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Performance of plasma p-tau217 for the detection of amyloid-β positivity in a memory clinic cohort using an electrochemiluminescence immunoassay

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Abstract

Background Plasma p-tau217 has emerged as the most promising blood-based marker (BBM) for the detection of Alzheimer Disease (AD) pathology, yet few studies have evaluated plasma p-tau217 performance in memory clinic settings. We examined the performance of plasma p-tau217 for the detection of AD using a high-sensitivity immunoassay in individuals undergoing diagnostic lumbar puncture (LP).

Methods Paired plasma and cerebrospinal fluid (CSF) samples were analysed from the TIMC-BRAiN cohort. Amyloid (Aβ) and Tau (T) pathology were classified based on established cut-offs for CSF $Aβ_{42}$ and CSF p-tau181 respectively. High-sensitivity electrochemiluminescence (ECL) immunoassays were performed on paired plasma/CSF samples for p-tau217, p-tau181, Glial Fibrillary Acidic Protein (GFAP), Neurofilament Light (NfL) and total tau (t-tau). Biomarker performance was evaluated using Receiver-Operating Curve (ROC) and Area-Under-the-Curve (AUC) analysis.

Results Of 108 participants (age: 69 ± 6.5 years; 54.6% female) with paired samples obtained at time of LP, 64.8% ($n = 70/108$) had Aβ pathology detected (35 with Mild Cognitive Impairment and 35 with mild dementia). Plasma p-tau217 was over three-fold higher in Aβ + (12.4 pg/mL; 7.3—19.2 pg/mL) vs. Aβ- participants (3.7 pg/mL; 2.8—4.1 pg/mL; Mann–Whitney U = 230, $p < 0.001$). Plasma p-tau217 exhibited excellent performance for the detection of Aβ pathology (AUC: 0.91; 95% Confidence Interval [95% CI]: 0.86–0.97)—greater than for T pathology (AUC: 0.83; 95% CI: 0.75–0.90; z = 1.75, p = 0.04). Plasma p-tau217 outperformed plasma p-tau181 for the detection of Aβ pathology (z = 3.24, $p < 0.001$). Of the other BBMs, only plasma GFAP significantly differed by Aβ status which significantly correlated with plasma p-tau217 in Aβ + (but not in Aβ-) individuals. Application of a two-point threshold at 95% and 97.5% sensitivities & specificities may have enabled avoidance of LP in 58–68% of cases.

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Conclusions Plasma p-tau217 measured using a high-sensitivity ECL immunoassay demonstrated excellent performance for detection of Aβ pathology in a real-world memory clinic cohort. Moving forward, clinical use of plasma p-tau217 to detect AD pathology may substantially reduce need for confirmatory diagnostic testing for AD pathology with diagnostic LP in specialist memory services.

Keywords Alzheimer disease, Cerebrospinal fluid, Blood-based markers, Amyloid, P-tau217, Cerebrospinal fluid, Diagnosis

Background

The increasing availability of Alzheimer Disease (AD) biomarkers has led to a paradigm shift towards the conceptualisation of AD as a clinical-biological diagnosis rather than one based on clinical phenotype alone [1, 2]. Detection of pathological hallmarks of AD—accumulation of amyloid β (Aβ) in plaques and hyper-phosphorylated tau in neurofibrillary tangles—in vivo using Aβ and p-tau in cerebrospinal fluid (CSF) [3] or using amyloid/tau-Positron Emission Tomography (PET) [4]—has enabled more accurate diagnosis for those living with AD.

More recently, there has been unprecedented progress in the development and validation of blood-based markers (BBMs) in AD [5]. BBMs are likely to have a transformative role in facilitating early and precise diagnosis as well as informing prognostication for those living with AD as they are cheaper, less invasive and more accessible than traditional diagnostic tools (CSF sampling/PET imaging). BBMs are also likely to have a crucial role in identifying individuals suitable for access to clinical trials and emerging disease-modifying therapies (DMTs) such as anti-amyloid immunotherapies [6–15]. Recent appropriate use guidelines for BBMs encourage their cautious use in specialised memory clinics—with confirmation of results using CSF/PET where possible [6].

Whilst Aβ42/Aβ40 is an established biomarker in CSF, its performance as a BBM is limited by peripheral Aβ production which reduces diagnostic sensitivity. Additionally, plasma Aβ42/Aβ40 also demonstrates low fold-change, putting high demand on analytic precision and stability over time [16]. Meanwhile, p-tau species have emerged as promising BBMs for AD detection, with several p-tau epitopes (p-tau181, p-tau217 and p-tau231)—changing at different stages of the AD continuum [17–20]. Currently, p-tau217 is the most promising BBM for detection of AD pathology [21–25] and may differentiate AD from other dementias [26–30]. Plasma p-tau217 can detect cortical Aβ accumulation and may mediate the association between Aβ plaques and subsequent tau tangle pathology [31–34]. Plasma p-tau217 may also have prognostic utility—levels are associated with future AD progression as measured by clinical decline and hippocampal/cortical atrophy [18, 20, 22, 29, 35].

There is growing evidence that plasma p-tau217 is an amyloid response measure – with increases in concentration beginning soon after CSF Aβ positivity occurs but before the cut-point for Aβ PET positivity has been reached. This makes plasma p-tau217 a promising early diagnostic measure of Aβ pathology in those presenting to specialist memory services [5, 36–38]. Importantly, p-tau217 has the potential to democratise AD diagnosis as it may offer a cost-effective and scalable diagnostic measure which could substantially reduce need for further invasive/expensive testing with CSF/PET – particularly in the context of specialist memory clinics [39, 40].

The performance of p-tau217 in detecting Aβ pathology may vary by platform used. Mass Spectrometry-based methods usually considered the gold standard are limited by high cost, availability and throughput. Recently, immunoassays for BBMs have been developed with excellent performance for the detection of Aβ pathology [41, 42]. In a head-to-head comparison of multiple p-tau assays in individuals with Mild Cognitive Impairment (MCI), several plasma p-tau217 immunoassays showed high and consistent accuracy for detection of Aβ [43, 44]. More broadly, immunoassays for p-tau217 have demonstrated excellent performance in detecting CSF Aβ status in several cohort studies [45, 46].

The accuracy of plasma p-tau217 is clearly established in cohort and population studies, but fewer studies have evaluated p-tau217 immunoassays in real-world memory clinic settings – one of the important contexts for BBM use in AD diagnosis [6]. There are several studies which clearly support the accuracy of p-tau217 immunoassays to detect Aβ pathology in clinical practice [44, 45, 47, 48]. However, further replication studies are needed to assess real-world clinical performance in different clinical contexts and across different platforms depending on access/availability and cost. Whilst many of these employ the digital ELISA Single molecule array (Simoa) platform, here we assessed the diagnostic performance of a commercially available (research-use only) high-sensitivity plasma p-tau217 electrochemiluminescence (ECL) immunoassay. This approach allows femtogram/mL measurement of analytes typically not detected by conventional ECL immunoassays [47, 49–51]. We assessed the use

of plasma p-tau217 measured using this platform for the detection of Aβ positivity in individuals presenting with early cognitive symptoms to a specialist memory service and undergoing diagnostic workup including LP for detection of AD pathology.

Methods

Study setting and participants

The current study used biological samples and participant data from the Tallaght University Hospital Institute of Memory and Cognition – Biobank for Research in Ageing and Neurodegeneration (TIMC-BRAiN), the protocol for which has been previously published [52]. The Regional Specialist Memory Centre (RSMC) at Tallaght University Hospital (TUH) in Dublin, Ireland assesses 400–500 patients annually experiencing cognitive symptoms. In the RSMC, patients are assessed in the first instance by an Advanced Nurse Practitioner (ANP) in memory and in addition to comprehensive clinical history and examination typically undergo cognitive testing including administration of the Addenbrooke's Cognitive Assessment III (ACE-III) and Frontal Assessment Battery (FAB), neuroimaging, routine investigations and where appropriate, diagnostic CSF sampling for AD biomarkers. All cases are discussed at an interdisciplinary case conference meeting led by consultants in geriatric medicine and neurology to inform diagnosis, further investigations and management as appropriate. CSF sampling is typically performed in individuals presenting with cognitive symptoms at MCI/mild dementia stage to detect or rule-out AD pathology as either a primary or co-pathology, depending on clinical phenotype and in accordance with recent guidelines [53].

Alongside routine assessment and workup, patients attending the RSMC are offered the opportunity to donate biological samples and clinical data to the TIMC-BRAiN Biobank. For the purposes of TIMC-BRAiN, each participant's diagnostic results are discussed at a dedicated monthly biobank diagnostic meeting where a final biobank diagnosis adjudicated by a consultant-led MDT. For consensus diagnosis, each participants diagnosis is divided into both functional status (Subjective Memory Complaints [SMCs]/MCI/Dementia) and a primary aetiological diagnosis (AD, Lewy Body Disease [LBD], Frontotemporal Dementia [FTD] etc.) as previously outlined [52]. For the current study, we included all TIMC-BRAiN participants who underwent diagnostic LP from January-December 2023 inclusive. Detailed demographic and clinical information was collected alongside cognitive assessment and final biobank diagnosis as previously reported [52].

