Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer’s disease

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Summary

Background Phosphorylated tau (p-tau) epitopes in cerebrospinal fluid (CSF) are accurate biomarkers for a pathological and clinical diagnosis of Alzheimer’s disease (AD) and are seen to be increased in preclinical stage of the disease. However, it is unknown if these increases transpire earlier, prior to amyloid-beta (Aβ) positivity as determined by position emission tomography (PET), and if an ordinal sequence of p-tau epitopes occurs at this incipient phase.

Methods We measured CSF concentrations of p-tau181, p-tau217 and p-tau231 in 171 participants across the AD continuum who had undergone Aβ ([18F]AZD4694) and tau ([18F]MK6240) position emission tomography (PET) and clinical assessment.

Findings All CSF p-tau biomarkers were accurate predictors of cognitive impairment but CSF p-tau217 demonstrated the largest fold-changes in AD patients in comparison to non-AD dementias and cognitively unimpaired individuals. CSF p-tau231 and p-tau217 predicted Aβ and tau to a similar degree but p-tau231 attained abnormal levels first. P-tau231 was sensitive to the earliest changes of Aβ in the medial orbitofrontal, precuneus and posterior cingulate before global Aβ PET positivity was reached.

Interpretation We demonstrate that CSF p-tau231 increases early in development of AD pathology and is a principal candidate for detecting incipient Aβ pathology for therapeutic trial application.

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Introduction

Neurofibrillary tangles (NFTs), primarily composed of abnormal hyperphosphorylated tau, are a key pathological hallmark of Alzheimer’s disease (AD). Increased concentrations of extracellular soluble phosphorylated tau (p-tau) and total tau (t-tau) constitute a reliable index of this intracellular process in mild cognitive impairment due to AD and AD dementia. NFTs are present many years after the deposition of extracellular amyloid-beta (Aβ) plaques but are more closely related to symptom onset and longitudinal studies have demonstrated that soluble p-tau and t-tau are increased already in preclinical disease (e.g., no cognitive impairment but evidence of AD pathology). Specifically, CSF concentrations of t-tau and p-tau are proposed to reflect neurodegeneration and tau pathology in the form of NFTs, respectively. However, these biomarkers remain at normal concentrations in other neurodegenerative disorders with substantial neurodegeneration, and also those with tau pathology. Preclinical studies provide compelling evidence supporting early brain amyloidosis as a driver of tau hyperphosphorylation in AD. Preclinical and AD neuronal culture models indicate that soluble Aβ oligomers (AβOs) induce tau hyperphosphorylation in multiple sites. Indeed, the link between early cerebral Aβ deposition and CSF p-tau elevations has been further supported by the fact that CSF tau species precede tau positron emission tomography (PET) abnormalities by a decade. Thus, it is suggested that CSF p-tau biomarkers likely indicate an active process of tau secretion, and one that is correlated with cerebral Aβ deposition in early disease.

CSF p-tau, in the context of AD, is often assumed to be the residue phosphorylated at threonine 181 (p-tau181). A multitude of mid-domain and C-terminal residues have also been described to be abnormally phosphorylated in the AD clinical spectrum and studies specifically assessing their comparative diagnostic performance remain limited. Recent reports have suggested that CSF p-tau217 might better discriminate AD from other neurodegenerative diseases than CSF p-tau181. In addition, CSF p-tau217 correlates better with Aβ and tau PET imaging than CSF p-tau181.
p-tau181. The findings support an emerging framework suggesting that certain CSF tau phosphorylation species predominate across the AD continuum.

We have recently reported that CSF p-tau231 increases early in the AD continuum and, therefore, may detect preclinical AD among cognitively unimpaired (CU) individuals. This is of fundamental importance for identifying individuals with subtle AD-related preclinical brain changes for therapeutic trials, as it is anticipated that disease-modifying drug candidates have a better opportunity to show effectiveness if initiated before symptom onset or even before overt Aβ plaque load has been achieved. Indeed, a recent study in transgenic mice suggests that early removal of Aβ seeds, before Aβ deposition becomes detectable, led to a significant reduction of Aβ accumulation and downstream pathologies. This emphasizes the need of a biomarker for detecting early Aβ seeds or the “pre-amyloid” phase. Therefore, it is imperative to investigate CSF p-tau epitopes associated with the earliest Aβ measures in CU to determine which of these early tau phosphorylation sites better reflects emerging Aβ pathology.

