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Diagnostic performance of plasma pTau₂₁₇, pTau₁₈₁, Aβ₁₋₄₂ and Aβ₁₋₄₀ in the LUMIPULSE automated platform for the detection of Alzheimer disease

Javier Arranz^{1,2,3}, Nuole Zhu^{1,3,4}, Sara Rubio-Guerra^{1,3}, Íñigo Rodríguez-Baz^{1,2}, Rosa Ferrer⁵, María Carmona-Iragui^{1,2,4}, Isabel Barroeta^{1,2,4}, Ignacio Illán-Gala^{1,4}, Miguel Santos-Santos^{1,4}, Juan Fortea^{1,2,4}, Alberto Lleó^{1,4}, Mireia Tondo^{5,6*} and Daniel Alcolea^{1,4*}

Abstract

Background Recently developed blood markers for Alzheimer's disease (AD) detection have high accuracy but usually require ultra-sensitive analytic tools not commonly available in clinical laboratories, and their performance in clinical practice is unknown.

Methods We analyzed plasma samples from 290 consecutive participants that underwent lumbar puncture in routine clinical practice in a specialized memory clinic (66 cognitively unimpaired, 130 participants with mild cognitive impairment, and 94 with dementia). Participants were classified as amyloid positive (A+) or negative (A-) according to CSF Aβ₁₋₄₂/Aβ₁₋₄₀ ratio. Plasma pTau₂₁₇, pTau₁₈₁, Aβ₁₋₄₂ and Aβ₁₋₄₀ were measured in the fully-automated LUMIPULSE platform. We used linear regression to compare plasma biomarkers concentrations between A+ and A- groups, evaluated Spearman's correlation between plasma and CSF and performed ROC analyses to assess their diagnostic accuracy to detect brain amyloidosis as determined by CSF Aβ₁₋₄₂/Aβ₁₋₄₀ ratio. We analyzed the concordance of pTau₂₁₇ with CSF amyloidosis.

Results Plasma pTau₂₁₇ and pTau₁₈₁ concentration were higher in A+ than A- while the plasma Aβ₁₋₄₂/Aβ₁₋₄₀ ratio was lower in A+ compared to A-. pTau₁₈₁ and the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio showed moderate correlation between plasma and CSF (Rho = 0.66 and 0.69, respectively). The areas under the ROC curve to discriminate A+ from A- participants were 0.94 (95% CI 0.92–0.97) for pTau₂₁₇, and 0.88 (95% CI 0.84–0.92) for both pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀. Chronic kidney disease (CKD) was related to increased plasma biomarker concentrations, but ratios were less affected. Plasma pTau₂₁₇ had the highest fold change (× 3.2) and showed high predictive capability in discriminating A+ from A-, having 4–7% misclassification rate. The global accuracy of plasma pTau₂₁₇ using a two-threshold approach was robust in symptomatic groups, exceeding 90%.

*Correspondence:

Mireia Tondo
mtondo@santpau.cat
Daniel Alcolea
dalcolea@santpau.cat

Full list of author information is available at the end of the article



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Conclusion The evaluation of blood biomarkers on an automated platform exhibited high diagnostic accuracy for AD pathophysiology, and pTau₂₁₇ showed excellent diagnostic accuracy to identify participants with AD in a consecutive sample representing the routine clinical practice in a specialized memory unit.

Keywords Plasma, Biomarkers, Alzheimer, Blood, Amyloid, Tau

What is already known on this topic

Blood biomarkers have shown high accuracy to detect AD pathophysiology. The feasibility of those biomarkers in different platforms and the influence of comorbidities in their concentrations needs to be further studied.

What this study adds

We analyzed the feasibility and diagnostic performance of blood AD biomarkers using a fully-automated platform in the setting of a memory clinic and assessed the impact of comorbidities on their diagnostic performance.

How this study might affect research, practice, or policy: The measurement of plasma AD biomarkers in an automated platform yields high accuracy to detect AD pathophysiology and would be easy to implement. Plasma pTau₂₁₇ was the single most accurate marker for clinical implementation.

Introduction

Early and accurate diagnosis is becoming an increasing priority with the recent developments of disease-modifying therapies for Alzheimer's Disease (AD). Pathophysiological biomarkers in cerebrospinal fluid (CSF) and positron emission tomography (PET) imaging with amyloid and tau tracers have extensively proven to be useful to detect the disease pathophysiology but are either expensive and/or invasive [1], which can delay the diagnosis and access to treatment.

