Comparing the Clinical Utility and Diagnostic Performance of Cerebrospinal Fluid P-Tau181, P-Tau217 and P-Tau231 Assays

Antoine Leuzy,¹,* Shorena Janelidze,¹ Niklas Mattsson-Carlgren,¹,²,³ Sebastian Palmqvist,¹,⁴ Dirk Jacobs⁵, Claudia Cicognola,¹ Erik Stomrud,¹,⁴ Eugeen Vanmechelen⁵, Jeffrey L. Dage,⁶ Oskar Hansson¹,⁴,*

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.
1Clinical Memory Research Unit, Department of Clinical Sciences, Lund University, Malmö, Sweden; 2Department of Neurology, Skåne University Hospital, Lund, Sweden; 3Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden; 4Memory Clinic, Skåne University Hospital, Lund, Sweden; 5ADx NeuroSciences NV, Technologiepark 4, Ghent, Belgium; 6Eli Lilly and Company, Indianapolis, IN, USA

*Corresponding author
Antoine Leuzy, PhD
E-mail: antoine.leuzy@med.lu.se
Or
Oskar Hansson, MD, PhD
E-mail: oskar.hansson@med.lu.se

Word count manuscript: 4500
Word count abstract: 310
Characters title: 119
References: 50
Tables: 2
Figures: 5

Study funding: The study was supported by the Swedish Research Council (2016-00906), the Knut and Alice Wallenberg foundation (2017-0383), the Marianne and Marcus Wallenberg foundation (2015.0125), the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson’s disease) at Lund University, the Swedish Alzheimer Foundation (AF-745911), the Swedish Brain Foundation (FO2019-0326), The Parkinson foundation of Sweden (1280/20), the Skåne University Hospital Foundation (2020-
O000028), Regionalt Forskningsstöd (2020-0314) and the Swedish federal government under the ALF agreement (2018-Projekt0279). The funding sources had no role in the design and conduct of the study; in the collection, analysis, interpretation of the data; or in the preparation, review, or approval of the manuscript. The precursor of $^{18}$F-flutemetamol was sponsored by GE Healthcare. The precursor of $^{18}$F-RO948 was provided by Roche.

### Disclosure

A. Leuzy, S. Janelidze, N. Mattsson-Carlgren, S. Palmqvist, C. Cicognola and E. Stomrud report no conflicts of interest. D. Jacobs is a current employee and E. Vanmechelen is co-founder of ADx NeuroSciences. J.L. Dage is a current employee of Eli Lilly and Company. O. Hansson has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, Biogen, Cerveau and Roche.

**Search terms:** Alzheimer’s disease [26], PET [122], Cerebrospinal Fluid [319]
Abstract

**Background and Objectives:** Phosphorylated tau (P-tau) in cerebrospinal fluid (CSF) is considered an important biomarker in Alzheimer’s disease (AD) and has been incorporated in recent diagnostic criteria. Several variants exist, including P-tau at threonines 181 (P-tau181), 217 (P-tau217) and 231 (P-tau231). However, no studies have compared their diagnostic performance or association to amyloid-β (Aβ) and Tau positron emission tomography (PET). Understanding which P-tau variant to use remains an important yet answered question. We aimed to compare the diagnostic accuracy of P-tau181, P-tau217 and P-tau231 in CSF for AD and their association with Aβ and Tau-PET.

**Methods:** 629 subjects from the Swedish BioFINDER-2 study were included (cognitively unimpaired, n=334; Aβ-positive mild cognitive impairment, n=84; AD dementia, n=119; and non-AD disorders, n=92). In addition to P-tau181 and P-tau217 measured using assays with the same detector antibodies from Eli Lilly (P-tau181*Lilly, P-tau217*Lilly) and P-tau231, we also included P-tau181 measurements from two commonly used assays (Innotest and Elecsys).

**Results:** Though all P-tau variants increased across the AD continuum, P-tau217*Lilly showed the greatest dynamic range (13-fold-increase vs 1.9-5.4-fold-increase for other P-tau variants for AD dementia vs non-AD). P-tau217*Lilly showed stronger correlations with Aβ- and Tau-PET (P<0.0001). P-tau217*Lilly exhibited higher accuracy than other P-tau variants for separating AD dementia from non-AD (AUC, 0.991 vs 0.906-0.982, P<0.0001) and for identifying Aβ- (AUC, 0.951 vs 0.816-0.924, P<0.0001) and Tau-PET positivity (AUC, 0.957 vs 0.836-0.938, P<0.0001). Finally, P-tau181*Lilly generally performed better than the other P-tau181 assays, (e.g., AD dementia vs non-AD, AUC, 0.976 vs 0.923, P<0.0001).

**Discussion:** CSF P-tau217*Lilly seem to be more useful than other included P-tau assays in the work-up of AD. Varied results across P-tau181 assays also highlights the importance of anti-tau antibodies for biomarker performance.
**Classification of evidence:** This study provides class II evidence that phosphorylated tau at threonine 217 provides higher diagnostic accuracy for diagnosis of AD dementia than P-tau at threonine 181 or 231.
Introduction

In addition to the extracellular deposition of amyloid-β (Aβ) plaques, Alzheimer’s disease (AD) is defined by the intracellular aggregation of tau in neurofibrillary tangles (NFTs), composed of abnormally hyperphosphorylated tau.\(^1\) Tau pathology is thought to be reflected in cerebrospinal fluid (CSF) levels of phosphorylated tau (P-tau). CSF P-tau has shown high prognostic accuracy for AD, and for predicting cognitive decline in cognitively unimpaired (CU) individuals and in patients with MCI due to AD.\(^2\)\(^,\)\(^3\) As CSF P-tau levels are higher in AD compared to other non-AD neurodegenerative disorders, including progressive supranuclear palsy (PSP), corticobasal syndrome (CBS), frontotemporal dementia (FTD), and vascular dementia (VaD), it has also proven of use in the differential diagnosis of AD versus other dementias.\(^4\)

Tau in CSF is largely present in the form of different fragments.\(^5\)\(^\text{-}^8\) Of these, N-terminal and mid-region variants are the most abundant. Further, there exist numerous sites where tau can undergo abnormal hyperphosphorylation.\(^9\) The most commonly used assays for P-tau, however, use antibodies targeting the mid-region of tau as well as an antibody targeting tau phosphorylated at threonine-181 (P-tau181).\(^10\) Besides P-tau181, increased levels of mid tau fragments phosphorylated at threonine-231 (P-tau231) appear to be an early occurrence in AD, preceding the formation of paired helical filaments.\(^11\) Though studies have shown that P-tau231 can accurately discriminate AD from CU individuals and patients with non-AD disorders, similar to P-tau181, a series of postmortem studies that examined both measures reported that CSF P-tau231 was better associated with neocortical fibrillary pathology than CSF P-tau181.\(^12\)\(^,\)\(^13\) Recently, P-tau fragments phosphorylated at threonine-217 (P-tau217) were also measured in CSF.\(^14\) Compared to P-tau181, P-tau217 showed stronger correlations with Aβ and tau positron emission tomography (PET) and more accurately distinguished AD dementia from non-AD neurodegenerative disorders.\(^14\) Additional work has shown that P-tau181 and P-tau217 are increased already in preclinical AD (Aβ-positive CU),
with these increases preceding Tau-PET positivity and even occurring prior to the threshold for Aβ-PET positivity.\textsuperscript{15,16}