In order to address this knowledge gap, we investigated whether CSF p-tau epitopes (p-tau181, p-tau217, and p-tau231) are capable of identifying early Aβ pathology at the clinical, preclinical and pre-amyloid phases of AD. We also tested the performance of CSF p-tau181, p-tau217 and p-tau231 as biomarkers to predict clinical outcomes and Aβ and tau PET status. Based on our previous data, we hypothesized that CSF p-tau231 associates with early Aβ pathology in brain regions that are affected early in the AD process. For these purposes, we performed voxel-wise analyses of the association between different CSF p-tau biomarkers and Aβ and tau PET in cognitively unimpaired (CU) and impaired (CI) individuals.

Methods

Study design

The main objective of this study was to investigate whether CSF p-tau epitopes can identify early Aβ deposition before Aβ PET positivity. We also aimed at comparing their performance to predict amyloid and tau pathologies indexed by PET in participants ranging within the AD continuum. This cross-sectional study was based on data from the Translational Biomarkers in Aging and Dementia (TRIAD) cohort, which is an observational and longitudinal biomarker-based study. TRIAD participants, mostly ranging in the AD spectrum, are followed yearly with detailed clinical and neuropsychological assessments, as well as with collection of fluid (blood, urine, saliva, and CSF) and acquisition of multiple imaging biomarkers. In addition to meeting standard diagnostic criteria, AD dementia participants had CDR between 1 and 2, subjects with mild cognitive impairment (MCI) had a CDR of 0.5 and CU subjects had a CDR of 0. MCI participants without Aβ pathology are considered as non-AD neurodegenerative disease, together with participants clinically diagnosed with frontotemporal dementia (FTD; clinical diagnosis of behavioral or semantic variant of FTD, CDR score >0.5 and Aβ PET negative). This article includes 171 participants (27 young, <30 years; 82 CU; 20 MCI; 21 AD; 21 non-AD) from which CSF and PET imaging were available on October 2019. For data description purposes, participants were sub-grouped according to their amyloid status as further described. For the imaging analysis, participants were initially evaluated according to their cognitive status as CU and CI.

Ethics

The TRIAD study was approved by The Research Ethics Board of the Montreal Neurological Institute as well as the Faculty of Medicine Research Ethics Office, McGill University, and all study participants provided written informed consent (Protocols: IUSMD 16-60 and 16-61).

CSF analysis

CSF samples were collected by syringe and transferred to polypropylene tubes for centrifugation at 20 °C, 2200 g for 10 min. Samples were then distributed into 1 mL aliquots in polypropylene vials (Fisher Scientific Inc. Catalog # 3741-WP1D-BR) and permanently stored at -80 °C pending analyses.

