau217$_{WashU}$ (R^2: 0.39) performed better than plasma p-tau217$_{Janssen}$ (R^2: 0.32) and plasma p-tau217$_{ALZpath}$ (R^2: 0.34, all $P_{diff} < 0.035$). When comparing plasma immunoassays, plasma p-tau217$_{Lilly}$ (R^2: 0.37) performed better than plasma p-tau217$_{Janssen}$ (R^2: 0.32, $P_{diff} = 0.018$), but not better than plasma p-tau217$_{ALZpath}$ (R^2: 0.34). Plasma p-tau217$_{Janssen}$ and plasma p-tau217$_{ALZpath}$ performed similarly ($P_{diff} = 0.210$).

Subgroups analyses in cognitively unimpaired and impaired individuals

All analyses were repeated when analysing cognitively unimpaired and cognitively impaired individuals separately. In general, the results were similar to the ones obtained when analysing the whole cohort, but a few differences were observed. In cognitively impaired individuals, no significant differences were observed between plasma %p-tau217$_{WashU}$, p-tau217$_{WashU}$, plasma p-tau217$_{Lilly}$ and plasma p-tau217$_{Janssen}$ regarding the rate of change in tau-PET values (R^2 between 0.50 and 0.54). Furthermore, all other plasma biomarkers performed significantly better in cognitively impaired individuals than plasma p-tau217$_{ALZpath}$ in this context ($P_{diff} < 0.012$). All results regarding

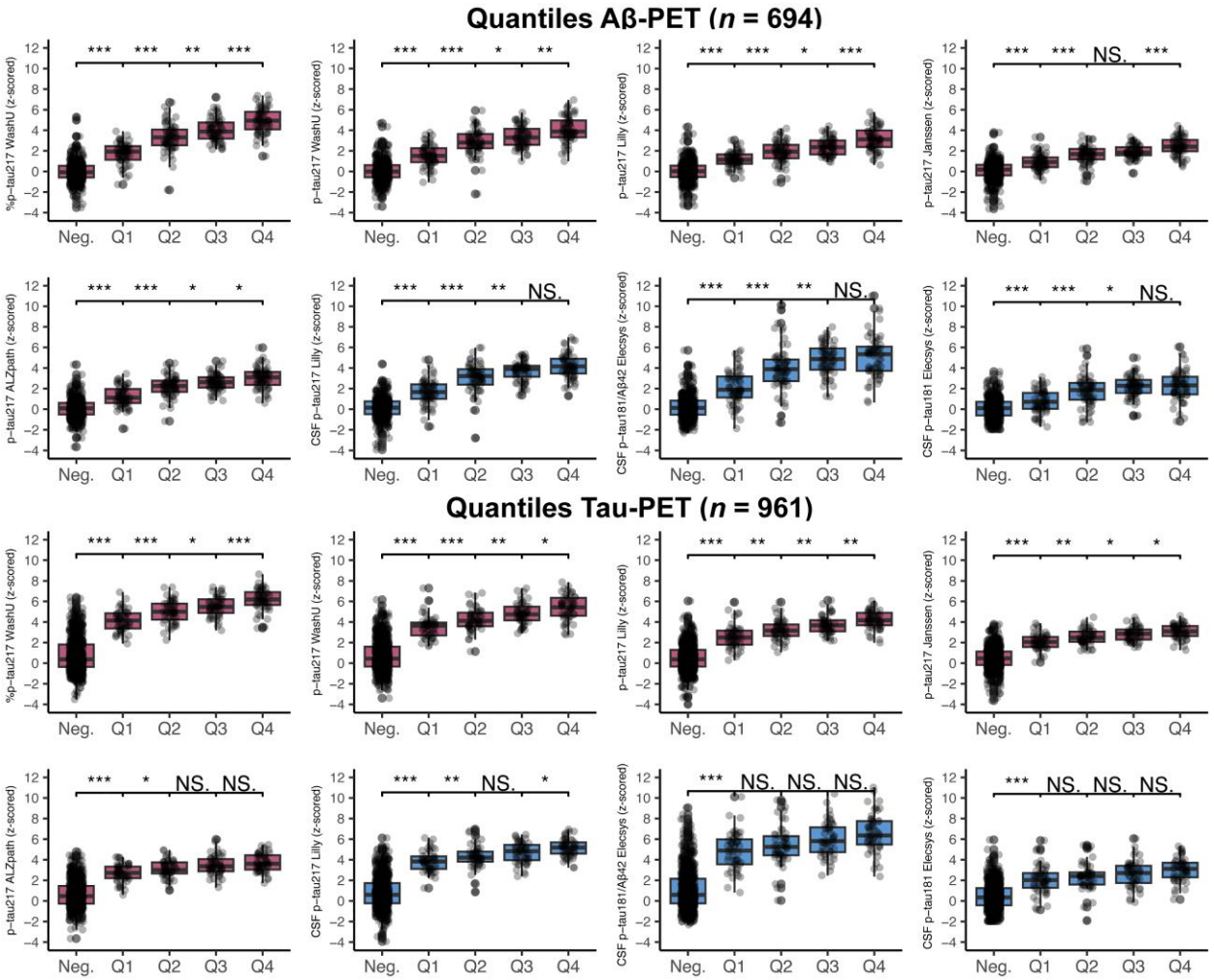

Figure 3 Quantile grouping for Aβ-PET and tau-PET. Boxes show the interquartile range, and the horizontal lines represent the medians. Negative participants were defined as falling below the predefined cut-offs (Aβ-PET: <1.033; tau-PET: <1.362). Neighbouring quantiles were compared with Wilcoxon signed-rank tests. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. Aβ = amyloid-β.

the subgroup analyses can be found in Supplementary Table 4 and Supplementary Figs 12–16.