Biological sampling and processing

Paired blood and CSF samples were obtained at time of diagnostic LP and stored for future analysis. Once diagnostic samples were collected by drip method, an additional 5 mL of CSF was collected in 2.5 mL sterile polypropylene tubes (Sarstedt Ltd; Cat No: 63.614.625) for processing and storage in the TIMC-BRAiN biobank. Biobank CSF samples were processed in a sterile manner on-site with centrifugation (*440 g×10 min*) and storage of cell-free CSF in 0.5 mL aliquots. Paired plasma samples at time of LP were obtained by aseptic venepuncture and collected in 9 mL K_2EDTA tubes (Greiner Bio One Ltd; Cat No: 455045), which were centrifuged (*1.8 g×10 min*) with plasma aliquoted into 0.5 mL sterile cryovials. Once processed, CSF/plasma are stored at -80 °C for future analysis. All paired CSF and plasma samples are processed by trained staff on-site within 30 min to minimise impact of sample handling on pre-analytical variability.

Diagnostic CSF analysis and definition of AD pathological biomarkers

All participants undergoing diagnostic LP have CSF analysed for $A\beta_{1\text{-}42}$, p-tau181 and t-tau as part of routine clinical care. Clinical samples were analysed either on the Roche Elecsys ® Immunoassay using a Cobas E801 analyser or using the Fujirebio Innotest ® as part of routine clinical practice. For the current study, as we were evaluating the performance of p-tau217 to detect Aβ and tau pathology, Aβ levels ≤ 1030 pg/mL (Elecsys®)/ ≤ 712.0 pg/mL (Innotest ®) on CSF diagnostic testing were considered indicative of Aβ pathology (A+) whilst p-tau 181 levels of ≥ 27 pg/mL (Elecsys®)/ ≥ 58.6 pg/mL (Innotest ®) were considered as indicative of tau pathology (T+). Cut-offs for the Elecsys® platform were based on local application of validated cut-off values from Roche Diagnostics [54, 55]. Innotest® cut-off values were derived based on consensus and external validation as part of the Irish Network for Biomarkers in Neurodegeneration which has been described elsewhere [56].

Electrochemiluminescence immunoassays

We used a high-sensitivity assay from MesoScaleDiscovery (MSD) (S-PLEX; Cat No: K51APFS) to assess levels of p-tau217 in both plasma and CSF samples. Briefly, the commercially available research use only S-PLEX assay uses ECL technology. Plates are coated with Streptavidin and a biotinylated antibody. Analytes of interest (in this case p-tau phosphorylated at threonine 217) are captured in standard sandwich format and a "TURBO BOOST®" mouse monoclonal antibody used for detection, which is enhanced with "TURBO-TAG®" reagents. Calibrator

controls supplied with the kit consists of p-tau217 (full-length recombinant phosphorylated tau – isoform tau441 – protein expressed in a human cell line).

Samples (paired plasma and CSF) were thawed on ice and measured across five individual plates according to the manufacturer's instructions. A plasma pool of 12 donors from TIMC-BRAiN selected for maximal spread of age, BMI, and symptom severity were created and analysed across all experiments to assess inter-assay Coefficients of Variation (CVs). Inter-assay CVs were calculated as the CV of the plasma pool across all five plates whilst intra-assay CVs were calculated from the CV of sample duplicates. Samples were randomised across the plates and the assessor blinded to the clinical status of each donor. Once completed, each plate was read on a MSD QuickPlex SQ 120 Analyser and Discovery Workbench 4.0 Software used to analyse results.

In parallel to analysing samples for p-tau217, separate plasma and CSF aliquots from each participant were analysed in single-plex for p-tau181 and in multi-plex for total tau (t-tau), Neurofilament Light (NfL) and Glial Fibrillary Acidic Protein (GFAP) using ultra-sensitive S-PLEX p-tau181 and S-PLEX neurology kits from MSD (K-156AGMS/ K-15639S) respectively. Experimental layout and analyses were identical as for p-tau217 and conducted according to manufacturer's instructions.

Statistics

Descriptive statistics consisted of means with standard deviation and medians with Interquartile Range (IQR) as appropriate. Between-group differences between disease stage (MCI or Dementia) or Aβ/tau positivity were carried out using t-tests and Mann–Whitney U tests. In order to assess the performance of p-tau217 in detecting Aβ or tau pathology as defined above (positive Aβ/ elevated ptau181 on CSF respectively – using established clinical cut-offs), Receiver Operating Characteristic (ROC) analysis was used and Area Under the Curve (AUC) with 95% Confidence Interval (95% CI) calculated. To examine for optimal cut-off a Youden index was computed and the cut-off with the maximal value taken for further analysis. In the first instance, we examined the performance of p-tau217 given clear existing evidence for its association with early Aβ pathology. In order to evaluate the performance of different cut-points, we employed a two-threshold approach for sensitivities and specificities of 90%, 95% and 97.5%, following previously published approaches aimed at integrating BBMs into clinical workflows [40]. In line with this approach, those scoring below the sensitivity threshold were deemed to have low likelihood of Aβ positivity whilst those with scores above specificity cut-offs were deemed to have high likelihood of Aβ positivity. Those in between the two thresholds were deemed to have intermediate likelihood of Aβ positivity. In order to evaluate how many LPs may have been avoided at each sensitivity/specificity level, we considered that those in the low and high likelihood categories would not undergo confirmatory LP and those in the intermediate category would require confirmatory LP.

We subsequently assessed the diagnostic performance of p-tau181 using the same method and compared AUCs of p-tau217 and p-tau181 using the DeLong test. For further analysis of GFAP, NfL and t-tau, we first assessed between-group differences in Aβ + ve vs Aβ -ve individuals. We subsequently performed correlational analysis (Spearman's R) to assess the relationship between these markers and plasma p-tau217, stratified by Aβ status. This was performed for all plasma and CSF biomarkers assessed. Across all analyses, an alpha level of $p < 0.05$ was considered statistically significant. Analysis was conducted in STATA v17.0 (StataCorp, Texas, USA) and GraphPad Prism v10.0 (Graphpad Software Inc, Boston, Massachusetts, USA).

Results

Participant characteristics & consensus diagnoses

Overall, 108 participants (age: 69 ± 6.5 years; 54.6% female) donated paired plasma and CSF samples at time of diagnostic LP. Of these, 64.8% ($n = 70/108$) had Aβ pathology detected on CSF – $n = 35$ with MCI and $n = 35$ with mild dementia. For those with MCI and positive Aβ on CSF, AD was determined to be the primary pathology in 34/35 cases whilst one individual with MCI and positive Aβ was awaiting further work-up. In those with dementia and positive Aβ, AD was judged to be the sole pathology causing dementia in 30 cases. Three individuals with positive Aβ were also met diagnostic criteria for Dementia with Lewy Bodies (DLB) and were determined to have AD-DLB dual pathology. Two individuals with dementia and positive Aβ (but negative CSF p-tau181) were judged by consensus to have probable behavioural variant FTD (bv-FTD)—one of whom was a pathogenic mutation carrier—with Aβ incidental/co-pathology.

The remaining participants (38/108; 35.2%) had negative Aβ on CSF testing. For those with MCI and negative Aβ ($n = 33$), 3 had consensus diagnosis of Lewy Body-MCI whilst 28 had non-AD MCI following diagnostic work-up. Two individuals with MCI were awaiting further investigations to determine aetiology. Of those with dementia and negative Aβ ($n = 5$), 2 were judged to have pure DLB without AD co-pathology and 3 were awaiting further investigations to determine aetiology. Demographic and clinical characteristics, in addition to immunoassay biomarker results presented by Aβ status, are provided below in Table 1.