All samples were analyzed for p-tau181, p-tau217 and p-tau231. P-tau181 and p-tau217 were quantified by novel single molecule array (Simoa) assay that have been previously described. Both single molecule array (Simoa) assay that have been previously described. In brief, rabbit polyclonal antibody specific for p-tau217 (#44-744, Invitrogen) and mouse AT270 mouse monoclonal antibody (MN1050; Invitrogen, Waltham, MA, USA) were used as capture, conjugated to paramagnetic beads (#103207, Quanterix). The mouse monoclonal antibody Tau12 (#806502, BioLegend) raised against the N-terminal epitope 6-18aa was used for detection. The assay calibrator was recombinant full-length tau-441 phosphorylated in vitro by glycogen synthase kinase 3β (#TO8-50FN, SignalChem). Calibrators and specimens were diluted with assay diluent (Tau 2.0 buffer; #101556, Quanterix). Analytical validation and assay measurement protocol for both CSF p-tau181 and CSF p-tau217 Simoa assays have been previously described. Both methods demonstrated intra and inter assay variation <8%. CSF p-tau231 was quantified using a research enzyme-linked immunosorbent assay (ELISA) assay using cis-conformational selective monoclonal antibody (ADx253, ADx NeuroSciences). Monoclonal mouse antibodies were generated using a 17-mer synthetic peptide, phosphorylated on hTau corresponding Thr231, spanning the tau region K₁₂₄KVAVVR(pT)P KPSPSSAK₂₄₀C.
was found to bind between R194 and G204 of tau441 or linear synthetic peptides and the ADx205 antibody ADx205 was recently fine mapped using overlapping form as pairing antibody in the p-epitope in the mid tau region was used in biotinylated tau mouse mAb (ADx205, ADx NeuroSciences) with previously described.40,42 The PET SUVR cutoff value of 1.55 (4 reconstruction on a 4D volume with 4 frames in injection and the OSEM algorithm was also used for MK6240 scans were acquired 90
FWHM.38 The inferior cerebellum and whole cerebellum gray matter were used as the reference regions for co-registration purposes. In addition, a Siemens High Resolution Research Tomograph (HRRT) was used for PET imaging acquisitions, which occurred ±80 days from the CSF collection date (median = 53 days). For Aβ PET, images were acquired 40–70 minutes post-injection of \(^{[18F]}\text{AZD4694}\) and scans were reconstructed using the ordered subset expectation maximization (OSEM) algorithm on a 4-dimensional volume with 3 frames (3×600s).35 For tau PET, \(^{[18F]}\text{MK6240}\) scans were acquired 90–110 minutes post-injection and the OSEM algorithm was also used for reconstruction on a 4D volume with 4 frames (4×300s).36 Additional pre-processing corrections were performed as described elsewhere.37 PET images were meninges and skull stripped, linearly and non-linearly registered to the ADNI template space and then spatially smoothed to achieve a final resolution of 8 mm FWHM.38 The inferior cerebellum and whole cerebellum gray matter were used as the reference regions for \(^{[18F]}\text{MK6240}\) and \(^{[18F]}\text{AZD4694}\), respectively.

Global Aβ PET used averaged SUVR of the precuneus, cingulate, inferior parietal, medial prefrontal, lateral temporal, and orbitofrontal cortices19 and positivity was visually defined by two neurologists blinded to clinical diagnosis. An additional Aβ PET SUVR cutoff value of 1.55 was estimated as previously described.40 Aβ PET SUVR was also converted to Centiloid units42 as previously described.40,42 The PET SUVR cutoff value of 1.55 corresponds to 24 Centiloids. Tau PET SUVR was globally estimated from a composite area including the transentorhinal (Braak stage I-II) and limbic (Braak III-IV) cortices.83-84 Tau positivity was defined as 2.5 standard deviations (SD) higher than the mean global SUVR of the young participants85 (in this study 2.5SD = 1.03 SUVR).

Statistical analysis
All non-imaging statistical analyses were performed using the R programming language (version 3.4.3). Data distribution was visually inspected using histograms and those variables that did not follow a normal distribution were log-transformed before parametric analysis when needed. Cross-sectional demographic and clinical data were assessed with linear models and \(\chi^2\) tests. Spearman rank correlation tests were applied to evaluate the association between biomarkers and correlation coefficients were compared using the R package “Cocor”. Linear regression models also tested the association between log-transformed CSF \(\tau\)-tau and other biomarkers always adjusting by age and gender. Similar models were also applied to evaluate group differences and, when necessary, Tukey honestly significant difference (HSD) test was used in the post hoc analysis. Receiver operating curves (ROC) provided both the area under the curve (AUC), for AD diagnosis or biomarker positivity, and the representative best value for accuracy at an optimal cutoff value. In addition to AUC, sensitivity and specificity, paired Delong’s test (\(\text{pROC} R\) package) was applied to statistically compare biomarker performance. Finally, imaging and CSF cutoffs were used to evaluate concordance between biomarkers.