Overall, findings indicate that increases in CSF P-tau occur in response to very early Aβ pathology and precede widespread tau aggregation. Thus far, however, there are no studies comparing P-tau181, P-tau217 and P-tau231 levels in relation to Aβ and Tau-PET across the symptomatic stages of AD, nor data directly comparing their diagnostic performance for separating AD dementia from non-AD neurodegenerative disorders and for identifying abnormal Aβ and Tau-PET status. Since CSF P-tau is an important biomarker in the work-up for AD and is incorporated in its diagnostic criteria,\textsuperscript{17} it is of great importance to determine which of these P-tau variants to use, especially since clinical heterogeneity and different stages in AD may be determined by heterogeneity in the post-translational modification (PTM) of tau.\textsuperscript{18} We herein aimed to address these questions using cross-sectional data from a well characterized cohort, ranging from Aβ-negative CU individuals to Aβ-positive CU and Aβ-positive patients with mild cognitive impairment (MCI) or AD dementia. In addition to comparing P-tau181 and P-tau217 measured using assays with the same detector antibodies from Eli Lilly (P-tau181\textsubscript{Lilly} and P-tau217\textsubscript{Lilly}) with P-tau231 measured using an assay with a phospho-specific cis-conformational monoclonal antibody (P-tau231\textsubscript{ADx}), we also compared P-tau181\textsubscript{Lilly} with P-tau181 measurements from two commonly used assays (Innotest[P-tau181\textsubscript{Innotest}] and Elecsys[P-tau181\textsubscript{Elecsys}]).

\textbf{Methods}

\textbf{Participants}

We included participants from the prospective and longitudinal Swedish BioFINDER 2 study (clinical trial no. NCT03174938), including CU participants and patients with mild cognitive impairment (MCI), AD dementia and non-AD neurodegenerative disorders. CU individuals were aged ≥60 years and did not have MCI or dementia.\textsuperscript{17} Patients with MCI fulfilled the DSM-5 criteria for mild neurocognitive disorder\textsuperscript{19} while patients with AD dementia fulfilled
the DSM-5 criteria for major neurocognitive disorder due to AD. Patients with non-AD disorders fulfilled diagnostic criteria for PSP or CBS, Parkinson’s disease with/without cognitive impairment, FTD and VaD. Further details pertaining to inclusion and exclusion criteria are described in the Supplement (Appendix e-1 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7). Groups were established without the use of biomarkers, but CU and MCI participants were subdivided based on Aβ-status, determined using CSF Aβ42/Aβ40 (Innotest, Fujirebio, Ghent, Belgium) and a cutoff of <0.089. We included only Aβ-positive AD dementia cases, in keeping with the research framework by the National Institute on Aging-Alzheimer’s Association. As Aβ-PET is by design performed only in CU individuals and in patients with MCI, CSF Aβ42/Aβ40 was thus chosen to have a common measure of Aβ pathology across all participants.

**Standard Protocol Approvals, Registrations, and Patient Consents**

All participants gave written informed consent. Ethical approval was given by the Regional Ethical Committee in Lund, Sweden. Approval for PET imaging was obtained from the Swedish Medical Products Agency and the local Radiation Safety Committee at Skåne University Hospital, Sweden.

**CSF P-tau181 and P-tau217 measurements (Eli Lilly)**

Analysis of CSF mid-domain P-tau181Lilly and P-tau217Lilly was performed at Eli Lilly and Company using the Meso Scale Discovery (MSD) platform, as previously described. The anti-P-tau217 antibody IBA413 and anti-P-tau181 antibody AT270 were used as capture antibodies in the P-tau181 and P-tau217 assays, respectively. Capture antibodies were conjugated with biotin (Thermo Scientific). Sulfo-tag (MSD) conjugated LRL antibody was used as a detector in both assays. The assays were calibrated using a recombinant tau (4R2N) protein that was phosphorylated in vitro using a reaction with glycogen synthase kinase-3 and characterized by mass spectrometry. Samples were analyzed in duplicates and the mean of
duplicates were used in statistical analysis. Ten samples from Aβ-negative CU individuals (1.59%) were below the limit of detection and were excluded, as were five AD subjects with very high P-tau217_Lilly levels (> 3 standard deviations above the mean).

**CSF P-tau231 measurements (ADx NeuroSciences)**

CSF P-tau231_{ADx} was measured at ADx NeuroSciences (Ghent, Belgium) with a research sandwich ELISA (version 1) according to the kit instructions. Phospho-specific cis-conformational monoclonal antibody ADx253 (T1H11) was used as a capture antibody and biotinylated pan-tau monoclonal antibody ADx205 (epitope region aa224-238) as a detector. The assay was calibrated using an in-house designed synthetic peptide combining both antibody epitopes and having the corresponding threonine231 phosphorylated and proline232 replaced by a homoproline, Pip, to reflect cis selectivity of ADx253. In all prior analyses, we observed a consistent low inter-plate variability well below 15%. Since pT231 quantifications require 80 microliter per single measurements—requiring at least 160 microliters per result—we opted to run the pT231 measurement in singlicate since the study was designed to explore the difference between the phospho-tau assays. Quality controls samples run on each plate, which were leftovers of CSF, confirmed high precision of these runs with coefficients of variation below 15%.
CSF P-tau181 measurements (Innotest and Elecsys)

For comparative purposes, we also included P-tau181 measured using the well known commercially available ELISA from Innotest (Fujirebio, Ghent, Belgium)\(^\text{10}\) (P-tau181\(_{\text{Innotest}}\)) and the fully automated Elecsys electrochemiluminescence immunoassay (Roche Diagnostics) (P-tau181\(_{\text{Elecsys}}\)) on a cobas e 601 analyzer (software version 05.02).\(^{26}\) For P-tau181\(_{\text{Innotest}}\), monoclonal capture/detection antibodies were HT7 (epitope region aa159-162) and AT270. For P-tau181\(_{\text{Elecsys}}\), a biotinylated monoclonal antibody specific for phosphorylation at threonine 181 (11H5V1) and a monoclonal Tau-specific antibody (PC1C6) were used (epitope region aa195-202). All samples were analyzed at the Clinical Neurochemistry Laboratory in Mölndal, Sweden.

Image acquisition and processing

Aβ and Tau-PET were performed using \(^{18}\)Fflutemetamol and \(^{18}\)FRO948, respectively, as described elsewhere.\(^{27,28}\) Briefly, dynamic (list-mode) studies were performed over the interval 90- to 100-min post-injection for \(^{18}\)Fflutemetamol and 70- to 90-min for \(^{18}\)FRO948. Standardized uptake value ratio (SUVR) images were created using the pons (\(^{18}\)Fflutemetamol) and inferior cerebellar cortex (\(^{18}\)FRO948) as reference regions. A high-resolution T1-weighted MRI was performed using a Siemens-3T MAGNETOM Prisma scanner for PET image co-registration and template normalization.

Regions of interest and cut-offs

Target regions of interest (ROIs) were chosen on the basis of previously published findings: a neocortical meta-ROI for Aβ-PET (prefrontal, lateral temporal, parietal, anterior cingulate, and posterior cingulate/precuneus)\(^{28,29}\) and, for Tau-PET, the entorhinal cortex (Braak I/II) a temporal meta-ROI (amygdala, inferior/middle temporal gyri, fusiform gyrus, and parahippocampal gyrus, approximating Braak III/IV)\(^{30}\) and a neocortical meta-ROI capturing late stage tau pathology (Braak V/VI).\(^{31}\) A priori cut-offs based on Gaussian mixture
modelling (Aβ-PET)\(^{14}\) and the mean SUVR within a given ROI plus 2.5 standard deviations among young Aβ-negative CU individuals (Tau-PET)\(^{25}\) were used to define positivity within these ROIs.