Measurement of AD biomarkers in blood through reliable high-throughput platforms would simplify the diagnostic process. This is now technically possible thanks to the development of sensitive technologies that can consistently quantify brain-derived molecules that are present in blood in very low concentrations [2–4]. Amyloid- β ($A\beta$) peptides and different isoforms of phosphorylated tau (pTau) in blood have shown high accuracy for detecting AD pathophysiology in previous research studies [5–12]. How all these plasma markers are affected by different comorbidities is also starting to be understood thanks to large well-characterized cohorts [13–15]. Thus, blood-based markers have the potential to be of great use in screening, early diagnosis, tracking progression, and ultimately, monitoring the efficacy of treatment [16–20]. Of all the plasma biomarkers evaluated, pTau₂₁₇ has demonstrated a promising profile in identifying amyloidosis,

showing the largest fold changes in symptomatic AD patients and the most predictive ability to identify cognitive decline [21–29]. In previous immunoassay studies CSF pTau₂₁₇ showed better correlation with amyloid-PET and tau-PET than pTau₁₈₁ [30]. Subsequent research has revealed comparable efficacy of pTau₂₁₇ in both plasma and CSF for the identification of AD neuropathology and for distinguishing pathophysiological AD from other neurodegenerative diseases [31–33]. However, most of the existing studies have assessed each of these plasma markers separately, measuring them on different platforms or through techniques not widely available in clinical laboratories, which limits their potential to be widely applied in the clinical routine. The implementation of blood AD markers in a fully-automated platform would facilitate their reproducibility and accessibility in clinical laboratories [34].

The fully-automated platform LUMIPULSE G, extensively used to measure CSF AD biomarkers in clinical laboratories world-wide, has recently launched developed assays to measure pTau₂₁₇, pTau₁₈₁, $A\beta_{1-42}$ and $A\beta_{1-40}$ in plasma. In this study, our aim was to assess the feasibility and diagnostic performance of pTau₂₁₇, pTau₁₈₁, $A\beta_{1-42}$ and $A\beta_{1-40}$ in plasma in the LUMIPULSE fully-automated platform in a cohort of well characterized consecutive individuals assessed in a memory clinic.

Methods

Study participants and clinical classification

We included all consecutive individuals who underwent lumbar puncture for the analysis of AD CSF biomarkers assessed at the Sant Pau Memory Unit (SPIN cohort, Barcelona, Spain) as part of their diagnostic work-up [35] between January 2021 and December 2021 (shown in flowchart in Supplementary Material (Fig. 1)). The study was approved by the Sant Pau Ethics Committee (Protocol code: EC/22/202/6880) following the standards for medical research in humans recommended by the Declaration of Helsinki. All participants or their legally authorized representative gave written informed consent to participate in biomarkers research studies.

At the time of CSF and plasma acquisition, participants had a diagnosis of dementia, mild cognitive impairment (MCI), or were cognitively unimpaired (CU). The clinical diagnosis was established after a thorough neurological