Voxel-wise analyses were performed using VoxelStats.45 Voxel-based linear regression models evaluated the correlation between log transformed CSF values and PET biomarkers adjusting for age, sex, and diagnosis, when necessary. Random field theory47 was used to correct the resulting \(t\) parametric maps for multiple comparisons (one-sided). To compare the effect of CSF biomarkers on the PET biomarkers, the adjusted R-squared values of the models were averaged within ROIs encompassing only the voxels that were significantly associated to all CSF biomarkers simultaneously. The ROIs include precuneus, posterior cingulate, frontal orbital gyri, post central gyri and medial frontal gyri for Aβ PET, whilst for tau PET they were the average of Braak I-II, Braak III-IV and Braak V-VI staging regions. CSF biomarkers were also plotted as a function of Aβ PET, which served as a proxy of disease progression. For that, Aβ PET values were given in Centiloid units and CSF biomarkers were first adjusted by age and sex and only then transformed in Z-score values based on the average and standard deviation of the CU population. Finally, a locally weighted polynomial regression method was employed, using the lowest function in R, with 0.6 smoother span.

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

Participants

This study included 171 participants, stratified as CU (n = 109 [23% Aβ PET+] and CI (n = 62 [66% Aβ PET+]) groups (Table 1), with cross-sectional CSF (p-tau181, p-tau217, p-tau231) and PET ([^18F]AZD4694 and[^18F] MK6240). The same participants were also categorized as CU (Aβ+/-), MCI (Aβ+), AD and non-AD (supplementary table 1). The mean age of the population was 62.77 years, with CI participants being older than CU (CU = 59.82; CI = 67.78; P < 0.001) owing to a proportion of the CU being < 30 years. As expected, the CI group had a lower MMSE scores, and higher Aβ PET and tau PET load as compared to the CU group (Table 1). APOE-ε4 carriers and males were also more common in the CI group. Older age was associated with higher levels of all CSF p-tau. There was no association between sex and p-tau biomarkers when adjusting for age and diagnosis.

CSF p-tau biomarkers biomarker defined groups

CSF p-tau biomarkers were highly correlated between each other, in the whole cohort, and in diagnostic categories groups (supplementary Figure 1a-c) with the strongest association being between CSF p-tau217 and CSF p-tau231 in whole cohort (r = 0.92; P < 0.001) and in the CI group (r = 0.93; P < 0.001). Within the CU group, the strongest association was found between CSF p-tau181 and CSF p-tau231 (r = 0.86; P < 0.001 [Spearman correlation]).

When assessing groups as either CU or CI, all CSF p-tau biomarkers were significantly increased in Aβ+ groups as compared to Aβ- groups. CSF p-tau231 (Figure 1a) and CSF p-tau217 (Figure 1b) were significantly increased in CU Aβ+ as compared to CI Aβ-, but this was not observed for CSF p-tau181 (Figure 1c). CSF p-tau217 was 6-fold higher in CI Aβ+ than in CU Aβ-, which was a significantly larger increase than other p-tau biomarkers (Pp-tau181=0.002; Pp-tau231=0.01; supplementary table 2). Similarly, CSF p-tau217 demonstrated the largest fold increase between CI Aβ+ and CI Aβ- (4.7-fold; P < 0.002; supplementary table 2). However, this was not significantly different to other CSF p-tau biomarkers.

CSF p-tau biomarkers concentrations were also visualized by specific diagnostic categories (Figure 1d–f) and fold change (supplementary table 2). This analysis further demonstrated that CSF p-tau217 had a superior fold change to other p-tau biomarkers (P < 0.02) when specifically comparing MCI Aβ+ and AD dementia with CU Aβ- (MCI Aβ+, 5.9-fold; AD, 7.1-fold) and non-AD neurodegenerative disorders (MCI Aβ+, 4.8-fold; AD, 5.8-fold). CSF Aβ42/40 groups differences are demonstrated in supplementary Figure 2.