Table 1 Baseline characteristics of included participants from the TIMC-BRAiN cohort

Characteristic	Aβ + ve (*n* = 70)	Aβ -ve (*n* = 38)
Age, Years (mean; SD)	69.6 ± 6.4	67.9 ± 6.6
Sex, Female (n; %)	40 (57.1%)	19 (50%)
Estimated Glomerular Filtration Rate (ml/min/1.73m^2)	74 (58–87)	73.5 (64.5–83.5)
Duration of Symptoms, Months (median; IQR)	24 (12–36)	22 (12–14)
Addenbrooke's Cognitive Assessment III (median; IQR)	68 (59–78)	78 (70–84)
Frontal Assessment Battery (median; IQR)	13 (11–16)	15 (12–17)
Clinical Stage (Consensus Diagnosis)		
Mild Cognitive Impairment (n; %)	35 (50%)	33 (87.8%)
Dementia (n; %)	35 (50%)	5 (13.2%)
Primary Pathology (Consensus Diagnosis)		
Alzheimer Disease (n; %)	67 (95.7%)	0
Lewy Body Disease/Dementia with Lewy Bodies (n; %)	0	5 (13.2%)
Frontotemporal Dementia (n; %)	2 (2.9%)	0
Other—Pending Further Investigations (n; %)	1 (1.4%)	5 (13.2%)
Other—Aetiology Undetermined (n; %)	0	28 (73.7%)
Plasma Biomarker Results (S-PLEX)		
p-tau217, pg/mL (median; IQR)	12.4 (7.3–19.2)	3.6 (2.8–4.1)
p-tau181, pg/mL (median; IQR)	2.1 (1.6–3.4)	1.4 (1.2–1.9)
t-tau, pg/mL (median; IQR)	22.8 (17.0–29.7)	19.1 (16.4–26.3)
NfL, pg/mL (median; IQR)	132.0 (75.4–188.5)	106.3 (69.9–188.5)
GFAP, pg/mL(median; IQR)	60.6 (46.8–88.5)	44.7 (29.1–69.9)
Cerebrospinal Fluid (CSF) Biomarker Results (S-PLEX)		
p-tau217, pg/mL (median; IQR)	659.2 (270.8–1094.3)	145.1 (94.8–250.8)
p-tau181, pg/mL (median; IQR)	43.3 (24.0–62.6)	11.8 (8.7–26.1)
t-tau, pg/mL (median; IQR)	145.5 (80.6–239.3)	102.6 (73.8–214.0)
NfL, ng/mL (median; IQR)	2.9 (1.7–3.8)	3.3 (1.7–4.2)
GFAP, ng/mL (median; IQR)	2.9 (2.0–5.4)	2.6 (2.0–4.7)

108 Individuals donated paired plasma and CSF at time of diagnosis. Values are provided as means with Standard Deviations (SD) or medians with Interquartile Ranges (IQRs) as indicated. Proportions are given as the total number of individuals from those with either Mild Cognitive Impairment (MCI) or dementia. Biomarker results are provided in pg/mL or ng/mL as appropriate

NfL Neurofilament Light, *GFAP* Glial Fibrillary Acidic Protein

Plasma p-tau217 accurately detects Alzheimer disease pathology in a memory clinic setting

Plasma P-tau217 was measured in all 108 study participants (Table 1). The lower limit of quantification (LLOQ) for the ECL assay was 0.85 pg/mL with detectable results for all samples tested. Median intra-assay CV for duplicates was 4.15% and inter-assay CV was 9.32%. No participant had a p-tau217 result above the upper limit of quantification (3,761 pg/mL).

Median plasma p-tau217 concentrations were over three-fold higher in Aβ + (12.4 pg/mL; 7.3—19.2 pg/mL) than Aβ- participants (3.6 pg/mL; 2.8—4.1 pg/mL; Mann–Whitney U = 230, $p < 0.001$) (Fig. 1). For the detection of Aβ pathology alone, p-tau217 demonstrated excellent performance with an AUC of 0.91 (95% CI: 0.86–0.97) (Fig. 1). By comparison, for detection of tau (T) pathology, p-tau217 concentrations were higher in T + (15.9 pg/mL; IQR: 10.1—20.9 pg/mL) than T- participants (7.5 pg/mL; IQR: 3.1 – 10.0 pg/mL) (Mann Whitney U = 460, $p < 0.001$) and exhibited an AUC of 0.83 (95% CI: 0.75—0.90). The performance of plasma p-tau217 was significantly better for detection of Aβ pathology compared to T pathology (DeLong test, z = 1.75, $p = 0.04$). Of note, there were no significant differences between Aβ—T + and Aβ—T- participants or between Aβ + T- and Aβ + T + participants (Fig. 1). On comparing those with MCI and dementia with Aβ pathology detected, individuals with dementia due to AD had significantly higher levels of p-tau217 in comparison to those with MCI due to AD (Fig. 1).

In line with findings for plasma p-tau217, CSF p-tau217 significantly differed in Aβ + ve vs Aβ-ve individuals (659.2 pg/mL; IQR: 270.8 – 1,094.3 in Aβ + vs. 145.1 pg/mL; 94.8–250.8 in Aβ-; U = 376.5, $p < 0.001$). Plasma p-tau217 exhibited significant positive correlations with CSF p-tau217 in Aβ positive (Spearman's $r = 0.58$,

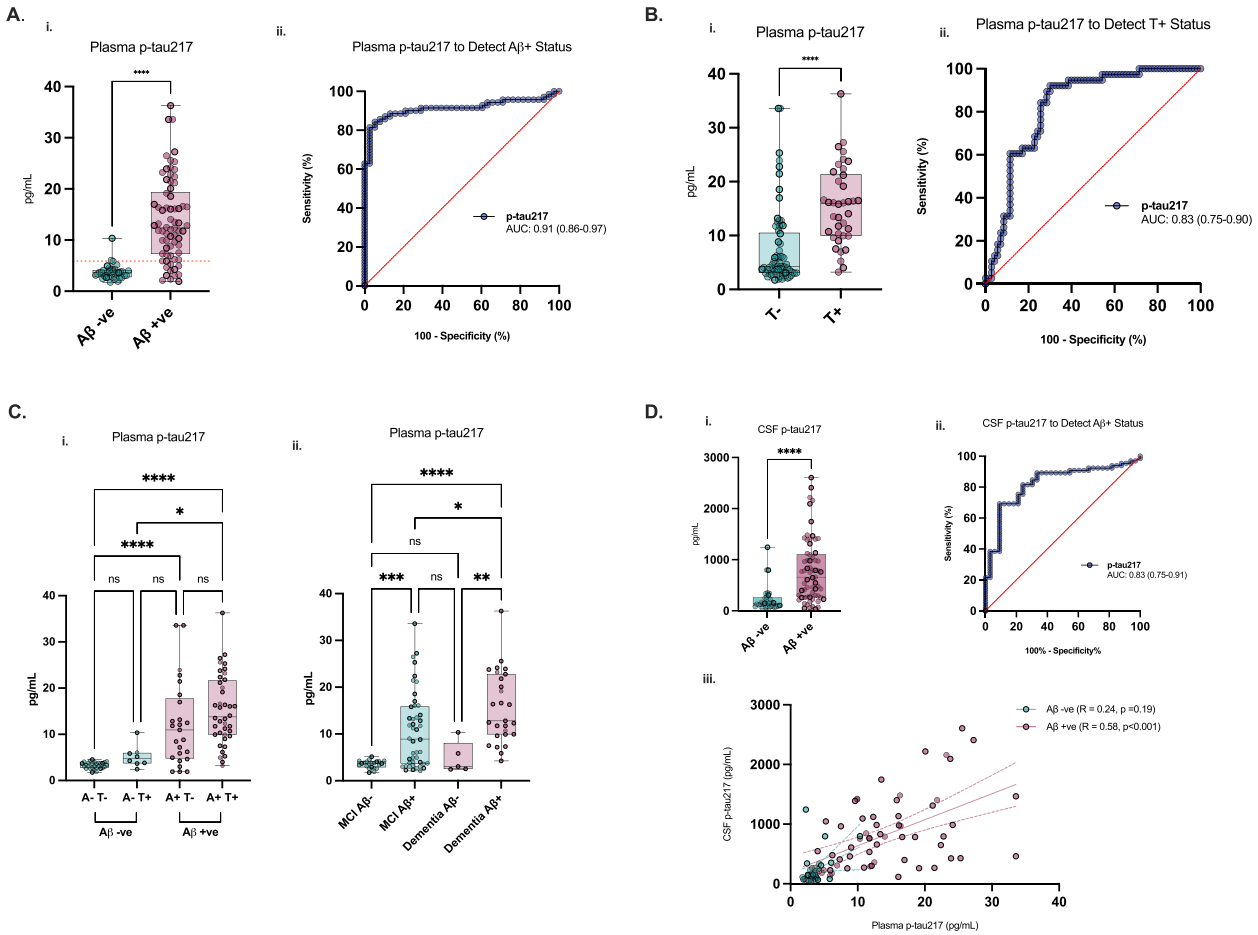


Fig. 1 P-tau217 Exhibits Excellent Performance for the Detection of Aβ Pathology in Individuals with MCI/Dementia. Plasma p-tau217 was measured in 108 individuals undergoing diagnostic lumbar puncture for the detection of Alzheimer Disease pathology. Paired plasma samples were analysed for p-tau217. **A** (i) P-tau217 was nearly four-fold higher in Aβ + vs Aβ- individuals (Mann–Whitney U = 230; $p < 0.001$). The red dotted line indicates the Youden optimised cut-off. (A) (ii) p-tau217 exhibited excellent performance in the detection of Aβ + status (Area-Under the Curve [AUC]: 0.91; 0.86–0.97). **B** (i) P-tau217 was significantly elevated in T + vs T- individuals. (ii) Performance of p-tau217 for detection of T + pathology alone gave an AUC of 0.83 (0.75–0.90) which was significantly lower than that for Aβ positivity (DeLong test, $p = 0.04$). **C** (i) Significant differences were not seen in concentrations of p-tau217 between A- T- and A- T + individuals or between A + T- and A + T + individuals supporting the role of p-tau217 as a marker of amyloid positivity. (ii)For Aβ + individuals, concentrations were significantly higher ($p = 0.03$) in individuals with dementia vs MCI due to AD. **D** (i) CSF p-tau217 was significantly higher in individuals with Aβ positivity. (ii) CSF p-tau217 had lower performance than plasma p-tau217 with an AUC 0.83 (0.75–0.91) with a trend for statistical significance ($p = 0.05$). (iii) Significant correlations were observed between CSF and plasma p-tau in individuals with Aβ positivity. *****p < 0.0001, ***p < 0.001, ** < 0.01, *p < 0.05, ns: non-significant; AUC: Area-Under-the-Curve*

$p < 0.001$) but not Aβ negative individuals. Overall, CSF p-tau217 had an AUC of 0.83 (95% CI: 0.75, 0.91) for the detection of Aβ positivity. Plasma p-tau217 outperformed CSF p-tau217 for the detection of Aβ positivity, with a trend for statistical significance observed (z = -1.6, $p = 0.05$, DeLong Test) (Fig. 1).