Replication in the Knight ADRC cohort

We replicated the main cross-sectional analyses in the Knight ADRC cohort ($n = 219$) when using Aβ-PET and cognition as the outcomes and the following predictors: plasma %p-tau217$_{WashU}$, plasma p-tau217$_{WashU}$, plasma p-tau217$_{Lilly}$, CSF p-tau181/Aβ42$_{Lumipulse}$ and CSF p-tau181$_{Lumipulse}$ (for demographics, see Supplementary Table 5). In this cohort, plasma %p-tau217$_{WashU}$ had a significantly higher AUC (AUC: 0.97) than plasma p-tau217$_{Lilly}$ (AUC: 0.93, $P_{diff} = 0.020$) for detecting Aβ-PET positive participants. Plasma %p-tau217$_{WashU}$ (AUC: 0.97) also performed significantly better than CSF p-tau181/Aβ$_{Lumipulse}$ (AUC: 0.87, $P_{diff} = 0.001$) (Fig. 6). When examining associations with baseline Aβ-PET values, plasma %p-tau217$_{WashU}$ showed a significantly better association (R^2: 0.61) than plasma p-tau217$_{WashU}$ (R^2: 0.54), plasma p-tau217$_{Lilly}$ (R^2: 0.46) and CSF p-tau181/Aβ$_{Lumipulse}$ (R^2: 0.42, all $P_{diff} < 0.004$). No differences were observed between biomarkers regarding their associations with cognition.

NULISA subsample

In the NULISA BioFINDER-2 subsample ($n = 463$; Supplementary Table 6), we repeated the cross-sectional analyses and validated them in the Knight ADRC cohort NULISA subsample ($n = 97$) with %p-tau217$_{WashU}$, p-tau217$_{WashU}$ and p-tau217$_{Lilly}$, which were available in both cohorts. Plasma %p-tau217$_{WashU}$ outperformed plasma p-tau217$_{NULISA}$ regarding Aβ-PET status (AUC$_{\%WashU}$: 0.96; AUC$_{NULISA}$: 0.93, $P_{diff} = 0.038$) and Aβ-PET load ($R^2_{\%WashU}$: 0.70; R^2_{NULISA}: 0.60, $P_{diff} = 0.002$) (Supplementary Fig. 17). Plasma %p-tau217$_{WashU}$ was also superior to plasma p-tau217$_{NULISA}$ regarding tau-PET status (AUC$_{\%WashU}$: 0.96; AUC$_{NULISA}$: 0.93, $P_{diff} = 0.013$) and tau-PET values ($R^2_{\%WashU}$: 0.43; R^2_{NULISA}: 0.34, $P_{diff} = 0.048$). In the Knight ADRC cohort, no differences were observed regarding Aβ-PET status, but plasma %p-tau217$_{WashU}$ showed a significantly larger association with Aβ-PET load than plasma p-tau217$_{NULISA}$ ($R^2_{\%WashU}$: 0.62; R^2_{NULISA}: 0.31, $P_{diff} = 0.001$) (Supplementary Fig. 18). In another BioFINDER-2 subsample ($n = 617$), we also compared the NULISA multiplex with their prototype singleplex assay. The two assays only showed a medium correlation (R^2: 0.49) (Supplementary Fig. 19). Overall, the multiplex assay outperformed the prototype singleplex assay, with a higher AUC for Aβ-PET (multiplex: 0.92, singleplex: 0.88, $P_{diff} = 0.046$) and showing