Performance of CSF p-tau in clinically defined groups

Next, we investigated the diagnostic accuracy of CSF p-tau biomarkers in differentiating clinical categories which was predetermined by underlying Aβ and tau PET status (supplementary table 3). In a ROC analysis, CSF p-tau181 was the best performer in distinguishing between CU and AD, when Aβ and tau PET status were unknown (AUC = 0.97; 95% CI, 0.95–0.99). This significantly outperformed CSF p-tau217 (P < 0.01 [DeLong’s]) but not CSF p-tau217 (P=0.13[DeLong’s]). All biomarkers had high accuracies (AUC > 0.96) in separating AD from non-AD and no biomarker was found to be statistically superior in this comparison. A noticeable decline in performance was observed for CSF p-tau181 when distinguishing MCI from non-AD (AUC = 0.83; 95% CI, 0.67–0.99) and MCI from CU (AUC = 0.81; 95% CI, 0.66–0.96) which was significantly different to p-tau231 and p-tau217 (P < 0.05

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Table 1: Demographics of the Translational Biomarkers in Aging and Dementia (TRIAD) cohort.

|                          | CU Aβ- (n = 83) | CU Aβ+ (n = 26) | CI Aβ- (n = 21) | CI Aβ+ (n = 41) |
|--------------------------|----------------|----------------|----------------|----------------|
| Females                  | 51 (61)        | 17 (65)        | 11 (52)        | 18 (43)        |
| Age (yr)                 | 55.48 (23.25)  | 72.20 (7.54)***| 67.24 (9.91)***| 67.86 (7.88)***|
| Education (yr)           | 15.56 (3.15)   | 14.42 (2.70)   | 14.94 (4.92)   | 15.42 (2.98)   |
| APOE-ε4 carriers         | 23 (27)        | 8 (30)         | 4 (19)         | 27 (67)***     |
| MMSE                     | 29.38 (0.90)   | 29.23 (0.86)   | 26.76 (2.10)** | 23.82 (6.09)** |
| Aβ PET SUVR              | 1.23 (0.10)    | 1.96 (0.42)**  | 1.25 (0.13)    | 2.38 (0.42)    |
| Tau PET SUVR             | 0.86 (0.10)    | 1.00 (0.15)    | 0.85 (0.13)    | 1.87 (0.61)    |
| p-tau231 (pg/mL)         | 8.71 (3.29)    | 22.14 (17.67)**| 9.64 (4.19)    | 34.32 (19.86)**|
| p-tau217 (pg/mL)         | 4.44 (2.88)    | 15.64 (17.63)**| 5.04 (2.30)    | 26.79 (16.87)**|
| p-tau181 (pg/mL)         | 198.83 (122.47)| 404.70 (213.81)| 219.96 (91.13)| 806.32 (508.79)**|

Data are mean (SD) or n (%); MMSE Mini-Mental State Examination. *P<0.05; **P<0.01; ***P<0.001.
No such changes were observed for other biomarkers demonstrating that CSF p-tau181 is not sensitive to early pathological changes but an accurate biomarker at late-stage disease.

**Associations between CSF p-tau biomarkers with tau PET**

Tau PET was performed in all 171 participants included in this study. High concentrations of all CSF p-tau biomarkers were associated with increased retention of \(^{18}\text{F}\)MK6240 composite Braak stage I-IV. A similar correlation coefficient was observed between CSF p-tau231 (\(r = 0.74, P < 0.001, \text{Figure 2a}\)) and CSF p-tau217 (\(r = 0.73, P < 0.001, \text{Figure 2b}\)) with Braak stage I-IV. In contrast, significantly inferior correlations (\(P_{\text{p-tau231 vs p-tau181}} = 0.004; P_{\text{p-tau217 vs p-tau181}} = 0.02\)) were observed for CSF p-tau181 (\(r = 0.66, P < 0.001, \text{Figure 2c}\)). No significant differences were found between the CSF biomarkers when predicting tau PET positivity and all demonstrated comparable AUC values (AUC = 0.86-0.93, \text{Figure 2d}). This included CSF A\(\beta\)42/40 which also had high accuracy to distinguish tau PET status (AUC 0.91; 95% CI, 86-97%).