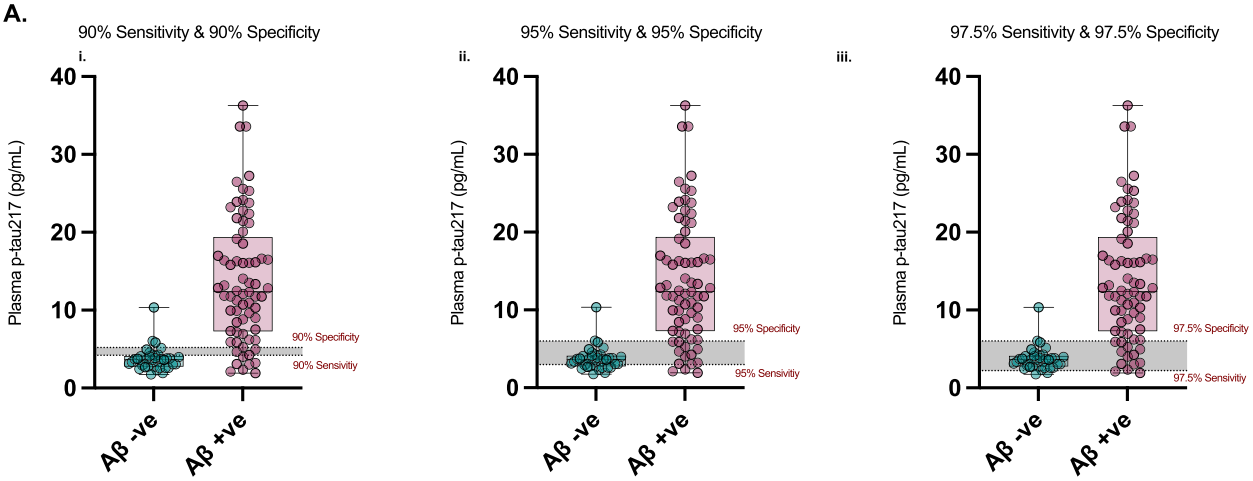
At the point with maximal Youden Index using a single-threshold approach applied to plasma p-tau217 for the detection of Aβ positivity—at a cut-off of 5.87 pg/mL—plasma p-tau217 had a sensitivity of 84.3% and a specificity of 94.7%. At these cut-off values in the current cohort, there were 2/38 (5.3%) "false positives" (CSF Aβ- participants with plasma p-tau217 values > 5.9 pg/mL) and 11/70 (15.7%) "false negatives" (CSF Aβ + individuals with plasma p-tau217 values < 5.9 pg/mL). The false positives included: (i) a participant with amnestic MCI and a p-tau-217 value just above the cut-off (6.1 pg/mL) and [14] a participant with strongly positive p-tau-217 result (10.4 pg/mL) with normal CSF $A\beta_{42}$ levels and clinical diagnosis solely consistent with DLB. Both of these had unimpaired renal function. The 11 false negatives (Aβ positivity on CSF but p-tau217 levels below cut-off) included: (i) 2 individuals with a consensus diagnosis of FTD, one of whom was a pathogenic mutation carrier,

and Aβ co-pathology/incidental pathology, (ii) 2 individuals with mild dementia due to AD (one individual Aβ + T + on CSF and one Aβ + T- on CSF) and (iii) 7 individuals with amnestic MCI attributable to AD pathology based on clinical phenotype and CSF results (2 of whom were Aβ + T + on CSF and 5 of whom were Aβ + T- on CSF). At this single point cut-off, plasma p-tau217 had a Positive Predictive Accuracy (PPA) of 97.18%, a Negative Predictive Accuracy (NPA) of 71.05% and an Overall Percent Agreement (OPA) of 87.96%.

To explore alternative thresholds of sensitivity and specificity on assay performance, we applied a previously published two-threshold approach [40]. Three distinct thresholds were considered: (i) 90% sensitivity & 90% specificity, [14] 95% sensitivity & 95% specificity and (iii) 97.5% specificity. Individuals below the sensitivity cut-off were deemed to have low likelihood of Aβ positivity and those above the specificity cut-off deemed to have high likelihood of Aβ positivity. Those falling between the two cut-offs were felt to have intermediate likelihood of Aβ positivity. Results are provided in Fig. 2 below in both graphical and tabular format. At 90% sensitivity and 90% specificity, the PPA, NPA and OPA (for p-tau217 positive and negative) were 95.24%, 81.08% and 90% respectively whilst at the 95% sensitivity and 95% specificity level, these values were 96.61%, 78.57% and 93.15% respectively. Finally for the 97.5% sensitivity and 97.5% specificity level, PPA was 96.61%, the NPA was 50% and OPA (for p-tau217 positive and negative) 93.5%. See Fig. 2.

If individuals deemed low or high likelihood based on these thresholds had not proceeded to LP, the potential number of LPs avoided in the current cohort would have been 93% (100/108) at the 90% sensitivity and 90% specificity threshold, 68% (73/108) at the 95% sensitivity and

A.



B.

Group	Total N	CSF Aβ -ve N (%)	CSF Aβ +ve N (%)
90% Sensitivity & 90% Specificity			
Low Risk (<4.2 pg/mL)	37	30 (81%)	7 (19%)
Intermediate (4.2 pg/mL-5.19 pg/mL)	8	5 (63%)	3 (17%)
High Risk (>5.19pg/mL)	63	3 (5%)	60 (95%)
95% Sensitivity & 95% Specificity			
Low Risk (<2.97 pg/mL)	14	11 (79%)	3 (21%)
Intermediate (2.97 pg/mL-5.99 pg/mL)	35	25 (71%)	10 (29%)
High Risk (>5.99pg/mL)	59	2 (3%)	57 (97%)
97.5% Sensitivity & 97.5% Specificity			
Low Risk (<2.21 pg/mL)	4	2 (50%)	2 (50%)
Intermediate (2.21 pg/mL-6.01 pg/mL)	45	34 (76%)	11 (24%)
High Risk (>6.01pg/mL)	59	2 (3%)	57 (97%)

C.

Performance Characteristic	%
90% Sensitivity & 90% Specificity	
PPA, upper reference point, %	95.24%
NPA, lower reference point, %	81.08%
OPA for p-tau217 positive and negative, %	90%
95% Sensitivity & 95% Specificity	
PPA, upper reference point, %	96.61%
NPA, lower reference point, %	78.57%
OPA for p-tau217 positive and negative, %	93.15%
97.5% Sensitivity & 97.5% Specificity	
PPA, upper reference point, %	96.61%
NPA, lower reference point, %	50.00%
OPA for p-tau217 positive and negative, %	93.65%

Fig. 2 Exploration of Two-Point Thresholds for Plasma p-tau217. **A** In order to examine different thresholds of sensitivity and specificity, we considered performance of plasma p-tau217 at three thresholds: (i) 90% sensitivity and 90% specificity; (ii) 95% sensitivity and 95% specificity; (iii) 97.5% sensitivity. Those above these specificity and below these sensitivity cut-offs were judged to have high risk and low risk of CSF-determined Aβ positivity respectively. Shaded areas indicate those in the intermediate category, with scores above the specified sensitivity cut-off but below the specificity cut off. **B** Tabular results obtained by applying these cut-offs indicating low, intermediate and high risk of CSF-determined Aβ, presented by CSF-defined Aβ status. **C** Positive Predictive Accuracy (PPA), Negative Predictive Accuracy (NPA) and Overall Percent Agreement for p-tau217 positive and negative participants are provided at each threshold

95% specificity threshold and 58% (63/108) at the 97.5% sensitivity and 97.5% specificity threshold.