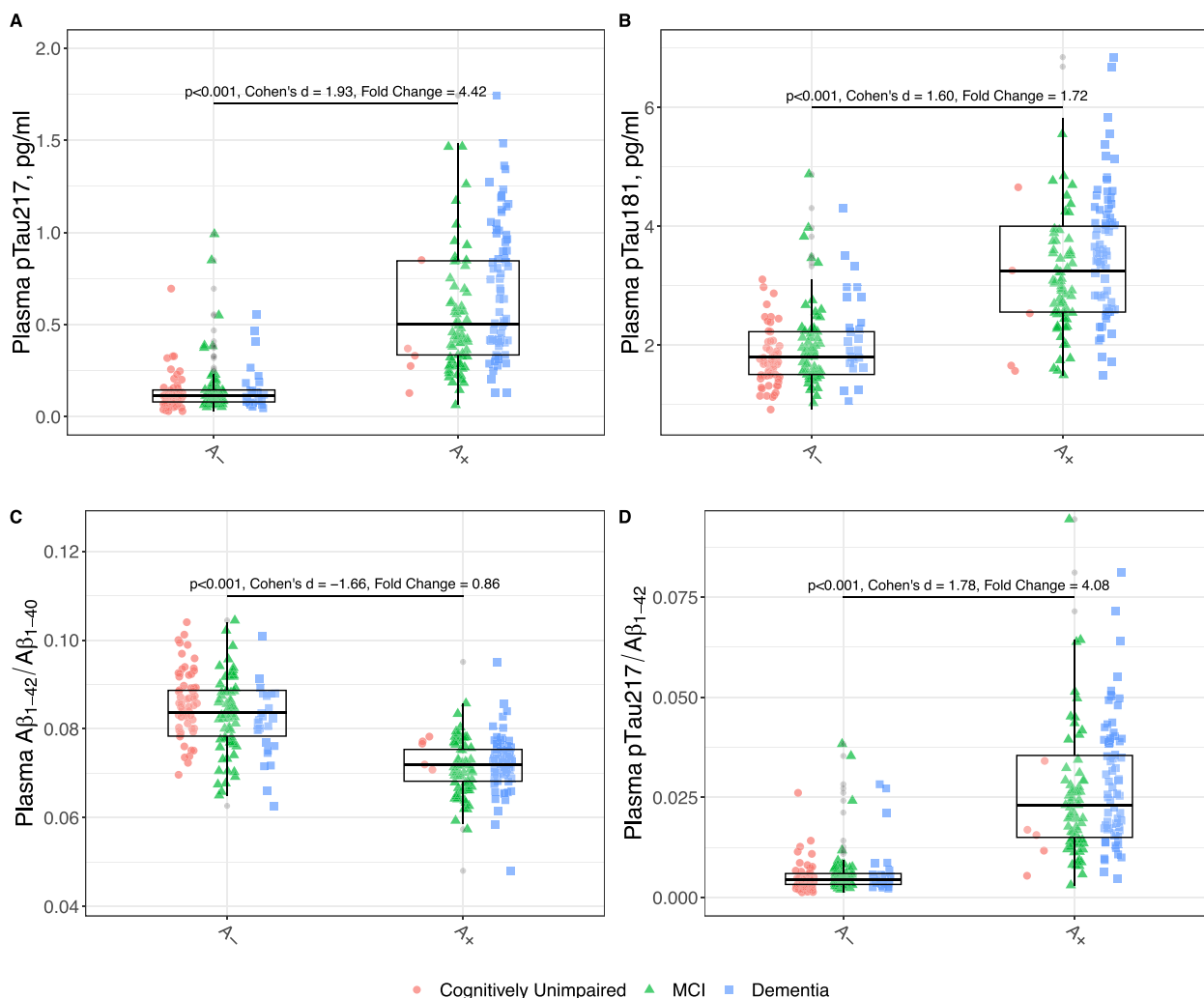


Fig. 1 Levels of plasma biomarkers and their ratios to the A status in CSF. All p-values are derived from multivariate linear model, adjusted for the effects of age, sex, *APOE ε4* status, chronic kidney disease stage, vascular risk factors and clinical stage. For better visualization, two outliers exceeding 2 pg/mL were excluded from the pTau₂₁₇ boxplot for the A+ group in the dementia stages. Not adjusted by other variables effect sizes are shown (Cohen's d). Plasma biomarkers Cohen's d and Fold Change calculated in log-transformed data. pTau₂₁₇: phosphorylated tau 217, pTau₁₈₁: phosphorylated tau 181. Aβ₁₋₄₂: Amyloid β₁₋₄₂. Aβ₁₋₄₀: Amyloid β₁₋₄₀. MCI: Mild cognitive impairment

and neuropsychological evaluation [35]. A more extensive description about how cognitively unimpaired participants were recruited and classified is provided in Supplementary Material (Text 1 & Fig. 1). To assess the impact of vascular risk factors and comorbidities, we collected information about the presence of high blood pressure, dyslipidemia, diabetes, obstructive sleep apnea and history of stroke. Participants were also classified according to the estimated glomerular filtrate rate (eGFR) in different stages (1–5) of chronic kidney disease (CKD) using CKD-EPI formula.

After a full evaluation that included analysis of AD CSF biomarkers, participants were classified according to their etiologic diagnosis, as Alzheimer disease (AD),

other neurodegenerative diseases (OtherDem), not neurodegenerative diseases (OtherNotDeg) or CU. A proportion of participants' diagnosis was classified as "uncertain" as they had an unclear etiologic diagnosis after a full initial evaluation and required clinical follow-up.