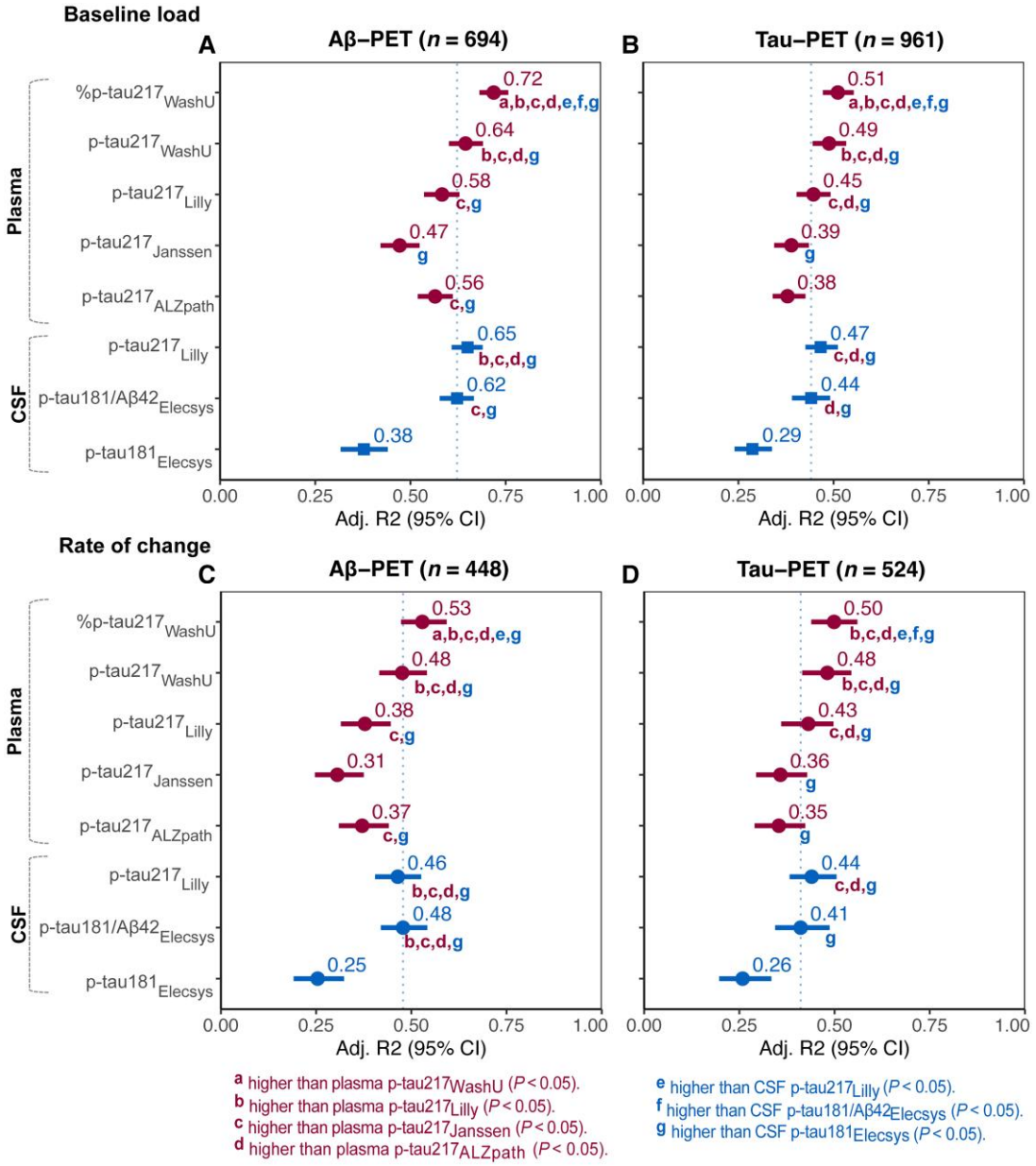

Figure 4 R^2 comparisons with Aβ-PET and tau-PET load as outcome. (**A**) R^2 comparisons for associations with baseline Aβ-PET load; (**B**) R^2 comparisons for associations with baseline tau-PET load; (**C**) R^2 comparisons for associations with the rate of change in Aβ-PET load; and (**D**) R^2 comparisons for associations with the rate of change in tau-PET load. Dots and squares represent R^2, and bars represent the 95% confidence interval (95% CI). The dashed line is drawn at CSF p-tau181/Aβ42$_{Elecsys}$, to facilitate comparison of the other tests with the current US Food and Drug Administration-approved test. Significant differences between assays were assessed through bootstrapping, and all *P*-values were false discovery rate corrected. Cross-sectional: PET SUVR ~ biomarker + age + sex. Longitudinal: individual rate of change in PET SUVR ~ biomarker + age + sex. Aβ = amyloid-β; SUVR = standardized uptake value ratio.

stronger associations with Aβ- and tau-PET load (Supplementary Fig. 20).

Discussion

In this study, we performed a head-to-head comparison of different key plasma p-tau217 tests, including both MS- and immunoassay-based methods. We compared blood and CSF tests to determine Aβ- and tau-PET status at baseline and how they were associated with baseline levels and the rate of change in Aβ- and tau-PET values and cognitive test scores. All plasma p-tau217 tests showed high AUCs and accuracies when identifying individuals with an abnormal Aβ- and tau-PET status. Across the different analyses, plasma %p-tau217$_{WashU}$ (measured with MS) showed consistently superior performance to all the immunoassay tests. Furthermore, plasma %p-tau217$_{WashU}$ performance was non-inferior or even superior to FDA-approved CSF measures across all analyses, as previously shown for Aβ- and tau-PET status,[7] but we now also show similar findings for either PET load or cognitive function both cross-sectionally and longitudinally. We also found that among the tested immunoassays, plasma p-tau217$_{Lilly}$ often performed better than plasma p-tau217$_{Janssen}$ and was non-inferior to the CSF tests in most models. Plasma p-tau217$_{Janssen}$ performed similarly to p-tau217$_{ALZpath}$ when using tau-PET as the outcome, but