Plasma p-tau217 outperforms p-tau181 for the detection of Aβ pathology

Plasma p-tau181 was measured in an identical fashion using a high-sensitivity ECL assay with an intra-assay CV of 7.9% and an inter-assay CV of 16%. Overall, median p-tau181 concentration was 1.7 times higher in Aβ + (2.3 pg/mL; IQR 1.7–4.2 pg/mL) vs Aβ- participants (1.4 pg/mL; IQR 1.2 – 2.0 pg/mL) (Mann–Whitney U = 661, $p < 0.001$). The AUC for p-tau181 for the detection of Aβ pathology was 0.72 (95% CI: 0.62—0.83) which was significantly lower than the performance of p-tau217 for detection of Aβ pathology (DeLong test, z = 3.24, $p < 0.001$) (See Fig. 3). P-tau181 concentrations did not significantly differ between A- T- vs A- T + individuals or between A + T- vs A + T + individuals. Similarly, within those Aβ- or Aβ + ve, concentrations of p-tau181 did not differ by MCI or dementia status. Significant correlations were observed between plasma p-tau217 and plasma ptau-181 in Aβ positive individuals (Spearman's R = 0.60, $p < 0.001$) but not in in Aβ negative individuals (R = 0.16, $p = 0.34$). Similarly, CSF p-tau181 was significantly correlated with plasma p-tau217 in Aβ positive (Spearman's R = 0.63, $p < 0.001$) but not in in Aβ negative (R = 0.10, $p = 0.60$) participants (Fig. 3).

Correlation between plasma p-tau217 and other markers in plasma and cerebrospinal fluid

In addition to measurement of p-tau217 and p-tau 181, GFAP, NfL and t-tau were measured in plasma and CSF in multiplex using the same high-sensitivity ECL platform. Intra- and inter-assay CVs were as follows for each assay—9.8%/14.5% for GFAP, 11.4%/16.3% for NfL and 3.9%/14.8% for t-tau.

On assessing GFAP, NfL and t-tau levels in CSF and plasma, only plasma GFAP differed significantly between Aβ positive and Aβ negative individuals (60.6 pg/mL; IQR: 46.8—88.5 in Aβ + vs 44.7 pg/mL; IQR: 29.1–69.9;

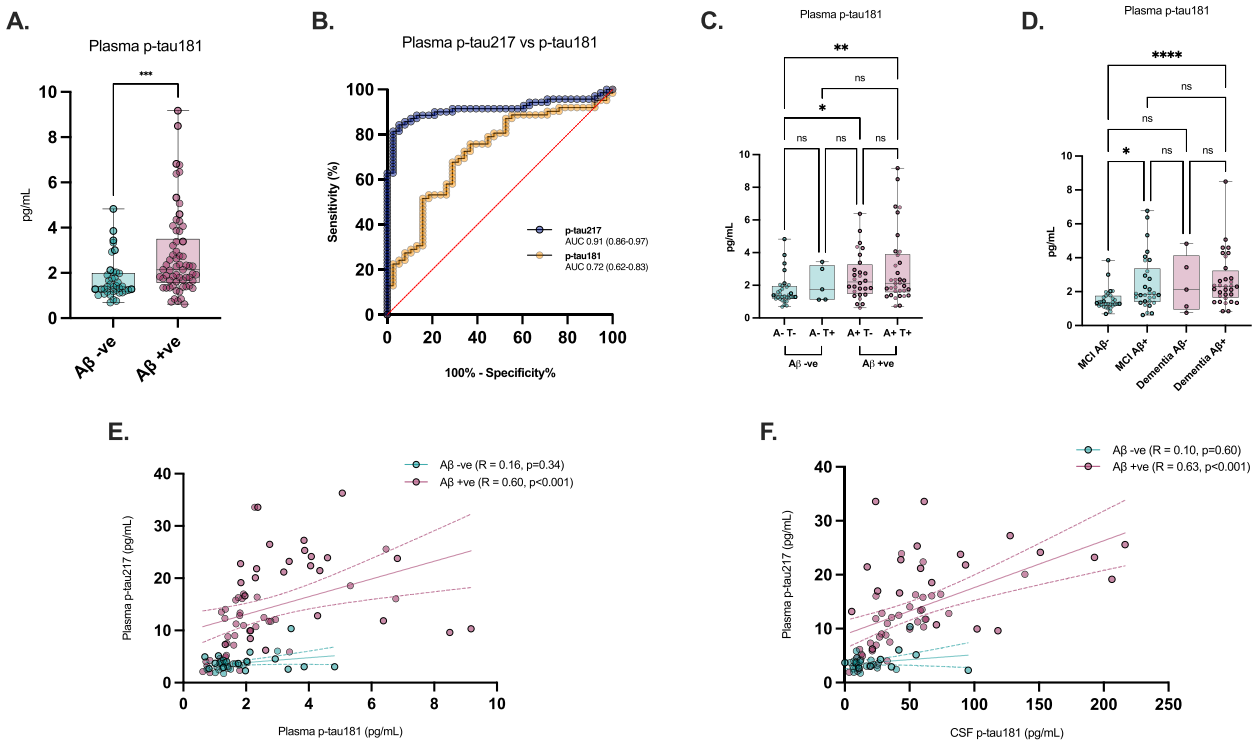


Fig. 3 P-tau217 Outperforms p-tau181 for the Detection of Aβ Positivity in Individuals with MCI/Dementia. Plasma samples were analysed for both p-tau181 and p-tau217. **A** P-tau181 was 1.7 times higher in Aβ + vs Aβ- individuals (Mann–Whitney U = 661; $p < 0.001$). **B** p-tau181 exhibited inferior performance than p-tau217 in the detection of Aβ + status (Area-Under the Curve [AUC]: 0.91; 0.86–0.97 for p-tau217 vs 0.72; 0.62–0.83, DeLong test $p < 0.001$). **C** Significant differences were not seen in concentrations of p-tau181 between A- T- and A- T + individuals or between A + T- and A + T + individuals. **D** There were no significant differences between dementia and MCI for either Aβ + or Aβ – groups. **E** Significant correlations were seen between plasma p-tau181 and plasma p-tau217 in Aβ + (Spearman's R = 0.60, $p < 0.001$) but not Aβ – (Spearman's R = 0.16, $p = 0.34$) individuals. **F** Significant correlations were seen between CSF p-tau181 and plasma p-tau217 again in Aβ + (Spearman's R = 0.63, $p < 0.001$) but not Aβ – (Spearman's R = 0.10, $p = 0.60$) individuals. *****p < 0.0001, ***p < 0.001, ** < 0.01, *p < 0.05, ns: non-significant; AUC: Area-Under-the-Curve*