Cognitively unimpaired participants were patients with no evidence of cognitive impairment after a thorough neuropsychological evaluation and healthy volunteers interested in research.

Sample collection and analysis

Blood samples were collected in EDTA-K2 tubes and subsequently centrifuged (2000 rpm × 10 min, 4°C)

within 2 h after extraction. Plasma was aliquoted and stored at -80°C until analysis. CSF samples were obtained through lumbar puncture, and were also centrifuged, aliquoted and stored at -80°C until analysis. Blood and CSF samples were collected simultaneously. Full protocol for CSF and blood sample collection in our center has been previously reported [35, 36].

All plasma samples were measured in the Lumipulse fully-automated platform G600II using commercially available kits (Fujirebio Europe, Ghent, Belgium) for pTau₁₈₁, A β ₁₋₄₂ and A β ₁₋₄₀ between July and August 2022 with the same lot of reagents. Plasma pTau₂₁₇ was analyzed between August and September 2023 in another aliquot of the same samples using a novel assay recently developed by Fujirebio. On the day of the analysis, plasma samples were brought to room temperature, mixed thoroughly, centrifuged for 5 min at 2000 g, and subsequently transferred to specific cuvettes for analysis in the Lumipulse platform.

CSF markers A β ₁₋₄₂, A β ₁₋₄₀, pTau₁₈₁ and tTau were used in the diagnostic assessment of patients and measured in routine runs scheduled twice a month throughout 2021 following previously reported methods [37]. According to CSF markers, all participants were classified as amyloid positive (A+, CSF A β ₁₋₄₂/A β ₁₋₄₀ < 0.062) or negative (A-), and as tau positive (T+, pTau₁₈₁ > 63 pg/mL) or negative (T-). Validation of these cutoff values has been described elsewhere [37]. To validate that our main findings would not be overly influenced by analytical error in CSF measurements, we performed additional sensitivity analyses excluding participants $\pm 10\%$ of the CSF cutoff points (Supplementary Material, Figs. 1–3).

DNA was extracted from full blood using standard procedures, and *APOE* was genotyped following previously reported methods [35]. Briefly, direct DNA sequencing of exon 4 was performed routinely for all participants in the SPIN cohort, followed by visual analysis of the resulting electropherogram to identify the two coding polymorphisms that encode the three possible *APOE* isoforms.

Statistical analysis

Data normality was assessed with the Shapiro–Wilk test. Non-normally distributed variables were log-transformed when necessary. Kruskal–Wallis and Wilcoxon rank sum test were used in continuous variables that were not normal distributed. Linear regression models and ANCOVA adjusted by age and sex were performed for group comparison. We used Chi Square test to assess differences in categorical variables and Fisher's exact test in group comparisons with small number of observations, and Spearman test to assess the correlation between plasma and CSF markers.

Diagnostic accuracy of plasma biomarkers was assessed through receiver operating characteristic (ROC) analysis. We calculated the areas under the curve (AUC) of individual markers and that of logistic regression models that combined them with each other and with clinical variables. A basic model that included Age, Sex and *APOE ϵ 4* status was used as a reference to assess the added diagnostic value of plasma markers. We compared the accuracy of individual markers and regression models using DeLong's test adjusted by multiple comparisons using Bonferroni method. We evaluated the sensitivity, specificity, and Youden's J index of a range of cutoffs to discriminate A+ from A- participants. We analyzed the concordance of pTau₂₁₇ with CSF amyloidosis. We followed a previously reported approach [38] to stratify our cohort in low, medium, and high risk of having CSF amyloidosis. Using predictive models, according to the risk of the participants of being A+, we selected conservative (97.5% sensitivity/specificity) and more lenient (95% sens/spec) cutoffs. We bootstrapped to assess the cutoff robustness. All tests were performed in R statistical software version 4.2.1. Alpha threshold was set at 0.05 for all analysis.

Results

Study participants and clinical classification

We included 290 participants who were syndromically classified as cognitively unimpaired (CU, $n=66$), having mild cognitive impairment (MCI, $n=130$) or a clinical diagnosis of dementia ($n=94$). Table 1 shows the etiologic diagnoses in each category, main demographic characteristics, and biomarker measures in each group. CU participants were younger than those with MCI ($p<0.001$) and those with dementia ($p<0.001$). There were more female participants (62%). The proportion of A+, A+ T+ and *APOE ϵ 4* positive increased according to the clinical stage. More extensive demographics details, including stratification by clinical diagnosis or A status, can be found in Supplementary Material (Tables 1 & 2).