**Statistical analyses**

Group differences in age adjusted CSF P-tau levels were assessed using pairwise analysis of variance (ANOVA) based comparisons of linear regression models. Associations between CSF P-tau isoforms and between P-tau isoforms and ROI-based Aβ and Tau-PET SUVR values were assessed using correlation analysis; differences between correlation coefficients were tested using a confidence interval-based approach with bootstrapping.\(^{32}\) Log-transformed biomarker and PET measures were used in regression analyses. Generalized additive models with cubic regression splines were used to compare the slopes of CSF P-tau isoforms (mean change from Aβ-negative CU) across different tau and Aβ-PET SUVR values. Differences between the estimated functions were assessed by means of bootstrapped confidence intervals. These were computed by repeatedly (n=10000) resampling the dataset (with replacement) and calculating the differences between spline fits. The discriminative performance of CSF P-tau measures was assessed using the area under the receiver operating characteristic curve (AUC), adjusted for age. Significant differences in AUC values were tested using DeLong statistics\(^{33}\) and Bonferroni correction was applied to account for multiple comparisons. In addition to AUC, sensitivity and specificity at the cut-off that resulted in the highest Youden index (sensitivity + specificity – 1) are reported. Analyses were performed in R, v.4.0.2, with significance set at \(P<0.05\), two-tailed. Voxelwise analyses examining the association between CSF P-tau levels and Aβ and Tau-PET were performed using multilinear models, as implemented in SPM12, adjusted for age and the interval between lumbar-puncture and PET scan.

**Data Availability**
Anonymized study data for the primary analyses presented in this report are available on request from any qualified investigator for purposes of replicating the results.

**Results**

**Participants**

We included 629 participants, including 334 CU controls (253 [76%] Aβ-negative and 81 [24%] Aβ-positive), 84 Aβ-positive MCI, 119 Aβ-positive AD dementia, and 92 with a non-AD neurodegenerative disorder (21 FTD, 40 Parkinson’s disease with or without cognitive impairment, 20 PSP/CBD and 11 VaD; overall, 15%(n=14) showed Aβ-positivity).

Demographic and clinical characteristics are summarized in Table 1. For a flow diagram of participants included in the study, see eFigure 1 (available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7).

**Correlations between P-tau isoforms**

A schematic overview of the included P-tau assays is provided in Figure 1. P-tau isoforms were strongly correlated across all subjects (range 0.853-0.977, all P<0.0001) (eFigure 2 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7). These associations were significant in Aβ-positive CU, Aβ-positive MCI and AD dementia, but not in Aβ-negative individuals. As moderate but significant correlations were observed between age and CSF P-tau levels (Tables e1 and e2 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7), age was accounted for when comparing CSF P-tau levels across Tau-PET based Braak stages and diagnostic groups.

**Correlations between CSF P-tau and Aβ and Tau-PET**

Correlations between CSF P-tau isoforms and Aβ and Tau-PET SUVR values in Braak ROIs are reported in the Supplement (eTable 3 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7). Correlations between the CSF P-tau species and Aβ
or Tau-PET did not differ significantly by APOE-status (ε4-carrier vs non-carrier), age (over/under median split age [70 years]) or sex (male vs female) (data not shown). Using Aβ-PET, P-tau217Lilly showed the strongest correlation with neocortical SUVR values in CU individuals (r=0.789, P<0.001). This correlation was significantly higher than those for P-tau181Innotest (r=0.497), P-tau181Lilly (r=0.737), P-tau181Elecsys (r=0.581) and P-tau231ADx (r=0.724) (P<0.0001). In Aβ-positive MCI, P-tau217Lilly also showed the strongest correlation (r=0.516, P<0.001) with Aβ-PET; this correlation was significantly stronger than those for P-tau181Innotest (r=0.312) and P-tau181Elecsys (r=0.314) (P<0.001). Findings from voxel-wise analyses were consistent with these ROI based results and also highlighted the stronger correlations between P-tau isoforms and Aβ-PET in CU individuals (eFigure 3 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7). When correlation analyses were repeated in CU individuals by Aβ-status, significant associations between CSF P-tau measures and Aβ-PET were found only in the Aβ-positive CU group (eTable 4 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7).

Using Tau-PET, CSF P-tau217Lilly was most strongly associated P-tau variant in CU participants, with the strongest correlation seen in the Braak I/II ROI (r=0.683). This correlation was significantly higher than those for P-tau181Innotest (r=0.485) (P<0.0001), P-tau181Lilly (r=0.640) (P<0.001), P-tau181Elecsys (r=0.546) (P<0.0001) and P-tau231ADx (r=0.605) (P<0.001). A similar pattern was seen when looking at Braak III/IV and V/VI. In Aβ-positive cognitively impaired (CI) individuals (i.e., Aβ-positive MCI and AD dementia patients, P-tau217Lilly showed the highest correlation to Tau-PET in the Braak III/IV ROI (r=0.592); this association was significantly higher than those P-tau181Innotest (r=0.272) (P<0.0001), P-tau181Lilly (r=0.486) (P<0.0001), P-tau181Elecsys (r=0.301) (P<0.001) and P-tau231ADx (r=0.393) (P<0.0001). This pattern also held when looking at Braak I/II and V/VI ROIs. In addition, P-tau181Lilly showed significantly higher correlations with Tau-PET across all Braak ROIs as compared to P-tau181Innotest, P-tau181Elecsys and P-tau231ADx. Voxelwise analyses (eFigure 4) supported these findings and in particular highlighted the stronger
associations of P-tau217\textsubscript{Lilly} to Tau-PET when compared to the associations between Tau-PET and other P-tau variants. Similar to findings using A\textbeta PET, associations between CSF P-tau measures and Tau-PET were significant only in the A\textbeta-positive CU group (eTable 4 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7) when repeating analyses by A\textbeta-status.

**CSF P-tau slopes as a function of A\textbeta and Tau-PET**

Spline models examining CSF P-tau concentrations across A\textbeta and Tau-PET are shown in Figure 2; confidence intervals for differences in P-tau biomarkers at specified SUVR values are detailed in the Supplement (eTable 5 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7). Using A\textbeta PET, the slope of P-tau217\textsubscript{Lilly} was significantly different from those of P-tau181\textsubscript{Innotest}, P-tau181\textsubscript{Lilly}, P-tau181\textsubscript{Elecsys} and P-tau231\textsubscript{ADx} in A\textbeta-positive CU. The slope of P-tau181\textsubscript{Lilly} differed significantly from those of P-tau181\textsubscript{Innotest} and P-tau181\textsubscript{Elecsys}; no significant difference was seen between confidence intervals for P-tau181\textsubscript{Lilly} and P-tau231\textsubscript{ADx}, however. The same pattern was seen for P-tau217\textsubscript{Lilly} when looking at A\textbeta-PET in A\textbeta-positive MCI. When analyses were performed separately in A\textbeta-positive and A\textbeta-negative CU individuals (eFigure 5, eTable 6 available from Dryad:https://doi.org/10.5061/dryad.4f4qrfjc7), increasing fold change with increasing SUVR values and separation of P-tau trajectories was largely confined to the A\textbeta-positive CU group.