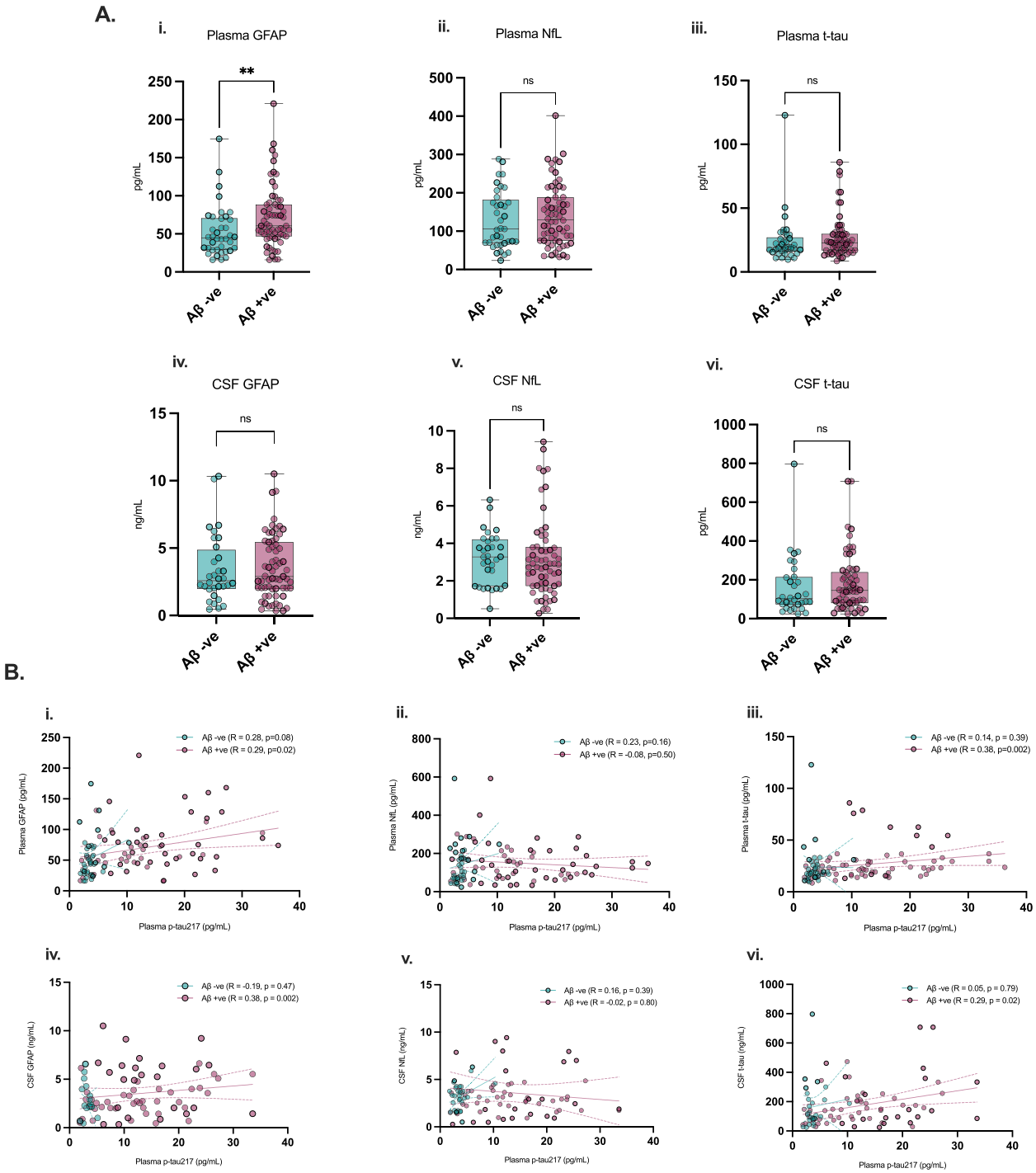


Fig. 4 Plasma P-tau217 is Significantly Correlated with Plasma GFAP, Plasma Total-tau and CSF Total-tau in Aβ + Individuals. Plasma and CSF samples were additionally analysed for Glial Fibrillary Acidic Protein (GFAP), Neurofilament Light (NfL) and Total tau (t-tau). **A** (i) Plasma GFAP significantly differed between Aβ + vs Aβ- individuals (U = 811, $p = 0.004$) (i-vi) None of the additional markers differed in Aβ + vs Aβ – individuals. **B** (i) Significant correlations were observed between plasma GFAP in Aβ + but not Aβ – individuals. (ii, v) No correlations were seen between plasma or CSF NfL and plasma p-tau217. (iv) CSF GFAP did not correlate with plasma p-tau217. (iii, vi) Significant correlations were seen between plasma p-tau217 and both plasma and CSF t-tau in Aβ + but not Aβ – individuals. *****p < 0.0001, ***p < 0.001, ** < 0.01, *p < 0.05, ns: non-significant*

Mann–Whitney U = 811, $p = 0.004$) (Fig. 4). There was a significant correlation between levels of plasma GFAP and plasma p-tau217 in Aβ + (Spearman's $r = 0.29$, $p = 0.02$) but not Aβ- individuals. On examining correlations between plasma p-tau217 and the other markers, significant correlations were observed between plasma p-tau217 and total tau levels in both plasma (Spearman's R = 0.38, $p = 0.002$) and CSF (Spearman's R = 0.29, $p = 0.02$) in Aβ + participants, but not in Aβ- participants (Spearman's R = 0.14, $p = 0.39$ for plasma t-tau; Spearman's R = 0.05, $p = 0.79$ for CSF t-tau) (See Fig. 4).

Discussion

In the current analysis of paired plasma and CSF obtained at time of diagnostic LP, we demonstrated excellent performance of a novel high-sensitivity plasma p-tau217 ECL immunoassay for the detection of Aβ positivity in individuals presenting to specialist memory services with MCI/mild dementia– one of the key contexts in which BBM are likely to be of future use. Importantly, the performance of our assay is in line with previous studies on the use of high-sensitivity plasma p-tau217 immunoassays for the detection of AD pathology [43, 46] and further supports the role of p-tau217 as the leading candidate BBM for detection of AD pathology.

In our study, p-tau217 had a higher AUC for detection of Aβ rather than tau pathology – a finding previously reported and supportive of p-tau217 as an amyloid response measure which increases following CSF positivity but preceding amyloid PET positivity [5]. In our data, the specificity of p-tau217 was greater than its sensitivity—with several "false negatives" having early amnestic MCI secondary to AD pathology. It is possible that changes in p-tau217 have not yet occurred for these individuals despite Aβ positivity on CSF testing. As only individuals who were symptomatic and are presenting to memory services were evaluated in the current cohort, the potential for false negatives may be significantly higher than estimated in community/longitudinal cohort contexts where there be many more true negatives – thus resulting in a lower sensitivity in the current context in comparison to community or cohort studies [57].

Whilst overall diagnostic performance of p-tau217 is consistent with previous reports, it is worth noting that the sensitivity of p-tau217 using an immunoassay in our clinical cohort was slightly lower than that seen using immunoassays in some community/population-based cohorts [46]. The only previous study that examined the same high-sensitivity ECL immunoassay as the current study reported an AUC of 0.98 for detection of "AD-like CSF" – namely both Aβ + and T + compared to A-T- CSF—which may explain why assay sensitivity in the current study was slightly lower as false negatives were mainly from Aβ + T—individuals with early amnestic MCI [47]. Future "round-robin" studies should compare the real-world sensitivities of different scalable immunoassays for p-tau217 in memory clinic cohorts to examine whether this is common across immunoassays or unique to the current study. Additionally, longitudinal studies in real-world memory clinic cohorts are required to tease-out the longitudinal relationships between CSF, plasma and PET biomarkers which will undoubtedly inform repeat-testing and follow-up strategies in the context of specialist memory clinics.

Another important consideration in this manner is if in clinical use, how information is given to patients about the implications of both positive and negative test results as BBMs are implemented into memory services. This may be increasingly important if BBMs are used to triage individuals for further work to determine eligibility for novel DMTs such as anti-amyloid immunotherapies and false negative results may erroneously limit access to anti-amyloid treatment. Further studies should evaluate different strategies and clinical pathways for further investigation, workup and follow-up pathways specifically in memory clinics—where BBMs are likely to significantly change these pathways.

Different strategies have been proposed on how best to integrate BBMs into clinical workflows. One proposal involve defining two different threshold values that maximise sensitivity and specificity. This strategy would allow a subset of positive and negative individuals via p-tau217 levels to be identified, with an intermediate category of individuals with levels between the proposed cut-points – people in this category would require further confirmatory diagnostic testing such as CSF or PET would be of use [40]. Application of this approach in the current cohort revealed that at 95% sensitivity and 95% specificity, 68% of LPs could have been potentially avoided and at a more stringent level of 97.5% sensitivity and 97.5% specificity, 58% may have been avoided. Optimal thresholds require larger real-world studies across different clinical contexts than the cohort examined here. Importantly, our data support the high specificity of plasma p-tau217 as a BBM to confirm the presence of Aβ pathology in specialist settings using a potential two-threshold approach, potentially meaning that individuals with early cognitive symptoms could avoid the need for further invasive or expensive diagnostic tests. Further studies may add novel insight into the sensitives of these assays in memory clinics and inform testing and clinical care pathways in these contexts.

Of note, whilst many immunoassays require relatively sophisticated equipment, staff and resource allocation (such as digital ELISA/Simoa platform), the assay evaluated here was a commercially available ECL

immunoassay. ECL technology is in widespread use in clinical contexts at present (currently the most common method by which CSF biomarkers are analysed). In particular, the use of automated ECL platforms – which are already in clinical use for other applications – have demonstrated excellent performance for the measurement of p-tau217. For instance, two recent pre-print manuscripts have highlighted the potential utility of the automated Lumipulse™ platform in memory clinic contexts [58, 59]. Similarly, recent reports have demonstrated excellent performance of the automated Roche Cobas™ platform for plasma p-tau217 [60]. Automated systems are more scalable than the ECL assay used in the current study – which was a research use only immunoassay requiring more manual steps and hence is more laborious in real-world clinical contexts than automated systems.

Our data is encouraging in suggesting that high-sensitivity ECL platforms are a viable option for clinical testing of plasma p-tau217. In the current data, p-tau217 outperformed plasma p-tau181 which only had an AUC of 0.73 in our analyses. Whilst the performance of this p-tau181 is much lower than that of p-tau217, it is in line with results from a previous head-to-head comparison of the MSD p-tau181 S-PLEX immunoassay in an MCI cohort similar to ours which reported an AUC of 0.64 for the detection of Aβ positivity [43]. Overall, our data supports the use of p-tau217 as the leading candidate BBM for detection of AD pathology. Interestingly, in the current study the correlation between plasma and CSF p-tau217 was not as strong as in previous reports. It is unclear why this is the case, however it is noteworthy that the AUC for CSF p-tau217 was lower than that for plasma – which trended towards statistical significance. Our data adds significant evidence to plasma as the optimal matrix for testing p-tau217 given its superior performance, and the lack of significance directly comparing CSF vs plasma may be due to the sample size under study in the current study.

Our study has several strengths. We demonstrate excellent performance of p-tau217 using a commercially-available ECL immunoassay. Further, our samples and data were obtained as part of routine diagnostic workup in a real-world memory clinic – one of the first contexts where BBMs are likely to be used. As part of this, participants were assigned a consensus diagnosis in line with diagnostic criteria and best practice. We assessed the performance of p-tau217 against CSF Aβ, which is the most common method currently used to define AD pathological change. Moving forward, more data such as ours from real-world clinical cohorts and importantly incorporating longitudinal follow-up will be required to further establish most appropriate use, precise cut-off values and diagnostic pathways for BBMs in memory clinic services.