At the voxel level, CSF biomarkers were associated with higher \(^{18}\text{F}\)MK6240 uptake in the inferior, medial and lateral temporal regions (\(T_{\text{all}} > 3.14; P_{\text{all}} < 0.05, \text{Figure 2E}\)), with associated areas overlapping between CSF biomarkers. No marked difference was observed between CSF p-tau231 and CSF p-tau217, whilst CSF p-tau181 indicated weaker associations narrowed to reduced regions if compared to the other biomarkers (\text{Figure 2e}). When groups were evaluated as CI (\text{Figure 2f}) and CU (\text{Figure 2g}) separately, broad associations were found in the CI group (\(T_{\text{CI}} > 3.25; P_{\text{CI}} < 0.05, \text{Figure 2f}\)), where high concentrations of CSF biomarkers were associated with high \(^{18}\text{F}\)MK6240 binding in the temporal lobes, cingulate regions, and orbitofrontal cortices, encompassing Braak stage regions I-VI. A stronger relationship between \(^{18}\text{F}\)MK6240 and CSF p-tau231 and CSF p-tau217 (\(T_{\text{CU}} > 3.16; P_{\text{CU}} < 0.05\)) were found in CU participants, with significant associations confined to temporal regions corresponding to Braak stage regions I-IV (\text{Figure 2g}).

**Associations between CSF p-tau biomarkers with A\(\beta\) PET**

A\(\beta\) PET was performed in all 171 participants included in this study. In a similar findings to tau PET, correlation coefficients where strongest between \(^{18}\text{F}\)AZD4694
global retention and CSF p-tau231 ($r = 0.81$, $P < 0.001$, Figure 3a) and CSF p-tau217 ($r = 0.79$, $P < 0.001$, Figure 3b). Once more, correlations were inferior for CSF p-tau181 ($r = 0.70$, $P < 0.001$, Figure 3c) which were significantly different to other p-tau biomarkers ($P_{p\text{-tau}231 \text{ vs } p\text{-tau}181} < 0.001$; $P_{p\text{-tau}217 \text{ vs } p\text{-tau}181} = 0.01$). CSF p-tau231 (AUC = 0.95; 95% CI, 0.92-0.98) and CSF p-tau217 (AUC = 0.95; 95% CI, 0.92-0.99) were significantly better predictors of $\text{A}^\beta$ PET status than CSF p-tau181 (AUC = 0.88; 95% CI, 0.81-0.94; both $P < 0.01$, Figure 3d). CSF $\text{A}^\beta_{42/40}$ was also a significantly better predictor of $\text{A}^\beta$ PET status than CSF p-tau181 (AUC= 0.95; 95% CI, 0.92-0.99; $P_{\text{A}^\beta_{42/40} \text{ vs } p\text{-tau}181} = 0.03$).

Results of the voxel-wise analysis showed high CSF biomarker levels being associated with high [F]AZD4694 binding in frontal, precuneus, posterior cingulate and temporal cortices ($T_{\text{all}} > 3.14$; $P_{\text{all}} < 0.05$, Figure 3e). Even though there was a topographical overlap of the significantly associated regions between the CSF biomarkers evaluated, a markedly stronger association was observed for CSF p-tau231 when the whole sample was analyzed (Figure 3e). In the CI group analyses, there was no difference between the associations of [F]AZD4694 with CSF p-tau231 and CSF p-tau-217 ($T_{\text{CI}} > 3.25$; $P_{\text{CI}} < 0.05$; Figure 3f). In contrast, in the CU group, there was an evident difference between CSF p-tau231 and the other biomarkers (Figure 3g), despite there not being an evident difference in the whole set of participants ($T_{\text{CU}} > 3.16$; $P_{\text{CU}} < 0.05$). Voxel-wise analysis including CSF $\text{A}^\beta_{42/40}$ as a comparator in CI and CU demonstrated in supplementary figure 4 and 5. As an additional comparison between biomarkers in the CU group, performing a ratio of p-tau231 and p-tau217 with CSF $\text{A}^\beta_{42}$ showed no statistically significance difference than p-tau alone. However, a numerical advantage was observed in CU participants (p-tau231/$\text{A}^\beta_{42}$, AUC = 0.96; 95% CI, 0.92-0.99; p-tau217/$\text{A}^\beta_{42}$, AUC=95%; 95% CI, 91-99%). A ratio of CSF $\text{A}^\beta_{42}$ with p-tau181 improved the prediction of amyloid status in CU participants ($p\text{-tau}181/\text{A}^\beta_{42}$, AUC=90%; 95% CI, 82-99%; $P_{p\text{-tau}181/\text{A}^\beta_{42} \text{ vs } p\text{-tau}181} = 0.03$).