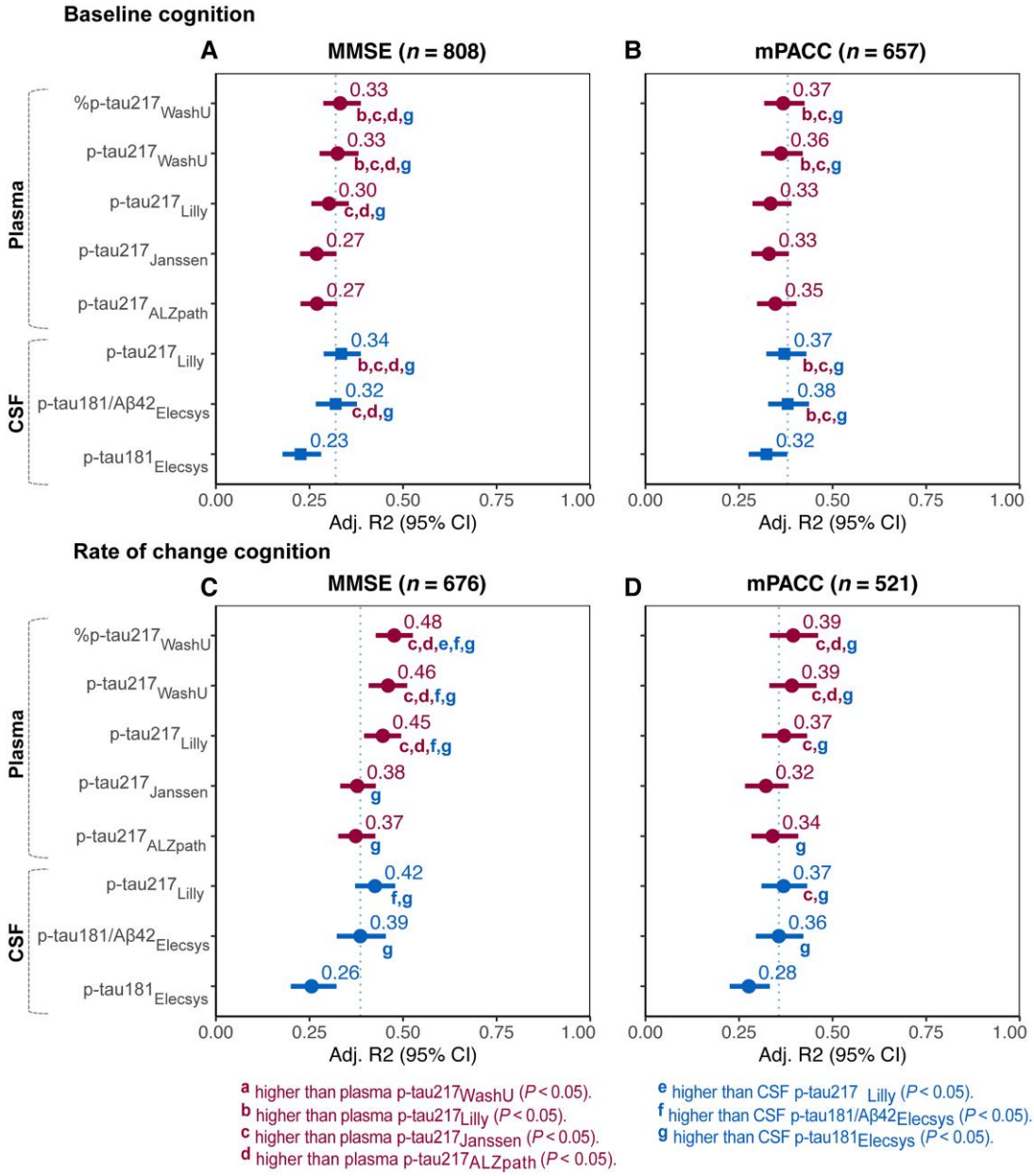

Figure 5 R^2 comparisons with cognition as outcome. (**A**) R^2 comparisons for associations with baseline MMSE scores; (**B**) R^2 comparisons for associations with baseline mPACC performance; (**C**) R^2 comparisons for associations with the rate of change in MMSE scores; and (**D**) R^2 comparisons for associations with the rate of change in mPACC performance. Dots and squares represent R^2, and bars represent the 95% confidence interval (95% CI). The dashed line is drawn at CSF p-tau181/Aβ42$_{Elecsys}$, to facilitate comparison of the other tests with the current US Food and Drug Administration-approved test. Significant differences between assays were assessed through bootstrapping, and all *P*-values were false discovery rate corrected. For the MMSE models, participants with non-AD dementia were excluded. For the mPACC models, participants with dementia were excluded. Cross-sectional: cognition ~ biomarker + age + sex + education. Longitudinal: individual rate of change in cognition ~ biomarker + age + sex + education. Aβ = amyloid-β; MMSE = Mini-Mental State Examination; mPACC = modified Preclinical Alzheimer Cognitive Composite.

p-tau217$_{ALZpath}$ often performed better when using Aβ-PET as the outcome. CSF p-tau181, however, was, in almost all cases, significantly inferior to the other biomarkers.

One of the main questions concerning the plasma assays is the distinction between MS and immuno-based approaches. MS methods allow simultaneous quantification of different peptides, but they often come at a higher cost owing to more expensive equipment and lower analysis throughput.[40] Immunoassays, in contrast, provide a cheaper alternative, although their results might be subject to higher measurement variability.[40,41] Previously, plasma % p-tau217$_{WashU}$ has demonstrated superior performance compared with immunoassays.[7,8] We have shown in a different cohort that plasma p-tau217$_{WashU}$ had a very high accuracy for discriminating between a normal and abnormal CSF Aβ status in 135 MCI patients and had the highest AUC to identify patients who progressed to dementia, performing significantly better than immunoassays.[8] In the present study, we replicated and extended these results with additional p-tau217 tests in a larger sample consisting of both cognitively unimpaired and cognitively impaired individuals. When discriminating between normal and abnormal Aβ-PET, no significant differences were observed between MS plasma p-tau217$_{WashU}$ (not the ratio) and plasma p-tau217$_{Lilly}$ in the present study, which was