Measures of pTau₂₁₇, pTau₁₈₁, A β ₁₋₄₂ and A β ₁₋₄₀ in plasma

All plasma measures for pTau₂₁₇, pTau₁₈₁, A β ₁₋₄₂ and A β ₁₋₄₀ were above their lower limit of quantification. The plasma concentration ranges in the study were 0.03 to 2.84 for pTau₂₁₇, 0.92 to 9.63 pg/mL for pTau₁₈₁, 14.06 to 50.84 pg/mL for A β ₁₋₄₂ and 180.41 to 569.89 pg/mL for A β ₁₋₄₀. Inter-assay coefficients of variation are shown in Supplementary Material (Table 3).

Correlation between plasma and CSF biomarkers

As per inclusion criteria, all participants had CSF biomarkers measures, and we explored the correlation between

Table 1 Demographics and plasma biomarker concentrations

	Cognitively Unimpaired, N = 66	MCI, N = 130	Dementia, N = 94	P value
Age	57 (12)	73 (11)	75 (9)	<0.001
Sex (Female)	46 (70%)	77 (59%)	57 (61%)	0.3
MMSE	29.0 (1.0)	26.0 (4.0)	22.0 (5.8)	<0.001
APOEϵ4	15 (23%)	32 (25%)	36 (38%)	0.042
Plasma pTau₂₁₇ (pg/mL)	0.11 (0.08)	0.23 (0.35)	0.46 (0.63)	<0.001
Plasma pTau₁₈₁ (pg/mL)	1.71 (0.55)	2.32 (1.28)	3.35 (1.86)	<0.001
Plasma Aβ₁₋₄₂ (pg/mL)	25.0 (4.6)	23.0 (5.0)	24.6 (6.2)	0.028
Plasma Aβ₁₋₄₀ (pg/mL)	290 (65)	301 (66)	319 (72)	<0.001
Plasma Aβ₁₋₄₂/Aβ₁₋₄₀	0.085 (0.010)	0.076 (0.014)	0.074 (0.008)	<0.001
A				<0.001
A +	5 (7.6%)	66 (51%)	69 (73%)	
AT				<0.001
A-T-	61 (92%)	64 (49%)	25 (27%)	
A +T-	2 (3.0%)	17 (13%)	12 (13%)	
A +T+	3 (4.5%)	49 (38%)	57 (61%)	
Etiology				<0.001
CU	56 (85%)	0 (0%)	0 (0%)	
AD	4 (6.1%)	43 (33%)	50 (53%)	
OtherNotDeg	1 (1.5%)	37 (28%)	11 (12%)	
OtherDem	3 (4.5%)	18 (14%)	20 (21%)	
Uncertain	2 (3.0%)	32 (25%)	13 (14%)	
VRF	39 (60%)	102 (82%)	75 (85%)	<0.001
HBP	24 (37%)	71 (57%)	49 (56%)	0.021
DM	11 (17%)	28 (23%)	20 (23%)	0.6
DLP	21 (32%)	64 (52%)	55 (63%)	0.001
eGFR_(mL/min/1.73m³)				<0.001
> 90	36 (64%)	29 (23%)	13 (14%)	
60–90	18 (32%)	91 (71%)	68 (72%)	
< 60	2 (3.6%)	8 (6.3%)	13 (14%)	

Median (IQR); n (%)

Kruskal–Wallis rank sum test was used to compare continuous variables that were not normally distributed; Pearson's Chi-squared test was used to compare categorical variables; Fisher's exact test was used to compare categorical variables with small number of observations

Unless otherwise specified, values are presented as median (IQR)