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Figure 3. Phosphorylated tau CSF epitopes and Aβ PET associations. Spearman rank correlations between CSF biomarkers and Aβ PET ([18F]AZD4694) across all groups show p-tau231 as having the highest correlation coefficient (a-c). The accuracy of CSF biomarkers in distinguishing Aβ PET status (positive vs negative) is evidenced by AUCs as shown in (d). T-parametric maps show the voxel-wise association between CSF biomarkers and [18F]AZD4694 in all participants (e) as well as within CI (f) and CU (g). Models were adjusted by age and sex and RFT was used to account for multiple comparisons (significant t-values > 3.1). The dot plot (h) shows the average slope (beta) values by ROI for each of the CSF biomarkers. The beta value was derived from linear models that had Aβ PET as the outcome measure, the CSF biomarkers were the predictors and the covariates were sex and age. These models were performed at the voxel level and the CSF p-tau beta values of each voxel were averaged within ROIs. Were included in the ROIs only the voxels that had a significant association between Aβ PET and all of the CSF biomarkers evaluated here. T-parametric maps (i) show the voxel-wise association between CSF biomarkers (p-tau231, p-tau217 and p-tau181) and [18F]AZD4694 in Aβ-negative CU subjects. Models were adjusted for age and sex and RFT was used to account for multiple comparisons (significant t-values > 3.1). In addition, CSF p-tau biomarkers were plotted (j) as a function of Aβ PET deposition (in Centiloids) in the CU group, which was used to infer AD pathology progression. Dashed line indicates the biomarker cut off for positivity. CU: cognitively unimpaired; CI: cognitively impaired.
positivity. No difference between the inflection point of these biomarkers was identified. Contrarily, CSF biomarkers showed to become abnormal at different pathological stages, with CSF p-tau231 status being positive at approximately Centiloid 37, followed by CSF p-tau181 at Centiloid 47 and CSF p-tau217 at Centiloid 50.

**CSF p-tau231 in emerging amyloid-beta pathology**

Considering the visually stronger association between CSF p-tau231 and [18F]AZD4694 within the CU group (Figure 3g), as compared to the other CSF biomarkers, we further investigated how these associations would be within the subjects in the earliest possible process of Aβ accumulation. Therefore, we repeated the voxel-wise analysis only in individuals classified as CU Aβ- (Figure 3i; supplementary Figure 7). CSF p-tau231 was the biomarker that best associated with [18F]AZD4694 uptake (TCU > 3.19; F CU < 0.05, Figure 3j) which was significantly superior to CSF p-tau217 and CSF p-tau181 biomarkers in this analysis. These associated areas were very focal and included the medial orbitofrontal, precuneus and posterior/isthmus cingulate cortices. In order to further support the findings suggesting that CSF p-tau231 best reflects the earliest Aβ dysmetabolism, we repeated locally-weighted polynomial regression analysis, and re-estimated at which point the relationship between the CSF p-tau231, CSF p-tau217 and CSF p-tau181 changes in relation to the disease within the CU group (Figure 3j). Again, no significant difference was observed between the inflection point of these biomarkers however, abnormality of CSF p-tau231 were observed far earlier (Centiloid 38.3) than whilst CSF p-tau217 and CSF p-tau181 (Centiloids >50). Thus, this analysis suggests CSF p-tau231 as the biomarker with the fastest preclinical increases which is the first to pres-
at CU stage of the AD continuum, only CSF p-tau231 and not CSF p-tau217 was significantly superior to CSF p-tau181. Furthermore, at the voxel-wise level, CSF p-tau231 demonstrated substantially higher associations with [18F]AZD4694 retention than CSF p-tau217 in CU individuals. We further performed a voxel-wise analysis in CU participants without prominent Aβ pathology (Aβ-) and demonstrated focal associations of CSF p-tau231 with emerging Aβ pathology in the medial orbitofrontal, precuneus and posterior/isthmus cingulate cortices which were stronger than what was observed for p-tau217 and absent for p-tau181. Previously, Palmqvist et al. showed that Aβ fibrils begin to accumulate early in core regions of default mode network (DMN), namely precuneus, posterior cingulate cortex, and orbitofrontal cortex. This finding was even reported in individuals with seemingly normal levels of Aβ and orbitofrontal cortex. This finding was even reported in individuals with seemingly normal levels of Aβ and orbitofrontal cortex.