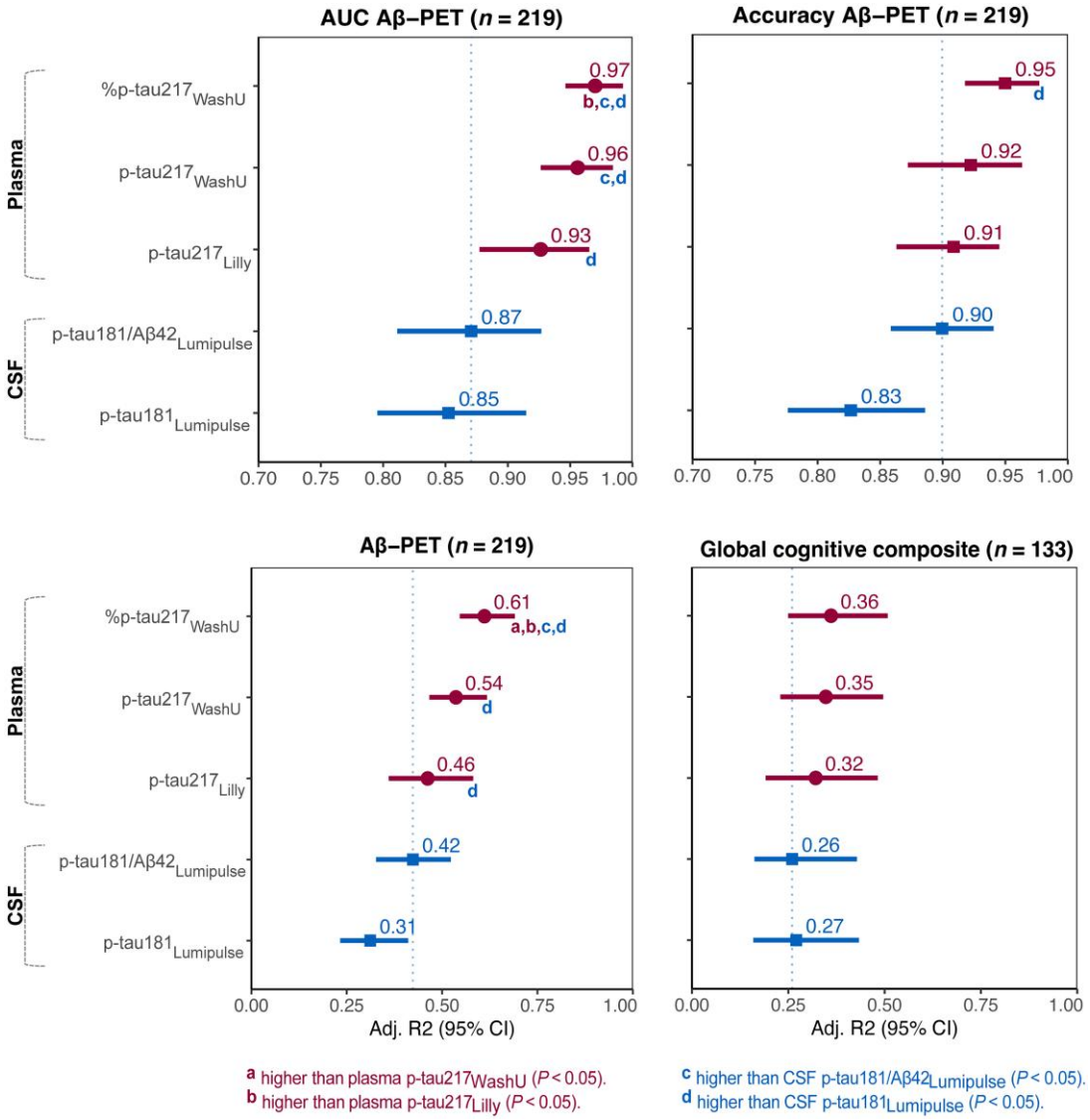

Figure 6 Replication in the Knight ADRC cohort. Dots and squares represent the area under the curve, accuracy or R^2, and bars represent the 95% confidence interval (95% CI). The dashed line is drawn at CSF p-tau181/Aβ42$_{Lumipulse}$, to facilitate comparison of the other tests with the current approved US Food and Drug Administration-approved test. The global cognitive composite was a composite of several cognitive tests, z-scored with cognitively unimpaired Aβ– individuals as reference group. Significant differences between assays were assessed through bootstrapping, and all *P*-values were false discovery rate corrected. Linear model Aβ-PET: Aβ-PET ~ biomarker + age + sex. Linear model global cognitive composite: cognition ~ biomarker + age + sex + education. Aβ = amyloid-β.

replicated in the validation cohort. Thus, the significant differences in performance observed here might be attributable to implementation of the ratio of phosphorylated to non-phosphorylated. For tau-PET load, however, both MS-based plasma %p-tau217$_{WashU}$ and plasma p-tau217$_{WashU}$ performed significantly better than immunoassay-based plasma p-tau217$_{Lilly}$ and plasma p-tau217$_{Janssen}$, suggesting that MS-based methods might be more precise than immunoassays, at least those included in this study, in identifying patients at later stages of the disease.