Using Tau-PET SUVR in the Braak I/II ROI in CU individuals, the slope of P-tau217\textsubscript{Lilly} differed significantly from those of P-tau181\textsubscript{Innotest}, P-tau181\textsubscript{Lilly}, P-tau181\textsubscript{Elecsys} and P-tau231\textsubscript{ADx} at SUVR values of 1.5 or greater. The slopes of P-tau181\textsubscript{Lilly} and P-tau231\textsubscript{ADx} also differed significantly from those of P-tau181\textsubscript{Innotest} and P-tau181\textsubscript{Elecsys}; no significant difference was seen between P-tau181\textsubscript{Lilly} and P-tau231\textsubscript{ADx}. The same pattern was seen when using the Braak III/IV ROI in A\textbeta-positive CI participants and using the Braak V/VI ROI (data not shown). Similar to the analyses with A\textbeta PET, greater fold change at higher SUVR
levels and separation of P-tau trajectories was largely confined to the Aβ-positive CU group (eFigure 5, eTable 6 available from Dryad:https://doi.org/10.5061/dryad.4f4qrjfc7).

**CSF P-tau levels by Tau-PET based Braak stages**

When dividing participants on the basis of their Tau-PET status in Braak ROIs (Figure 3) (i.e. [18F]RO948 negative (Braak 0) or abnormal retention in the Braak I/II ROI only; or abnormal retention in the Braak III/IV ROI (but not V/VI) or Braak V/VI, fold change (relative to the mean of the Braak 0 group) was highest for P-tau217Lilly (Braak III/IV, 4.69[95% CI, 4.15-5.24]; V/VI, 6.93[95% CI, 6.21-7.65]) followed by P-tau181Lilly (Braak III/IV, 2.93[95% CI, 2.63-3.23], V/VI, 3.78[95% CI, 3.41-4.15]), P-tau231ADx (Braak III/IV, 2.39[95% CI, 2.19-2.59]; V/VI, 2.93[95% CI, 2.66-3.20]), P-tau181Elecsys (Braak III/IV, 1.88[95% CI, 1.71-2.04], Braak V/VI, 2.23[95% CI, 2.04-2.44]) and P-tau181Innotest (Braak III/IV, 1.71[95% CI, 1.57-1.85], Braak V/VI, 1.98[95% CI, 1.82-2.14]).

**CSF P-tau levels by diagnostic group**

By comparison to all Aβ-negative participants, CSF P-tau levels were increased in Aβ-positive CU, MCI and AD (Figure 4). In Aβ-positive MCI and AD, the mean fold increases (compared to Aβ-negative CU) were between 7.36[95% CI, 6.26-8.47] and 13.27[95% CI, 12.04-14.51] for P-tau217Lilly. By comparison, P-tau181Lilly showed a mean fold increase of between 3.38[95% CI, 2.98-3.79] and 5.35[95% CI, 4.89-5.81] while P-tau231ADx showed mean fold increases of between 2.40[95% CI, 2.57-3.18] and 3.96 [95% CI, 3.68-4.25]. Mean fold increases were between 1.72[95% CI, 1.59-1.86] and 2.14 [95% CI, 1.99-2.30] for P-tau181Innotest and between 1.88[95% CI, 1.71-2.05] and 2.47 [95% CI, 2.27-2.67] for P-tau181Elecsys. In Aβ-positive CU, the greatest fold increase was seen for P-tau217Lilly (4.78[95% CI, 4.02-5.54), followed by P-tau181Lilly (2.61[95% CI, 2.29-2.92]) and P-tau231ADx (2.39[95% CI, 2.15-2.63]), P-tau181Elecsys (1.66[95% CI, 1.53-1.79]) and P-tau181Innotest (1.58[95% CI, 1.44-1.68]).
**Diagnostic accuracy of CSF P-tau isoforms**

Receiver operating characteristic curves and associated AUC values are shown in Figure 5. AUC values—along with sensitivity and specificity estimates at cut-offs that resulted in the highest Youden index—are reported in Table 2. The diagnostic performance of CSF P-tau for AD dementia vs Aβ-negative CU (Figure 5A) and non-AD neurodegenerative disorders (Figure 5B) was highest using P-tau217Lilly. For both contrasts, AUC values for P-tau217Lilly were significantly higher than those for P-tau181Innotest and P-tau181Elecsys (\(P<0.0001\)). For the separation of AD dementia from non-AD neurodegenerative disorders, the AUC value for P-tau217Lilly was significantly higher than that for P-tau181Lilly (\(P<0.05\)). For both contrasts, AUC values for P-tau181Lilly were significantly higher than those for P-tau181Innotest and P-tau181Elecsys (\(P<0.0001\)).

When differentiating Aβ-PET positive from Aβ-PET negative participants (Figure 5C), P-tau217Lilly outperformed P-tau181Innotest (\(P<0.0001\)), P-tau181Lilly (\(P<0.001\)) and P-tau181Elecsys (\(P<0.001\)). Using Tau-PET status in the Braak III/IV (Figure 5D) and V/VI (Figure 5E) ROIs, AUCs for P-tau217Lilly were significantly higher than those for P-tau181 and P-tau231ADx (\(P<0.0001\)). Using both Aβ and Tau-PET, the diagnostic performance of P-tau181Lilly was superior to that of P-tau181Innotest and P-tau181Elecsys (\(P<0.0001\)). Using Tau-PET, AUC values for P-tau231ADx were significantly higher than those for P-tau181Innotest (Braak III/IV, \(P<0.0001\); Braak V/VI, \(P<0.001\)), P-tau181Elecsys (Braak III/IV, \(P<0.0001\); Braak V/VI, \(P<0.01\)) and P-tau181Lilly (Braak III/IV, \(P<0.01\); Braak V/VI, \(P<0.05\)). The AUC value of P-tau181Elecsys was significantly higher than that for P-tau181Innotest using the Braak III/IV ROI (\(P<0.05\)) but not when using the Braak V/VI ROI.

**Classification of evidence**
This study provides class II evidence that phosphorylated tau at threonine 217 provides higher diagnostic accuracy for diagnosis of AD dementia than P-tau at threonine 181 or 231.

**Discussion**

Consistent with previous work using these assays, levels CSF P-tau181\textsubscript{Lilly} and P-tau217\textsubscript{Lilly} were progressively higher across both the AD continuum (i.e., moving from Aβ-positive CU through Aβ-positive AD dementia)\textsuperscript{14,15} and Tau-PET Braak stages.\textsuperscript{14} Further, in agreement with a previous study, we found that P-tau217\textsubscript{Lilly} had significantly higher correlations with Aβ and Tau-PET as compared to P-tau181\textsubscript{Lilly} \textsuperscript{14} and extended this finding to show that the correlation was also significantly higher than for P-tau181\textsubscript{Innotest}, P-tau181\textsubscript{Elecsys} and P-tau231\textsubscript{ADx}. Previously, using CSF samples taken prior to baseline Tau-PET in Aβ-positive CU, 56% of subjects showed positive P-tau217\textsubscript{Lilly} levels, compared with only 25% for P-tau181\textsubscript{Lilly}.\textsuperscript{15} Combined with mass spectrometry findings in AD showing an increased degree of phosphorylation at threonine 217 compared with position 181,\textsuperscript{16,34} these results were interpreted as suggesting that phosphorylation at position 217 may be more pronounced by comparison to other sites. Though the differences were modest, stronger correlations observed with PET would also prove consistent with findings showing that threonine 217 phosphorylation was considerably increased in AD as compared to threonine 181\textsuperscript{8,35} and with the preferential phosphorylation of tau at specific sites across the different stages of AD.\textsuperscript{16,36} In addition, by comparison to studies using P-tau181 measurements from commercial assays such as P-tau181\textsubscript{Innotest} and P-tau181\textsubscript{Elecsys},\textsuperscript{37} larger effect sizes were seen when using P-tau181\textsubscript{Lilly}, P-tau217\textsubscript{Lilly} and P-tau231\textsubscript{ADx}.