Limitations

There are several limitations to our study. In the first instance, our study is a single-centre study and only considered patients presenting to a single memory service. Further, our study did not include longitudinal analysis of biomarkers and so cannot comment of temporal sequence of changes between CSF and plasma biomarkers. This may be particularly important in examining the individuals in our study with MCI and Aβ positivity on CSF despite plasma p-tau217 below the cut-off and in designing future clinical pathways for appropriate follow-up and further diagnostic workup in individuals assessed using BBMs in memory clinics.

Conclusion

In conclusion, we assessed the real-world clinical performance of a novel high-sensitivity plasma p-tau217 immuno-assay for the detection of Aβ pathology in a real-world memory clinic setting. Plasma p-tau217 measured by this method demonstrated excellent performance with an AUC of 0.91 for detection of Aβ pathology and outperformed plasma p-tau181 for the detection of Aβ. Of note, plasma p-tau217 was significantly correlated with CSF p-tau217 levels as well as the concentration of plasma GFAP. Further studies will continue to evaluate the real-world clinical utility of high-sensitivity ECL immuno-assays for the detection of Aβ pathology which may be a scalable and affordable platform for BBM assessment in routine clinical use.

Abbreviations

Aβ	Amyloid Beta
ACE-III	Addenbrooke's Cognitive Assessment III
AD	Alzheimer's Disease
ANP	Advanced Nurse Practitioner
AUC	Area-Under-the-Curve
BBM	Blood-Based Marker
BMI	Body Mass Index
bvFTD	Behavioural Variant Fronto-Temporal Dementia
CI	Confidence Interval
CSF	Cerebrospinal Fluid
CVs	Coefficients of Variation
DLB	Dementia with Lewy Bodies
DMTs	Disease-Modifying Therapies
ECL	Electrochemiluminescence
EDTA	Ethylene-Diamine-Tetra-Acetic Acid
FAB	Frontal Assessment Battery
FTD	Fronto-Temporal Dementia
GFAP	Glial Fibrillary Acidic Protein
IQR	Inter-Quartile Range
LP	Lumbar Puncture
LBD	Lewy Body Disease
LLOQ	Lower Limit of Quantification
MCI	Mild Cognitive Impairment
MDT	Multi-Disciplinary Team
MSD	MesoScaleDiscovery
NfL	Neurofilament Light
PET	Positron Emission Tomography
ROC	Receiver Operating Characteristic
RSMC	Regional Specialist Memory Centre
SD	Standard Deviation

Simoa	Single Molecule Array
SMCs	Subjective Memory Complaints
T	Tau
TIMC-BRAiN	Tallaght Institute of Memory and Cognition Biobank for Research in Ageing and Neurodegeneration
TUH	Tallaght University Hospital
t-Tau	Total Tau

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Authors' contributions
AHD, HD and SPK are responsible for the overall design, administration and conduct of the study. AHD, HD, AO wrote the manuscript and analysed data. AO, LM, GS, AM, E Kileen, C Gallagher, ND, EC, SL, CY, C Gaffney, PD, PC, RE, CM, JJ, GK, E Kelly, AF and SO were involved in participant recruitment, biobanking, curation of clinical data and running of the TIMC-BRAiN biobank. CO and NMB advised on and supervised laboratory experiments performed by AHD. All authors have read and approved the final manuscript. All authors were involved in informing the aims and design of the study.

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Availability of data and materials
Due to risk of patient identification, the data accompanying this article are not openly available to the public. Requests for anonymised data will be facilitated by request to the corresponding author (dyera@tcd.ie).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
TIMC-BRAiN has received full ethical approval from the St. James's Hospital/Tallaght University Hospital Joint Research Ethics Committee (Project ID: 2159), which operates in compliance with the European Communities Regulations 2004, ICH Good Clinical Practice Guidelines and the Declaration of Helsinki. All participants provided consent to participate in the study which received full ethical approval from the St. James's Hospital/Tallaght University Hospital Joint Research Ethics Committee (Project ID: 2159).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Dubois B, Villain N, Frisoni GB, Rabinovici GD, Sabbagh M, Cappa S, et al. Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. Lancet Neurol. 2021;20(6):484–96.
2. Dolphin H, Dyer AH, Morrison L, Shenkin SD, Welsh T, Kennelly SP. New horizons in the diagnosis and management of Alzheimer's disease in older adults. Age Ageing. 2024;53(2):5.
3. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016;15(7):673–84.
4. Shimohama S, Tezuka T, Takahata K, Bun S, Tabuchi H, Seki M, et al. Impact of Amyloid and Tau PET on changes in diagnosis and patient management. Neurology. 2023;100(3):e264–74.
5. Arslan B, Zetterberg H, Ashton NJ. Blood-based biomarkers in Alzheimer's disease - moving towards a new era of diagnostics. Clin Chem Lab Med. 2024;62:1063–9.
6. Hansson O, Edelmayer RM, Boxer AL, Carrillo MC, Mielke MM, Rabinovici GD, et al. The Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease. Alzheimers Dement. 2022;18(12):2669–86.
7. Teunissen CE, Verberk IMW, Thijssen EH, Vermunt L, Hansson O, Zetterberg H, et al. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. Lancet Neurol. 2022;21(1):66–77.
8. Zetterberg H, Blennow K. Moving fluid biomarkers for Alzheimer's disease from research tools to routine clinical diagnostics. Mol Neurodegener. 2021;16(1):10.
9. Sims JR, Zimmer JA, Evans CD, Lu M, Ardayfio P, Sparks J, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. JAMA. 2023;330(6):512–27.
10. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in early Alzheimer's disease. N Engl J Med. 2023;388(1):9–21.
11. Gauthier S, Therriault J, Pascoal T, Rosa-Neto P. Impact of p-tau181 and p-tau217 levels on enrollment for randomized clinical trials and future use of anti-amyloid and anti-tau drugs. Expert Rev Neurother. 2020;20(12):1211–3.
12. Gonzalez-Ortiz F, Kac PR, Brum WS, Zetterberg H, Blennow K, Karikari TK. Plasma phospho-tau in Alzheimer's disease: towards diagnostic and therapeutic trial applications. Mol Neurodegener. 2023;18(1):18.
13. Mattsson-Carlgren N, Collij LE, Stomrud E, PichetBinette A, Ossenkoppele R, Smith R, et al. Plasma biomarker strategy for selecting patients with Alzheimer disease for antiamyloid immunotherapies. JAMA Neurol. 2024;81(1):69–78.
14. Rissman RA, Langford O, Raman R, Donohue MC, Abdel-Latif S, Meyer MR, et al. Plasma Aβ42/Aβ40 and phospho-tau217 concentration ratios increase the accuracy of amyloid PET classification in preclinical Alzheimer's disease. Alzheimers Dement. 2024;20(2):1214–24.
15. Dyer AH, Dolphin H, Shenkin SD, Welsh T, Soysal P, Roitto HM, et al. Emerging disease modifying therapies for older adults with Alzheimer disease: perspectives from the EuGMS special interest group in dementia. Eur Geriatr Med. 2023;14(5):919–23.
16. Cullen NC, Janelidze S, Mattsson-Carlgren N, Palmqvist S, Bittner T, Suridjan I, et al. Test-retest variability of plasma biomarkers in Alzheimer's disease and its effects on clinical prediction models. Alzheimers Dement. 2023;19(3):797–806.
17. Barthélemy NR, Li Y, Joseph-Mathurin N, Gordon BA, Hassenstab J, Benzinger TLS, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. Nat Med. 2020;26(3):398–407.
18. Ashton NJ, Janelidze S, Mattsson-Carlgren N, Binette AP, Strandberg O, Brum WS, et al. Differential roles of Aβ42/40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring. Nat Med. 2022;28(12):2555–62.
19. Ashton NJ, Benedet AL, Pascoal TA, Karikari TK, Lantero-Rodriguez J, Brum WS, et al. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. EBioMedicine. 2022;76:103836.
20. Bayoumy S, Verberk IMW, den Dulk B, Hussainali Z, Zwan M, van der Flier WM, 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(1):198.