However, there are also notable differences between the different immunoassays. For instance, the immunoassays included in this head-to-head comparison did not always showcase equal performance. Although all immunoassays performed very well, plasma p-tau217$_{Lilly}$ and plasma p-tau217$_{ALZpath}$ both had a significantly higher AUC for Aβ-PET status and showed stronger associations with baseline and longitudinal Aβ-PET load than plasma p-tau217$_{Janssen}$. Plasma p-tau217$_{Lilly}$ additionally showed significantly stronger associations with tau-PET load and with the longitudinal accumulation of both Aβ- and tau pathology and was more strongly associated with the rate of change in cognition than both plasma p-tau217$_{Janssen}$ and plasma p-tau217$_{ALZpath}$. A recent study comparing plasma p-tau217$_{Janssen}$ with p-tau217$_{ALZpath}$ found that both assays were highly associated with Aβ- and tau-PET loads and with PET status in a sample consisting of cognitively unimpaired and cognitively impaired individuals ($n = 294$).[13] Another previous study in a different cohort of ours ($n = 147$) compared plasma p-tau217$_{Lilly}$ with plasma p-tau217$_{Janssen}$ in MCI patients and found that they were similarly associated with Aβ status in CSF and annual change in MMSE scores.[12] Although plasma p-tau217$_{Janssen}$ performed similarly to other assays in these studies, we found that when comparing plasma p-tau217$_{Janssen}$ with other assays, its performance was slightly, but significantly,

worse in its associations with Aβ-PET. The present study population consists of a larger sample, which increases the power to detect more subtle differences in assay performances. Some other assay differences might also be at play; the p-tau217$_{Janssen}$ assay additionally targets p-tau212.[27] This cross-reactivity might contribute to its overall slightly inferior performance compared with plasma p-tau217$_{Lilly}$. Another difference between the immunoassays is that the detection antibody used in the Lilly assay does not detect big tau, which is mainly released from peripheral sources, unlike the other immunoassays used in the present study. In the NULISA sub-analyses, we saw that the multiplex assay performed similarly to the Lilly and ALZpath p-tau217 immunoassays. However, when comparing their multiplex assay directly with their singleplex assay, the multiplex assay performed better. The better performance of the multiplex compared with the singleplex assay might be explained, in part, by the latter being still in a prototype stage, which might benefit from further optimization. It is noteworthy that, in all analyses, CSF p-tau181$_{Elecsys}$ showed the worst performance of all the fluid biomarker tests. In contrast, the plasma p-tau217 measures, in addition to CSF p-tau217$_{Lilly}$, showed stronger associations with disease pathology and cognition, suggesting the superiority of p-tau217 in both plasma and CSF over p-tau181 in CSF. This is in line with previous results[17,42] and might be important for clinical use of fluid biomarkers.

Recently, the Global CEO Initiative on Alzheimer's disease defined acceptable performance of blood biomarker tests of Aβ pathology.[15] The authors suggested that a test should have a sensitivity and a specificity of ≥90% in cognitively impaired individuals to be used as a stand-alone confirmatory test where a subsequent test is not needed confirm to Aβ status. Furthermore, the recent update of the criteria for diagnosis and staging of Alzheimer's disease led by the Alzheimer's Association suggested that a stand-alone biomarker test should exhibit an accuracy of 90%.[43] In the present study, only %ptau217$_{WashU}$ fulfilled all three of these requirements when detecting Aβ-PET status. This was also the case when analysing cognitively unimpaired and cognitively impaired separately (Supplementary Table 4). The other biomarkers, although having most AUCs ≥0.90, did not reach a specificity, sensitivity or accuracy of 90%. In the previously mentioned studies by Janelidze *et al.*,[8] Therriault *et al.*[13] and Groot *et al.*,[12] similar results were observed for the immunoassays when predicting amyloid positivity: high AUCs, but no specificity, sensitivity or accuracy >90%. In this context, p-tau217 immunoassays might instead be used as triaging tests where a positive plasma test result must be confirmed by a second high-performing test, such as Aβ-PET.[15] Alternatively, a two cut-off approach can be used for the p-tau217 immunoassays where test results below a lower cut-off or above a higher cut-off are regarded as confirmatory of a negative or positive Aβ status, respectively.[33,44] This might also improve their sensitivity, specificity and accuracy. However, patients with intermediate results, which are between the two cut-offs, need to undergo a subsequent more high-performing test.[33] To decide which tests can be used as confirmatory tests or triaging tests with a one or two cut-off approach must be determined by future systematic reviews in which results from many different cohorts need to be incorporated.

This study has important strengths over previous studies. First, it included a large sample size consisting of both cognitively unimpaired and impaired individuals. Furthermore, we conducted a comprehensive comparison of multiple different key p-tau217 assays, including both MS- and immuno-based assays, and evaluated their performance against several and clinically relevant outcomes, with highly consistent results. We studied how the different p-tau217 tests were associated with baseline and the rate of change in Aβ- and tau-PET values and cognitive test scores, which has not been done before. Nonetheless, this work is not without limitations. First, Aβ-PET was not available in patients with a dementia diagnosis by study design, hence this patient group was not included in analyses using Aβ-PET as an outcome. Second, the sample was further reduced when examining longitudinal changes in AD pathology, because not all participants with baseline Aβ-PET had follow-up measures available, although it still consisted of a considerably large sample ($n = 448$). Considering ongoing efforts for disease-modifying therapies in individuals at early stages of the disease, with the aim of disease prevention, it is important to assess how plasma p-tau217 performs as a biomarker also in cognitively unimpaired individuals alone, as we have done in the present study. Third, as shown previously,[45] %p-tau217 was not affected by kidney function, whereas mild associations were seen with all other p-tau217 biomarkers (when not used as a ratio to non-phosphorylated tau). Further replication of these findings in other cohorts is warranted, because performance could be affected by the prevalence of amyloid positivity in the cohort, in addition to other demographic characteristics and the presence of comorbidities. This is already suggested by the slightly better performance of the MS assay in the Knight ADRC cohort, which has a high prevalence of cognitively unimpaired Aβ-negative participants. Therefore, it is likely that the difference between assay performances depend on the relative distribution of participants at different stages of amyloid deposition, or the prevalence of participants with other neurological disorders. Finally, replications will be required to comprehend fully how blood-based biomarkers perform among different populations, for example with different ethnicities or in a more general population with a higher prevalence of comorbidities.[45-47] Biomarker performance and their accessibility and feasibility outside the research setting should also be investigated further. Here, it will be important to examine the additional plasma p-tau217 assays (from other vendors) that might become commercially available in the future and batch-to-batch variability.