Using spline-based analyses, we compared the slopes of P-tau isoforms in relation to continuous Aβ and Tau-PET SUVR values. These analyses were performed in CU individuals and in cognitively impaired Aβ-positive subjects. Though greater PET SUVR values were associated with higher CSF P-tau concentrations for all isoforms, no significant differences were seen in the courses of P-tau181\textsubscript{Lilly} and P-tau231\textsubscript{ADx}. By contrast,
comparison of confidence intervals showed that the slope of P-tau217\textsubscript{Lilly} diverged from those of P-tau181\textsubscript{Lilly} and P-tau231\textsubscript{ADx} across a range of SUVR values, particularly in the CU group when using Tau-PET in the Braak I/II ROI. These findings are consistent with increases in the active production of soluble tau in the presence of aggregated Aβ\textsuperscript{8} and, possibly, with the idea that the relative phosphorylation of tau at specific sites varies across the course of AD\textsuperscript{16}. Though findings with Tau-PET in the CI group suggest a plateau in the course of all three isoforms—possibly due a process though which phosphorylation rates are reduced due sequestration by hyperphosphorylated aggregates\textsuperscript{38,39}—phosphorylation of threonine 217 may continue to increase later into the disease course, similar, for example, to what has been reported for P-tau205\textsuperscript{16}. This, combined with P-tau217\textsubscript{Lilly} possibly showing a higher specificity for AD\textsuperscript{14} may explain the higher AUC values seen for P-tau217\textsubscript{Lilly}. Though a tau-centric hypothesis ascribing a primary role to tau\textsuperscript{40} has been proposed as an alternative to the view that AD is caused by the accumulation of Aβ in the brain\textsuperscript{41} both spline- and correlation-based sensitivity analyses in CU individuals by Aβ-status showed there to be little association with Aβ and Tau-PET in Aβ-negative CU individuals.

A very recent study focused on characterizing the patterns of change in P-tau231\textsubscript{ADx} and P-tau181\textsubscript{Elecsys} in preclinical AD\textsuperscript{42}. In ROC analyses, they found that P-tau231\textsubscript{ADx} had statistically significant higher predictive accuracies than P-tau181\textsubscript{Elecsys} for discriminating Aβ-positive from Aβ-negative CU individuals. Moreover, P-tau231\textsubscript{ADx} showed an AUC that was higher than that of P-tau181\textsubscript{Elecsys}. Though our findings showing that P-tau231\textsubscript{ADx} had higher AUCs compared to P-tau181\textsubscript{Elecsys} and P-tau181\textsubscript{Innotest} are consistent with this, our results also suggest that P-tau231\textsubscript{ADx} is similar to P-tau181\textsubscript{Lilly}. In a related study by Karikari et al.,\textsuperscript{43} N-Ptau217 showed higher diagnostic performance for identifying Aβ pathology and AD at the MCI stage compared to established P-tau181 assays (P-tau181\textsubscript{Innotest} and P-tau181\textsubscript{Lumipulse}), but not compared to N-Ptau181. Possibly complicating this comparison, however, is the comparatively small number of prodromal AD cases. Though our results cannot be directly compared because of differences in the assays used for P-tau217, studies thus far suggest,
overall, that P-tau217 assays are generally more sensitive. Further studies directly comparing these assays are required, however, as well as whether P-tau231\textsubscript{ADx} and P-tau181\textsubscript{Lilly} begin to increase at the same time point or if P-tau231\textsubscript{ADx} starts to increase earlier in order to help establish the temporal dynamics of these different measures. Here, longitudinal studies comparing P-tau181, P-tau217 and P-tau231 will prove crucial.

Clinical utility in terms of fold change with respect to levels in Aβ-negative CU individuals varied across the investigated CSF P-tau measures. Though sharing the same P-tau181 specific antibody (AT270), the Innotest and Lilly P-tau181 assays had different total tau (i.e., not binding to the phosphorylation site) antibodies and showed large differences in fold change. This indicates the importance of tau isoforms and/or fragmentation with respect to clinical utility: should fragmentation occur in the region of the protein where the two total-tau antibodies bind, this could lead to the measurement of different pools of tau present in CSF. This hypothesis is reinforced by the fact that P-tau181\textsubscript{Innotest} and P-tau181\textsubscript{Elecsys} assays showed similar performance despite differing in both P-tau181-specific and total tau antibodies; presumably, this reflects these assays measuring the same tau isoform/fragment. The influence of the total tau antibody on the clinical utility of P-tau181 is thus significant, as P-tau181\textsubscript{Lilly} showed about double the fold change as P-tau181\textsubscript{Innotest} and P-tau181\textsubscript{Elecsys} in AD. One explanation for the differences in fold-change is also the possibility of a different binding affinity of the total tau antibody for tau, which could lead to differences in the measured signal with increasing protein concentrations. However, the Lilly assays showed that P-tau217 showed greater fold change compared to P-tau181 when using the same total tau antibody in combination with different phosphorylation-specific antibodies. The variability observed in the fold-change of the measurements with P-tau181 assay cannot therefore be fully explained by technical differences of the assay or antibody affinity. One could speculate that the binding of antibodies to different phospho-epitopes could lead to conformational changes in the protein and therefore different affinity of the total tau antibody, but further studies are needed to demonstrate this. Similarly, we do not yet know if
P-tau231 will be better or worse in a head-to-head comparison to P-tau217 as the P-tau231\textsubscript{ADx} assay uses a different total tau antibody.

Recent mass spectrometry-based work\textsuperscript{44} addressing Tau PTMs has shown that P-tau181, P-tau217 and P-tau231 appear to be indicators of early AD pathology based on Braak NFT staging of post-mortem brain tissue.\textsuperscript{18} In a related study exploring the biochemical link between measures of Aβ and tau phosphorylation, however, a somewhat different conclusion was reached: while soluble P-tau181, P-tau217 and P-tau231 were highly correlated to Aβ levels,\textsuperscript{45} the highest degree of tau phosphorylation was observed in the insoluble fractions of AD brain tissue, suggesting that correlations with Aβ and tau aggregates may be more complex than simple linear relations. Despite this recent progress in understanding the link between abnormal PTMs and the aggregation of tau in AD, additional studies are required to understand how such abnormal PTMs are reflected in predominantly C-terminally truncated tau.\textsuperscript{8,46} Current findings nevertheless highlight the importance of mapping PTMs in order to better understand the pathophysiology of AD; moreover, increased insight into the role of PTMs will facilitate the identification of novel therapeutic targets and improve AD diagnostics.