As Aβ accumulates toward Aβ positivity, during neurodegeneration and cognitive abnormalities phases, CSF p-tau epitopes have largely reached a plateau.

Limitations

We are aware of limitations to this study. Firstly, to definitively describe an ordinal sequence of CSF p-tau biomarkers, from pre-amyloid to symptomatic phases of the disease, longitudinal studies are required. Further to this, our main finding of early of pre-amyloid changes of p-tau231 is based on 83 CU Aβ- individuals and should be replicated in an independent cohort which is not enriched for AD with exclusion criteria’s. Using the ratio of Aβ42 to p-tau did not largely change our results but produced higher AUC’s, which should be further investigated in larger cohort sizes or utilized if the optimal p-tau is not available, as highlighted in this study. Furthermore, the use of cross-sectional Aβ PET Centiloids as a proxy of time in the disease was employed and while a gradual Aβ accumulation is observed though AD development, it is not certain that a greater Aβ PET SUVR is indicative of more advanced disease state. CSF p-tau biomarkers in this study are compared on two different platforms, with differing detection and capture antibody configurations: N-terminally derived CSF p-tau181 and CSF p-tau217 on the Simoa platform, Mid-terminal CSF p-tau231 by ELISA. Thus, it cannot be ruled out that subtle changes in biomarkers performances are determined by platform differences or antibody superiority. Yet, we believe this is unlikely given that our most promising finding is derived from a traditionally less sensitive technique. In addition, the ADx253 antibody utilized in the p-tau231 ELISA was determined to simulate cis-selectivity however it cannot be determined the specific abundance of the toxic cis p-tau231 species in this assay format.
In conclusion, this study documents the pronounced preclinical increases of CSF p-tau biomarkers but specifically highlights CSF p-tau231 which begins to associate with Aβ deposition before the threshold of Aβ PET positivity has occurred and strongly associates with known early Aβ accumulating regions in the DMN. This finding further supports a model of active soluble tau release by cells into the CSF which is related to early Aβ deposition, likely induced by early Aβ seeds. Thus, CSF p-tau231 is a novel candidate for detecting early and emerging Aβ pathology in individuals for the recruitment into therapeutic trials, act as a target engagement biomarker to monitor the effect of Aβ therapeutics or supports the notion of therapeutically targeting tau epitopes in preclinical disease before tau aggregation can be visualized by tau PET.

Declaration of interests
EVM is the Chief scientific officer in ADx Neurosciences. ES and CF are employees of ADx NeuroSciences. SG has served as a consultant for TauRx, Biogen Canada Member and Roche Canada. HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Sumaned, Siemens Healthineers, Pinten Therapeutics and CogRx, and has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen. Alzheimer’s Association Global Biomarker Standardization Consortium chair and cofounder of Brain Biomarker Solutions in gothenburg AB (BBS) which is a part of the GU ventures Incubator program. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. CF, and ES and are employee of ADx NeuroSciences, Gent, Belgium, EVM is a co-founder of ADx NeuroSciences. The other authors declare no competing interest.

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

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103836.

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