21. Mielke MM, Dage JL, Frank RD, Algeciras-Schimnich A, Knopman DS, Lowe VJ, et al. Performance of plasma phosphorylated tau 181 and 217 in the community. Nat Med. 2022;28(7):1398–405.
22. Gonzalez-Ortiz F, Ferreira PCL, González-Escalante A, Montoliu-Gaya L, Ortiz-Romero P, Kac PR, et al. A novel ultrasensitive assay for plasma p-tau217: Performance in individuals with subjective cognitive decline and early Alzheimer's disease. Alzheimers Dement. 2024;20(2):1239–49.
23. Groot C, Cicognola C, Bali D, Triana-Baltzer G, Dage JL, Pontecorvo MJ, 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(1):67.
24. Janelidze S, Palmqvist S, Leuzy A, Stomrud E, Verberk IMW, Zetterberg H, et al. Detecting amyloid positivity in early Alzheimer's disease using combinations of plasma Aβ42/Aβ40 and p-tau. Alzheimers Dement. 2022;18(2):283–93.
25. Teunissen CE, Thijssen EH, Verberk IMW. Plasma p-tau217: from "new kid" to most promising candidate for Alzheimer's disease blood test. Brain. 2020;143(11):3170–2.
26. Hanes J, Kovac A, Kvartsberg H, Kontsekova E, Fialova L, Katina S, et al. Evaluation of a novel immunoassay to detect p-tau Thr217 in the CSF to distinguish Alzheimer disease from other dementias. Neurology. 2020;95(22):e3026–35.
27. Karikari TK, Emeršič A, Vrillon A, Lantero-Rodriguez J, Ashton NJ, Kramberger MG, et al. Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. Alzheimers Dement. 2021;17(5):755–67.
28. Leuzy A, Janelidze S, Mattsson-Carlgren N, Palmqvist S, Jacobs D, Cicognola C, et al. Comparing the clinical utility and diagnostic performance of CSF P-Tau181, P-Tau217, and P-Tau231 assays. Neurology. 2021;97(17):e1681–94.
29. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. JAMA. 2020;324(8):772–81.
30. Thijssen EH, La Joie R, Strom A, Fonseca C, Iaccarino L, Wolf A, et al. Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. Lancet Neurol. 2021;20(9):739–52.
31. Cogswell PM, Lundt ES, Therneau TM, Wiste HJ, Graff-Radford J, Algeciras-Schimnich A, et al. Modeling the temporal evolution of plasma p-tau in relation to amyloid beta and tau PET. Alzheimers Dement. 2024;20(2):1225–38.
32. Mattsson-Carlgren N, Janelidze S, Bateman RJ, Smith R, Stomrud E, Serrano GE, et al. Soluble P-tau217 reflects amyloid and tau pathology and mediates the association of amyloid with tau. EMBO Mol Med. 2021;13(6):e14022.
33. Milà-Alomà M, Ashton NJ, Shekari M, Salvadó G, Ortiz-Romero P, Montoliu-Gaya L, et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease. Nat Med. 2022;28(9):1797–801.
34. Therriault J, Vermeiren M, Servaes S, Tissot C, Ashton NJ, Benedet AL, et al. Association of phosphorylated tau biomarkers with amyloid positron emission tomography vs tau positron emission tomography. JAMA Neurol. 2023;80(2):188–99.
35. Mattsson-Carlgren N, Salvadó G, Ashton NJ, Tideman P, Stomrud E, Zetterberg H, et al. Prediction of longitudinal cognitive decline in preclinical Alzheimer disease using plasma biomarkers. JAMA Neurol. 2023;80(4):360–9.
36. Doré V, Doecke JD, Saad ZS, Triana-Baltzer G, Slemmon R, Krishnadas N, et al. Plasma p217+tau versus NAV4694 amyloid and MK6240 tau PET across the Alzheimer's continuum. Alzheimers Dement (Amst). 2022;14(1):e12307.
37. Leuzy A, Smith R, Cullen NC, Strandberg O, Vogel JW, Binette AP, et al. Biomarker-based prediction of longitudinal tau positron emission tomography in Alzheimer disease. JAMA Neurol. 2022;79(2):149–58.
38. Mattsson-Carlgren N, Janelidze S, Palmqvist S, Cullen N, Svenningsson AL, Strandberg O, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. Brain. 2020;143(11):3234–41.
39. Brum WS, Hansson O. Cost-effective Alzheimer's disease detection using plasma p-tau217-based risk stratification. Nat Aging. 2023;3(9):1053–4.
40. Brum WS, Cullen NC, Janelidze S, Ashton NJ, Zimmer ER, Therriault J, 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(9):1079–90.
41. Therriault J, Woo MS, Salvadó G, Gobom J, Karikari TK, Janelidze S, et al. Comparison of immunoassay- with mass spectrometry-derived p-tau quantification for the detection of Alzheimer's disease pathology. Mol Neurodegener. 2024;19(1):2.
42. Teunissen CE, Kimble L, Bayoumy S, Bolsewig K, Burtscher F, Coppens S, et al. Methods to discover and validate biofluid-based biomarkers in neurodegenerative dementias. Mol Cell Proteomics. 2023;22(10):100629.
43. Janelidze S, Bali D, Ashton NJ, Barthélemy NR, Vanbrabant J, Stoops E, et al. Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease. Brain. 2023;146(4):1592–601.
44. Mendes AJ, Ribaldi F, Lathuiliere A, Ashton NJ, Janelidze S, Zetterberg H, et al. Head-to-head study of diagnostic accuracy of plasma and cerebrospinal fluid p-tau217 versus p-tau181 and p-tau231 in a memory clinic cohort. J Neurol. 2024;271(4):2053–66.
45. Ashton NJ, Puig-Pijoan A, Milà-Alomà M, Fernández-Lebrero A, García-Escobar G, González-Ortiz F, et al. Plasma and CSF biomarkers in a memory clinic: Head-to-head comparison of phosphorylated tau immunoassays. Alzheimers Dement. 2023;19(5):1913–24.
46. Ashton NJ, Brum WS, Di Molfetta G, Benedet AL, Arslan B, Jonaitis E, et al. Diagnostic accuracy of a plasma phosphorylated Tau 217 immunoassay for Alzheimer disease pathology. JAMA Neurol. 2024 Mar 1;81(3):255–63.
47. Kivisäkk P, Fatima HA, Cahoon DS, Otieno B, Chacko L, Minooei F, et al. Clinical evaluation of a novel plasma pTau217 electrochemiluminescence immunoassay in Alzheimer's disease. Sci Rep. 2024;14(1):629.
48. Mundada NS, Rojas JC, Vandevrede L, Thijssen EH, Iaccarino L, Okoye OC, et al. Head-to-head comparison between plasma p-tau217 and flortaucipir-PET in amyloid-positive patients with cognitive impairment. Alzheimers Res Ther. 2023;15(1):157.
49. Hawerkamp HC, Dyer AH, Patil ND, McElheron M, O'Dowd N, O'Doherty L, et al. Characterisation of the pro-inflammatory cytokine signature in severe COVID-19. Front Immunol. 2023;14:1170012.
50. Kivisäkk P, Carlyle BC, Sweeney T, Trombetta BA, LaCasse K, El-Mufti L, et al. Plasma biomarkers for diagnosis of Alzheimer's disease and prediction of cognitive decline in individuals with mild cognitive impairment. Front Neurol. 2023;14:1069411.
51 Spanos M, Shachar S, Sweeney T, Lehmann HI, Gokulnath P, Li G, et al. Elevation of neural injury markers in patients with neurologic sequelae after hospitalization for SARS-CoV-2 infection. iScience. 2022;25(8):104833.
52. Dyer AH, Dolphin H, O'Connor A, Morrison L, Sedgwick G, McFeely A, et al. Protocol for the Tallaght University Hospital Institute for memory and cognition-biobank for research in ageing and neurodegeneration. BMJ Open. 2023;13(12):e077772.
53. Frisoni GB, Festari C, Massa F, Cotta Ramusino M, Orini S, Aarsland D, et al. European intersocietal recommendations for the biomarker-based diagnosis of neurocognitive disorders. Lancet Neurol. 2024;23(3):302–12.
54. Roche. The Power of Elecsys® in Alzheimer's disease. 2024. Available from: https://diagnostics.roche.com/global/en/article-listing/health-topics/neurology/the-power-of-elecsys-in-alzheimers-disease.html.
55. Doecke JD, Ward L, Burnham SC, Villemagne VL, Li Q-X, Collins S, et al. Elecsys CSF biomarker immunoassays demonstrate concordance with amyloid-PET imaging. Alzheimers Res Ther. 2020;12(1):36.
56. Miller AM, Begley E, Coen R, Doyle M, Dunne J, Hutchinson S, et al. Clinical consensus guidelines on the application of cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: recommendations of the Irish network for biomarkers in neurodegeneration. Ir Med J. 2016;109(10):483.
57. DeMarco ML, Algeciras-Schimnich A, Budelier MM. Blood tests for Alzheimer's disease: the impact of disease prevalence on test performance. Alzheimers Dement. 2024;20(5):3661–63.

58. Arranz J, Zhu N, Rubio-Guerra S, Rodríguez-Baz Í, Ferrer R, Carmona-Iragui M, et al. Diagnostic performance of plasma pTau 217, pTau 181, Aβ 1-42 and Aβ 1-40 in the LUMIPULSE automated platform for the detection of Alzheimer disease. Alzheimers Res Ther. 2024;16(1):139. https://doi.org/10.1186/s13195-024-01513-9.
59. Andrea P, Virginia Q, Chiara T, Chiara T, Diego B, Cristina M, et al. Plasma p-tau217 in Alzheimer's disease: Lumipulse and ALZpath SIMOA head-to-head comparison. medRxiv. 2024:2024.05.02.24306780.
60. Palmqvist S, Stomrud E, Cullen N, Janelidze S, Manuilova E, Jethwa A, et al. An accurate fully automated panel of plasma biomarkers for Alzheimer's disease. Alzheimers Dement. 2023;19(4):1204–15.

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