Strengths of our study include the large number of subjects spanning the AD continuum, within subject measurements of multiple CSF P-tau isoforms and their comparison to the widely used P-tau181 from Innotest and Elecsys, and the availability of Aβ and Tau-PET imaging. Moreover, the use of Mid-fragments across all P-tau measures allowed for a more direct comparison of P-tau biomarkers. This study has limitations, however. First, our inferences as to the ordering of changes in P-tau isoforms over the course of AD are based on cross-sectional data whereas longitudinal studies are needed to accurately address this question. Second, we did not have Aβ-PET in the AD dementia group. Though earlier work indicated that Aβ pathology may reach a plateau during the dementia phase of AD,\textsuperscript{47} recent findings suggest that this may not be the case.\textsuperscript{48} As such, we were not able to examine the effect of higher Aβ-PET SUVR values on P-tau isoforms but were nonetheless...
able to identify the significantly higher dynamic range of P-tau217\textsubscript{Lilly} using the available Aβ-PET from non-demented participants. Though we acknowledge the lack of Aβ-PET across all groups as a limitation, very high concordance is seen between CSF Aβ42/Aβ40 and Aβ-PET.\textsuperscript{49} As such, the two measures provide similar information with respect to defining Aβ-status. As we were interested in the relationship between P-tau and the amount of fibrillary brain Aβ, however, we chose Aβ-PET as this measure reflects the cumulative burden of accumulated Aβ pathology while CSF Aβ42/40 reflects the production and clearance of Aβ42 and Aβ40 at a given time point.\textsuperscript{50} Lastly, though our study used the same assay for P-tau231\textsubscript{ADx} and P-tau181\textsubscript{Elecsys} as used in the study by Suárez-Calvet et al.,\textsuperscript{42} assays for P-tau181 and P-tau217 differed. In order to more definitively address the ordering of P-tau biomarkers, future work comparing phosphorylation epitopes will require the use of assays that are as similar as possible using head-to-head designs and validation in independent datasets.

In conclusion, we found that CSF P-tau217\textsubscript{Lilly} more strongly correlated with Aβ and Tau-PET, showed greater increases as compared to P-tau181\textsubscript{Innotest}, P-tau181\textsubscript{Lilly}, P-tau181\textsubscript{Elecsys} and P-tau231\textsubscript{ADx} in AD dementia and across Tau-PET Braak stages. Moreover, CSF P-tau217\textsubscript{Lilly} showed greater discriminative accuracy for AD dementia, as compared to CSF P-tau181\textsubscript{Innotest}, P-tau181\textsubscript{Lilly}, P-tau181\textsubscript{Elecsys} and P-tau231\textsubscript{ADx}. These results suggest that CSF P-tau217\textsubscript{Lilly} should be the preferred P-tau variant to use for AD diagnostics and for tracking disease progression (e.g., as an outcome in clinical AD trials).
### Appendix 1. Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antoine Leuzy, PhD</td>
<td>Lund University, Malmö, Sweden</td>
<td>Design and conceptualized study; analyzed the data; drafted the manuscript for intellectual content</td>
</tr>
<tr>
<td>Shorena Janelidze, PhD</td>
<td>Lund University, Malmö, Sweden</td>
<td>Design and conceptualized study; revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Niklas Mattsson-Carlsgren, MD, PhD</td>
<td>Lund University, Malmö, Sweden</td>
<td>Design and conceptualized study; revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Sebastian Palmqvist, MD, PhD</td>
<td>Lund University, Malmö, Sweden; Skåne University Hospital, Lund, Sweden</td>
<td>Design and conceptualized study; revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Dirk Jacobs, PhD</td>
<td>ADx NeuroSciences NV, Ghent, Belgium</td>
<td>Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Claudia Cicognola, MD, PhD</td>
<td>Lund University, Malmö, Sweden; Skåne University Hospital, Lund, Sweden</td>
<td>Interpreted the data; revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Erik Stomrud, MD, PhD</td>
<td>Lund University, Malmö, Sweden; Skåne University Hospital, Lund, Sweden</td>
<td>Major role in the acquisition of data; revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Eugen Vanmechelen, PhD</td>
<td>ADx NeuroSciences NV, Ghent, Belgium</td>
<td>Major role in the acquisition of data; interpreted the data;</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Institution(s)</td>
<td>Contributions</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Jeffrey L. Dage, PhD</td>
<td>Eli Lilly and Company, Indianapolis, IN, USA</td>
<td>Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Oskar Hansson, MD, PhD</td>
<td>Lund University, Malmö, Sweden; Skåne University Hospital, Lund, Sweden</td>
<td>Design and conceptualized study; analyzed the data; drafted the manuscript for intellectual content</td>
</tr>
</tbody>
</table>
References


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

Figure 1. A schematic overview of the included P-tau assays

Schematic illustration of full-length tau-441, including N-terminal, proline rich region, microtubuli binding domain and C-terminal. Anti-tau antibodies are indicated for each of the five included P-tau assays under the respective epitope region.
Figure 2. CSF P-tau slopes as a function of Aβ and Tau-PET SUVR

CSF P-tau levels (expressed as mean fold change relative to the mean of Aβ-negative CU participants) are shown against global Aβ PET neocortical SUVR across all CU participants (A) and in Aβ-positive MCI (B). Panels C and D show corresponding plots for Tau-PET in all CU participants (Braak I/II) and in all Aβ-positive cognitively impaired participants (Braak III/IV) (i.e., Aβ-positive MCI and AD dementia combined). Generalized additive models with cubic regression splines were used to compare the slopes of CSF P-tau isoforms across different Aβ and Tau-PET SUVR values. Shaded grey areas indicate 95% confidence intervals.
**Figure 3.** CSF P-tau across PET based Braak stages

Levels of CSF P-tau181\textsubscript{Innotest} (A), P-tau181\textsubscript{Lilly} (B), P-tau181\textsubscript{Elecsys} (C), P-tau217\textsubscript{Lilly} (D) and P-tau231\textsubscript{ADx} (E) are expressed relative to the mean of participants showing no abnormal Tau-PET SUVR values in any of the investigated regions of interest (Braak 0, n=437). Tau positivity in Braak stages III/VI was established using apriori cutoffs based on the mean SUVR within a given ROI plus 2.5 standard deviations (SD) among Aβ-negative young controls. Solid grey horizontal lines indicate age-adjusted group comparisons: AD dementia higher than all groups ($P<0.001$); Aβ-positive MCI higher than CU and non-AD ($P<0.001$); Aβ-positive CU higher than Aβ-negative CU and non-AD ($P<0.001$). In order to facilitate comparison between P-tau measures, y-axes were scaled to the maximum fold change seen across biomarkers.
Figure 4. CSF P-tau across diagnostic groups

Levels of CSF P-tau181\textsubscript{Innotest} (A), P-tau181\textsubscript{Lilly} (B), P-tau181\textsubscript{Elecsys} (C), P-tau217\textsubscript{Lilly} (D) and P-tau231\textsubscript{ADx} (E) are expressed relative to the mean of Aβ-negative participants (n=253). Solid grey horizontal lines indicate age-adjusted group comparisons: AD dementia higher than all groups (P<0.001); Aβ-positive MCI higher than CU and non-AD (P <0.001); Aβ-positive CU higher than Aβ-negative CU and non-AD (P<0.001). In order to facilitate comparison between P-tau measures, y-axes were scaled to the maximum fold change seen across biomarkers. In order to facilitate comparison between P-tau measures, y-axes were scaled to the maximum fold change seen across biomarkers. Non-AD, non-Alzheimer’s disease neurodegenerative disorders; Aβ- CU, Aβ-negative cognitively unimpaired; Aβ+ CU, Aβ-positive cognitively unimpaired; Aβ+ MCI, Aβ-positive mild cognitive impairment; AD dem., Alzheimer’s disease dementia.
Figure 5. Receiver operating characteristic plots for CSF P-tau