Conclusion

In summary, all plasma p-tau217 biomarkers in this study consistently and reliably detected abnormalities in Aβ-PET and tau-PET. However, associations with Aβ-PET and tau-PET load and with cognition differed between the different plasma and CSF tests. Plasma %p-tau217 measured with MS was superior to the immunoassay-based plasma p-tau217 tests. We also observed differences in performance between immunoassays, in which p-tau217$_{Lilly}$ was superior to p-tau217$_{Janssen}$ and p-tau217$_{ALZpath}$, but the differences were, in general, rather minor. Overall, plasma p-tau217 assays have great potential as diagnostic biomarkers for Alzheimer's disease and might provide a less invasive yet equally well-performing alternative to CSF markers.

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 if data transfer agrees 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.

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Competing interests

N.W., G.S., S.J., N.M.C., D.B., A.O.D., H.K., A.A., C.A.R., T.L.S.B., J.C.M., L.I., J.T., N.A., B.A., K.B. and A.P.B. report no competing interests. N.R.B. is co-inventor on a US patent application 'Methods to detect novel tau species in CSF and use thereof to track tau neuropathology in Alzheimer's disease and other tauopathies', and 'CSF phosphorylated tau and Amyloid beta profiles as biomarkers of tauopathies'. N.R.B. is co-inventor on a non-provisional patent application 'Methods of Diagnosing and Treating Based on Site-Specific Tau Phosphorylation'. N.R.B. receives royalty income based on technology (blood plasma assay and methods of diagnosing AD with phosphorylation changes) licensed by Washington University to C2N Diagnostics. G.T.B. is an employee of Johnson and Johnson Innovative Medicine. S.E.S. has received consultancy/speaker fees from Eisai, Eli Lilly and Novo Nordisk. C.C. has received research support from: GSK and EISAI. C.C. is a member of the scientific advisory board of Circular Genomics and owns stocks. C.C. is a member of the scientific advisory board of Admit. H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk 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). N.R.B. and R.J.B. are co-inventors on a non-provisional patent application: 'Methods of diagnosing and treating based on site-specific tau phosphorylation'. R.J.B. is a co-inventor on US patent applications: 'Methods to detect novel tau species in CSF and use thereof to track tau neuropathology in Alzheimer's disease and other tauopathies' and 'CSF phosphorylated tau and amyloid beta profiles as biomarkers of tauopathies'. R.J.B. co-founded C2N Diagnostics. Washington University and R.J.B. have equity ownership interest in C2N Diagnostics and receive royalty income based on technology (stable isotope labelling kinetics, blood plasma assay and methods of diagnosing Alzheimer's disease with phosphorylation changes) that is 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. has received research funding from Avid Radiopharmaceuticals, Janssen, Roche/Genentech, Eli Lilly, Eisai, Biogen, AbbVie, Bristol Myers Squibb and Novartis. O.H. has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, C2N Diagnostics, Eli Lilly, Eisai, Fujirebio, GE Healthcare, and Roche. In the past 2 years, he has received consultancy/speaker fees from Alzpath, BioArctic, Biogen, Bristol Meyer Squibb, Eisai, Eli Lilly, Fujirebio, Merck, Novartis, Novo Nordisk, Roche, Sanofi and Siemens.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Hansson O, Blennow K, Zetterberg H, Dage J. Blood biomarkers for Alzheimer's disease in clinical practice and trials. *Nat Aging*. 2023;3:506-519.
2. Hansson O, Edelmayer RM, Boxer AL, et al. The Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease. *Alzheimers Dement*. 2022;18:2669-2686.
3. Hansson O. Biomarkers for neurodegenerative diseases. *Nat Med*. 2021;27:954-963.