Receiver operating characteristic curves are shown for the following groups: AD dementia vs Aβ-negative CU (A), AD dementia vs non-AD disorders (B), Aβ PET positive vs negative (C), and Tau-PET positive vs negative using the Braak III/IV (D) and V/VI (E) ROIs.
Table 1. Demographic, clinical and biomarker characteristics by diagnostic group

<table>
<thead>
<tr>
<th></th>
<th>Aβ- CU (n=253)</th>
<th>Aβ+ CU (n=81)</th>
<th>Aβ+ MCI (n=84)</th>
<th>AD dementia (n=119)</th>
<th>Non-AD (n=92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N, % female</td>
<td>132 (52%)</td>
<td>41 (51%)</td>
<td>45 (54%)</td>
<td>64 (54%)</td>
<td>44 (48%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>60.58 (14.93)</td>
<td>71.59 (8.45)</td>
<td>71.21 (8.66)</td>
<td>77.34 (7.03)</td>
<td>69.98 (9.32)</td>
</tr>
<tr>
<td>Education, years</td>
<td>12.68 (3.22)</td>
<td>12.05 (3.71)</td>
<td>13.28 (4.92)</td>
<td>12.39 (4.48)</td>
<td>12.57 (3.62)</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.98 (1.20)</td>
<td>28.83 (1.29)</td>
<td>26.58 (1.97)</td>
<td>20.14 (4.37)</td>
<td>25.93 (4.04)</td>
</tr>
<tr>
<td>Aβ positivity, N, %</td>
<td>0 (0%)</td>
<td>81 (100%)</td>
<td>84 (100%)</td>
<td>119 (100%)</td>
<td>14 (15%)</td>
</tr>
<tr>
<td>APOE ε4 positivity, N, %</td>
<td>94 (37%)</td>
<td>56 (69%)</td>
<td>64 (76%)</td>
<td>85 (71%)</td>
<td>28 (30%)</td>
</tr>
<tr>
<td>CSF P-tau181&lt;sub&gt;Innotest&lt;/sub&gt;, pg/mL</td>
<td>39.89 (13.60)</td>
<td>62.33 (21.27)</td>
<td>68.93 (25.45)</td>
<td>88.63 (35.71)</td>
<td>39.91 (15.80)</td>
</tr>
<tr>
<td>CSF P-tau181&lt;sub&gt;Lilly&lt;/sub&gt;, pg/mL</td>
<td>38.49 (15.31)</td>
<td>99.20 (53.24)</td>
<td>128.62 (70.30)</td>
<td>216.27 (112.26)</td>
<td>47.56 (23.00)</td>
</tr>
<tr>
<td>CSF P-tau181&lt;sub&gt;Elecsys&lt;/sub&gt;, pg/mL</td>
<td>16.37 (5.22)</td>
<td>27.23 (9.93)</td>
<td>30.82 (13.28)</td>
<td>40.37 (18.19)</td>
<td>17.00 (6.12)</td>
</tr>
<tr>
<td>CSF P-tau217&lt;sub&gt;Lilly&lt;/sub&gt;, pg/mL</td>
<td>48.82 (25.47)</td>
<td>206.26 (146.62)</td>
<td>316.64 (218.35)</td>
<td>616.50 (358.60)</td>
<td>67.58 (52.44)</td>
</tr>
<tr>
<td>CSF P-tau231&lt;sub&gt;ADx&lt;/sub&gt;, pg/mL</td>
<td>9.87 (4.41)</td>
<td>23.47 (10.53)</td>
<td>28.21 (13.72)</td>
<td>40.15 (16.22)</td>
<td>10.57 (4.91)</td>
</tr>
<tr>
<td>Aβ PET - Neocortical SUVR</td>
<td>0.47 (0.03)</td>
<td>0.67 (0.14)</td>
<td>0.75 (0.16)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tau-PET - Braak I/II SUVR</td>
<td>1.09 (0.12)</td>
<td>1.31 (0.25)</td>
<td>1.62 (0.44)</td>
<td>1.99 (0.37)</td>
<td>1.14 (0.19)</td>
</tr>
<tr>
<td>Tau-PET - Braak III/IV SUVR</td>
<td>1.14 (0.09)</td>
<td>1.25 (0.24)</td>
<td>1.49 (0.42)</td>
<td>2.13 (0.66)</td>
<td>1.15 (0.10)</td>
</tr>
<tr>
<td>Tau-PET - Braak V/VI SUVR</td>
<td>1.05 (0.08)</td>
<td>1.07 (0.13)</td>
<td>1.18 (0.29)</td>
<td>1.51 (0.42)</td>
<td>1.04 (0.09)</td>
</tr>
</tbody>
</table>
Though Aβ PET was only considered as a continuous value, earlier work has established an SUVR > 0.53 as abnormal.\textsuperscript{14} Cut-offs for Tau-PET SUVR were > 1.48 (Braak I/II), > 1.36 (Braak III/IV) and > 1.35 (Braak V/VI).\textsuperscript{25}
<table>
<thead>
<tr>
<th>Performance (95% CI)</th>
<th>Cut-off</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AD dementia vs Aβ- CU</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>^{a} CSF P-tau181\textsubscript{Innotest}</td>
<td>57.73</td>
<td>0.83 (0.78 – 0.88)</td>
<td>75.44 (59.65 – 84.21)</td>
<td>81.89 (74.90 – 93.83)</td>
</tr>
<tr>
<td></td>
<td>^{b} CSF P-tau181\textsubscript{Lilly}</td>
<td>86.59</td>
<td>0.93 (0.91 – 0.96)</td>
<td>85.09 (77.19 – 92.98)</td>
<td>89.30 (82.30 – 94.65)</td>
</tr>
<tr>
<td></td>
<td>^{c} CSF P-tau181\textsubscript{Elecsys}</td>
<td>25.28</td>
<td>0.85 (0.80 – 0.90)</td>
<td>75.44 (64.04 – 85.09)</td>
<td>85.60 ( 76.13 – 93.00)</td>
</tr>
<tr>
<td></td>
<td>^{d} CSF P-tau217\textsubscript{Lilly}</td>
<td>194.32</td>
<td>0.94 (0.91 – 0.97)</td>
<td>86.84 (78.07 – 92.98)</td>
<td>90.53 (84.36 – 95.88)</td>
</tr>
<tr>
<td></td>
<td>^{e} CSF P-tau231\textsubscript{ADx}</td>
<td>20.14</td>
<td>0.93 (0.89 – 0.96)</td>
<td>84.21 (76.32 – 92.11)</td>
<td>90.53 (81.07 – 95.06)</td>
</tr>
<tr>
<td><strong>AD dementia vs non-AD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>^{a} CSF P-tau181\textsubscript{Innotest}</td>
<td>55.75</td>
<td>0.88 (0.84 – 0.93)</td>
<td>81.58 (71.05 – 90.35)</td>
<td>86.67 (76.67 – 94.44)</td>
</tr>
<tr>
<td></td>
<td>^{b} CSF P-tau181\textsubscript{Lilly}</td>
<td>99.87</td>
<td>0.96 (0.94 – 0.98)</td>
<td>91.23 (81.58 – 98.25)</td>
<td>95.56 (85.56 – 100)</td>
</tr>
<tr>
<td></td>
<td>^{c} CSF P-tau181\textsubscript{Elecsys}</td>
<td>25.44</td>
<td>0.89 (0.85 – 0.94)</td>
<td>79.82 (65.79 – 89.47)</td>
<td>91.11 (80.00 – 98.89)</td>
</tr>
<tr>
<td></td>
<td>^{d} CSF P-tau217\textsubscript{Lilly}</td>
<td>190.87</td>
<td>0.98 (0.96 – 0.99)</td>
<td>93.86 (87.72 – 98.25)</td>
<td>96.67 (91.11 – 100)</td>
</tr>
<tr>
<td></td>
<td>^{e} CSF P-tau231\textsubscript{ADx}</td>
<td>18.76</td>
<td>0.96 (0.94 – 0.98)</td>
<td>92.98 (82.46 – 98.25)</td>
<td>91.11 (82.22 – 98.89)</td>
</tr>
<tr>
<td>Aβ PET + vs Aβ PET - (Neocortical ROI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>a CSF P-tau181&lt;sub&gt;Innotest&lt;/sub&gt;</td>
<td>53.87</td>
<td>0.74 (0.69 – 0.80)</td>
<td>66.23 (51.66 – 76.16)</td>
<td>76.98 (67.17 – 89.06)</td>
<td>a vs b, ****</td>
</tr>
<tr>
<td>b CSF P-tau181&lt;sub&gt;Lilly&lt;/sub&gt;</td>
<td>71.49</td>
<td>0.83 (0.79 – 0.88)</td>
<td>73.51 (64.90 – 80.79)</td>
<td>87.92 (81.51 – 93.58)</td>
<td>a vs d, ****</td>
</tr>
<tr>
<td>c CSF P-tau181&lt;sub&gt;Elecsys&lt;/sub&gt;</td>
<td>23.64</td>
<td>0.77 (0.72 – 0.82)</td>
<td>66.23 (55.63 – 76.82)</td>
<td>83.02 (72.45 – 98.82)</td>
<td>a vs e, ****</td>
</tr>
<tr>
<td>d CSF P-tau217&lt;sub&gt;Lilly&lt;/sub&gt;</td>
<td>137.55</td>
<td>0.86 (0.82 – 0.90)</td>
<td>72.85 (63.58 – 80.79)</td>
<td>90.57 (83.40 – 96.60)</td>
<td>b vs c, ****</td>
</tr>
<tr>
<td>e CSF P-tau231&lt;sub&gt;ADx&lt;/sub&gt;</td>
<td>16.45</td>
<td>0.85 (0.80 – 0.89)</td>
<td>76.82 (67.55 – 84.11)</td>
<td>85.28 (78.49 – 91.70)</td>
<td>b vs d, ****</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tau-PET + vs Tau-PET - (Braak III/IV ROI)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a CSF P-tau181&lt;sub&gt;Innotest&lt;/sub&gt;</td>
<td>59.37</td>
<td>0.80 (0.76 – 0.84)</td>
<td>72.73 (57.14 – 81.82)</td>
<td>75.27 (69.37 – 87.96)</td>
<td>a vs b, ****</td>
</tr>
<tr>
<td>b CSF P-tau181&lt;sub&gt;Lilly&lt;/sub&gt;</td>
<td>99.82</td>
<td>0.92 (0.89 – 0.94)</td>
<td>84.42 (75.32 – 92.21)</td>
<td>87.31 (78.34 – 94.31)</td>
<td>a vs d, ****</td>
</tr>
<tr>
<td>c CSF P-tau181&lt;sub&gt;Elecsys&lt;/sub&gt;</td>
<td>24.25</td>
<td>0.83 (0.79 – 0.87)</td>
<td>76.62 (62.32 – 85.06)</td>
<td>75.71 (69.15 – 89.06)</td>
<td>a vs e, ****</td>
</tr>
<tr>
<td>d CSF P-tau217&lt;sub&gt;Lilly&lt;/sub&gt;</td>
<td>235.21</td>
<td>0.94 (0.91 – 0.96)</td>
<td>87.01 (81.82 – 92.86)</td>
<td>90.37 (85.78 – 93.00)</td>
<td>b vs c, ****</td>
</tr>
<tr>
<td>e CSF P-tau231&lt;sub&gt;ADx&lt;/sub&gt;</td>
<td>17.10</td>
<td>0.89 (0.86 – 0.92)</td>
<td>88.96 (74.03 – 95.45)</td>
<td>75.93 (69.58 – 90.37)</td>
<td>b vs e, ****</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tau-PET + vs Tau-PET - (Braak V/VI ROI)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a CSF P-tau181&lt;sub&gt;Innotest&lt;/sub&gt;</td>
<td>69.41</td>
<td>0.82 (0.77 – 0.88)</td>
<td>74.03 (62.34 – 87.01)</td>
<td>82.02 (67.60 – 88.76)</td>
<td>a vs b, ****</td>
</tr>
<tr>
<td>b CSF P-tau181&lt;sub&gt;Lilly&lt;/sub&gt;</td>
<td>127.22</td>
<td>0.91 (0.88 – 0.95)</td>
<td>88.31 (80.52 – 96.10)</td>
<td>86.79 (78.65 – 91.20)</td>
<td>a vs d, ****</td>
</tr>
<tr>
<td>c CSF P-tau181&lt;sub&gt;Elecsys&lt;/sub&gt;</td>
<td>29.19</td>
<td>0.84 (0.78 – 0.89)</td>
<td>77.92 (63.64 – 88.31)</td>
<td>80.52 (67.98 – 91.01)</td>
<td>a vs e, ****</td>
</tr>
<tr>
<td>d CSF P-tau217&lt;sub&gt;Lilly&lt;/sub&gt;</td>
<td>326.04</td>
<td>0.94 (0.91 – 0.96)</td>
<td>90.91 (83.12 – 97.40)</td>
<td>90.26 (85.02 – 93.63)</td>
<td>b vs c, ****</td>
</tr>
<tr>
<td>e CSF P-tau231&lt;sub&gt;ADx&lt;/sub&gt;</td>
<td>26.95</td>
<td>0.89 (0.84 – 0.93)</td>
<td>85.71 (74.03 – 97.40)</td>
<td>81.84 (64.42 – 89.51)</td>
<td>b vs e, ****</td>
</tr>
</tbody>
</table>
P-values (Bonferroni corrected) are for the comparison of AUC values across P-tau measures. Superscripted letters (a-e) indicate the different P-tau measures. * = P<0.05; ** = P<0.01; *** = P<0.001; **** = P<0.00001;
Comparing the Clinical Utility and Diagnostic Performance of Cerebrospinal Fluid P-Tau181, P-Tau217 and P-Tau231 Assays
Antoine Leuzy, Shorena Janelidze, Niklas Mattsson-Carlsgren, et al.
Neurology published online September 7, 2021
DOI 10.1212/WNL.000000000012727

This information is current as of September 7, 2021

Updated Information & Services
including high resolution figures, can be found at:
http://n.neurology.org/content/early/2021/09/07/WNL.000000000012727.full

Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
Alzheimer’s disease
http://n.neurology.org/cgi/collection/alzheimers_disease
Cerebrospinal Fluid
http://n.neurology.org/cgi/collection/cerebrospinal_fluid
Class II
http://n.neurology.org/cgi/collection/class_ii
PET
http://n.neurology.org/cgi/collection/pet

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
http://www.neurology.org/about/about_the_journal#permissions

Reprints
Information about ordering reprints can be found online:
http://n.neurology.org/subscribers/advertise