4. Sims JR, Zimmer JA, Evans CD, et al. Donanemab in early symptomatic Alzheimer disease: The TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA*. 2023;330:512-527.
5. van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. 2022;388:9-21.
6. Ashton NJ, Janelidze S, Mattsson-Carlgren N, et al. Differential roles of Aβ42/40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring. *Nat Med*. 2022;28:2555-2562.
7. Barthélemy NR, Salvadó G, Schindler SE, et al. Highly accurate blood test for Alzheimer's disease is similar or superior to clinical cerebrospinal fluid tests. *Nat Med*. 2024;30:1085-1095.
8. Janelidze S, Bali D, Ashton NJ, et al. Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease. *Brain*. 2023;146:1592-1601.
9. Mattsson-Carlgren N, Collij LE, Stomrud E, et al. Plasma biomarker strategy for selecting patients with Alzheimer disease for antiamyloid immunotherapies. *JAMA Neurol*. 2024;81:69-78.
10. Mattsson-Carlgren N, Salvadó G, Ashton NJ, et al. Prediction of longitudinal cognitive decline in preclinical Alzheimer disease using plasma biomarkers. *JAMA Neurol*. 2023;80:360-369.
11. Ashton NJ, Puig-Pijoan A, Milà-Alomà M, et al. Plasma and CSF biomarkers in a memory clinic: Head-to-head comparison of phosphorylated tau immunoassays. *Alzheimers Dement*. 2023;19:1913-1924.
12. 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;14:67.
13. Therriault J, Ashton NJ, Pola I, et al. Comparison of two plasma p-tau217 assays to detect and monitor Alzheimer's pathology. *eBioMedicine*. 2024;102:105046.
14. 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*. 2021;13:198.
15. Schindler SE, Galasko D, Pereira AC, et al. Acceptable performance of blood biomarker tests of amyloid pathology – recommendations from the global CEO initiative on Alzheimer's disease. *Nat Rev Neurol*. 2024;20:426-439.
16. Salvadó G, Horie K, Barthélemy NR, et al. Disease staging of Alzheimer's disease using a CSF-based biomarker model. *Nat Aging*. 2024;4:694-708.
17. Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA*. 2020;324:772-781.
18. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA research framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14:535-562.
19. Ossenkoppele R, Smith R, Ohlsson T, et al. Associations between tau, Aβ, and cortical thickness with cognition in Alzheimer disease. *Neurology*. 2019;92:e601-e612.
20. Hahn A, Schain M, Erlandsson M, et al. Modeling strategies for quantification of in vivo ^{18}F-AV-1451 binding in patients with tau pathology. *J Nucl Med*. 2017;58:623-631.
21. Barthélemy NR, Saef B, Li Y, et al. CSF tau phosphorylation occupancies at T217 and T205 represent improved biomarkers of amyloid and tau pathology in Alzheimer's disease. *Nat Aging*. 2023;3:391-401.
22. 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;7:263-269.
23. Barthélemy NR, Horie K, Sato C, Bateman RJ. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *J Exp Med*. 2020;217:e20200861.
24. 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. *Alzheimers Dement*. 2022;18:283-293.
25. 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;14:989-997.
26. Doré 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:e12307.
27. 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:e12204.
28. Ashton NJ, Brum WS, Di Molfetta G, et al. Diagnostic accuracy of a plasma phosphorylated tau 217 immunoassay for Alzheimer disease pathology. *JAMA Neurol*. 2024;81:255-263.
29. Ibanez L, Liu M, Beric A, et al. Benchmarking of a multi-biomarker low-volume panel for Alzheimer's disease and related dementia research. *medRxiv*. [Preprint]. https://doi.org/10.1101/2024.06.13.24308895
30. Hansson O, Batrla R, Brix B, et al. The Alzheimer's Association international guidelines for handling of cerebrospinal fluid for routine clinical measurements of amyloid β and tau. *Alzheimers Dement*. 2021;17:1575-1582.
31. Hansson O, Seibyl J, Stomrud E, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-β PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement*. 2018;14:1470-1481.
32. 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*. 2021;13:68.
33. Brum WS, Cullen NC, Janelidze S, et al. A two-step workflow based on plasma p-tau217 to screen for amyloid β positivity with further confirmatory testing only in uncertain cases. *Nat Aging*. 2023;3:1079-1090.
34. Leuzy A, Smith R, Ossenkoppele R, et al. Diagnostic performance of RO948 F 18 tau positron emission tomography in the differentiation of Alzheimer disease from other neurodegenerative disorders. *JAMA Neurol*. 2020;77:955-965.
35. Cho H, Choi JY, Hwang MS, et al. In vivo cortical spreading pattern of tau and amyloid in the Alzheimer disease spectrum. *Ann Neurol*. 2016;80:247-258.
36. Leuzy A, Smith R, Cullen NC, et al. Biomarker-based prediction of longitudinal tau positron emission tomography in Alzheimer disease. *JAMA Neurol*. 2022;79:149-158.
37. Klunk WE, Koeppe RA, Price JC, et al. The centiloid project: Standardizing quantitative amyloid plaque estimation by PET. *Alzheimers Dement*. 2015;11:1-15.e1–4.
38. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189-198.
39. Borland E, Edgar C, Stomrud E, Cullen N, Hansson O, Palmqvist S. Clinically relevant changes for cognitive outcomes in preclinical and prodromal cognitive stages: Implications for clinical Alzheimer trials. *Neurology*. 2022;99:e1142-e1153.
40. Therriault J, Woo MS, Salvadó G, et al. Comparison of immunoassay- with mass spectrometry-derived p-tau

quantification for the detection of Alzheimer's disease pathology. *Mol Neurodegener*. 2024;19:2.
41. Korecka M, Shaw LM. Mass spectrometry-based methods for robust measurement of Alzheimer's disease biomarkers in biological fluids. *J Neurochem*. 2021;159:211-233.
42. 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*. 2021;78:1108-1117.
43. Jack CR Jr, Andrews JS, Beach TG, et al. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. *Alzheimers Dement*. 2024;20:5143-5169
44. Palmqvist S, Tideman P, Mattsson-Carlgren N, et al. Blood biomarkers to detect Alzheimer disease in primary care and secondary care. *JAMA*. 2024;332:1245-1257.
45. Janelidze S, Barthélemy NR, He Y, Bateman RJ, Hansson O. Mitigating the associations of kidney dysfunction with blood biomarkers of Alzheimer disease by using phosphorylated tau to total tau ratios. *JAMA Neurol*. 2023;80:516-522.
46. Pichet Binette A, Janelidze S, Cullen N, et al. Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical performance. *Alzheimers Dement*. 2023;19:1403-1414.
47. Palmqvist S, Tideman P, Cullen N, et al. Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures. *Nat Med*. 2021;27:1034-1042.