CU Cognitively Unimpaired, MCI Mild cognitive impairment, AD Alzheimer disease, OtherNotDeg Other not degenerative, OtherDem Other diseases, VRF one or more vascular risk factors, HBP High blood pressure, DM Diabetes mellitus, DLP Dyslipidemia, eGFR Estimated glomerular filtration rate

both matrices. The correlation between plasma and CSF was moderate for pTau₁₈₁ (Rho=0.66, $p < 0.001$) and low for A β ₁₋₄₂ (Rho=0.26, $p = 0.007$), and A β ₁₋₄₀ (Rho 0.11, $p = 0.06$). When using ratios, the correlation was moderate for A β ₁₋₄₂/A β ₁₋₄₀ (Rho=0.69, $p < 0.001$). The correlation between plasma pTau₂₁₇ and CSF pTau₁₈₁ was high (Rho=0.75, $p < 0.001$). Both plasma pTau₂₁₇ and pTau₁₈₁ correlated with age (Rho=0.42, $p < 0.001$ and Rho=0.45, $p < 0.001$ respectively). Detailed correlations within clinical subgroups and partial correlations are shown in Supplementary Material (Figs. 4 and 5).

Association between plasma biomarkers and amyloid status in CSF

We assessed the differences in plasma biomarkers between CSF amyloid positive and amyloid negative individuals considering other variables in a multivariate model. We studied the effect of age, sex, APOE status (APOE $\epsilon 4$ +), renal function measured by the estimated glomerular filtration rate (eGFR), vascular risk factors (presence of at least one of the following: high blood pressure, diabetes mellitus, dyslipidemia, history of stroke, obstructive sleep apnea with CPAP) and clinical

status (CU, MCI and Dementia). As shown in Fig. 1, the log transformed multivariate model confirmed that the A+ group had higher plasma concentrations of pTau₂₁₇ (fold-change 4.42, *p* < 0.001) and pTau₁₈₁ (fold-change 1.72, *p* < 0.001) compared to the A- group. Similar results were seen using the ratios pTau₂₁₇/Aβ₁₋₄₂ and Aβ₁₋₄₂/Aβ₁₋₄₀. The plasma pTau₂₁₇/Aβ₁₋₄₂ ratio was higher in A+ compared to A- (fold-change 4.08, *p* < 0.001). The plasma Aβ₁₋₄₂/Aβ₁₋₄₀ ratio was lower in A+ compared to A- (fold-change 0.86, *p* < 0.001). We found lower plasma concentrations of Aβ₁₋₄₂ in A+ compared to the A- group (fold-change 0.92, *p* < 0.001) and no differences in Aβ₁₋₄₀ concentrations between A status (not shown). To assess the influence of the values close to CSF Aβ₁₋₄₂/Aβ₁₋₄₀ cutoff, we conducted a sensitivity analysis by excluding participants with values close to the cutoff point (± 10%) (Supplementary Material, Figs. 1– 3). We found no significant influence of these observations in our analysis.

Effect of other variables on plasma biomarkers

We assessed whether log transformed plasma pTau₂₁₇, pTau₁₈₁, Aβ₁₋₄₂ and Aβ₁₋₄₀ were affected by other variables

in the multivariate model. As seen in Fig. 2, amyloid positivity was the variable with the largest effect on all plasma markers. We also observed that decreased renal function was associated with higher concentrations of pTau₂₁₇ (*p* = 0.019) and pTau₁₈₁ (*p* < 0.001) and higher Aβ₁₋₄₂/Aβ₁₋₄₀ ratio (*p* < 0.001). Male sex was associated with higher pTau₂₁₇ (*p* = 0.033) and pTau₁₈₁ (*p* = 0.0009), and age was associated with lower Aβ₁₋₄₂/Aβ₁₋₄₀ ratio. Our model had an adjusted R² value of 0.62 for pTau₂₁₇, 0.52 for pTau₁₈₁ and 0.48 for Aβ₁₋₄₂/Aβ₁₋₄₀. All the presented coefficients were obtained from the model in which all the listed variables were included. Complete forest plot shown in Supplementary Material (Fig. 6).

To further investigate the association of pTau₂₁₇ and pTau₁₈₁ with renal function, we performed a subanalysis stratifying by estimated glomerular filtration rate. We found that pTau₁₈₁ concentration in plasma was higher as renal function decreased (<60 vs 60-90 mL/min/1.73m² and <60 vs. >90 mL/min/1.73m², *p* < 0.001). Aβ₁₋₄₂ and Aβ₁₋₄₀ concentrations in plasma were also higher as renal function decreased (*p* < 0.001). We also observed marginally significant differences in pTau₂₁₇ concentrations in plasma samples from patients with low renal function

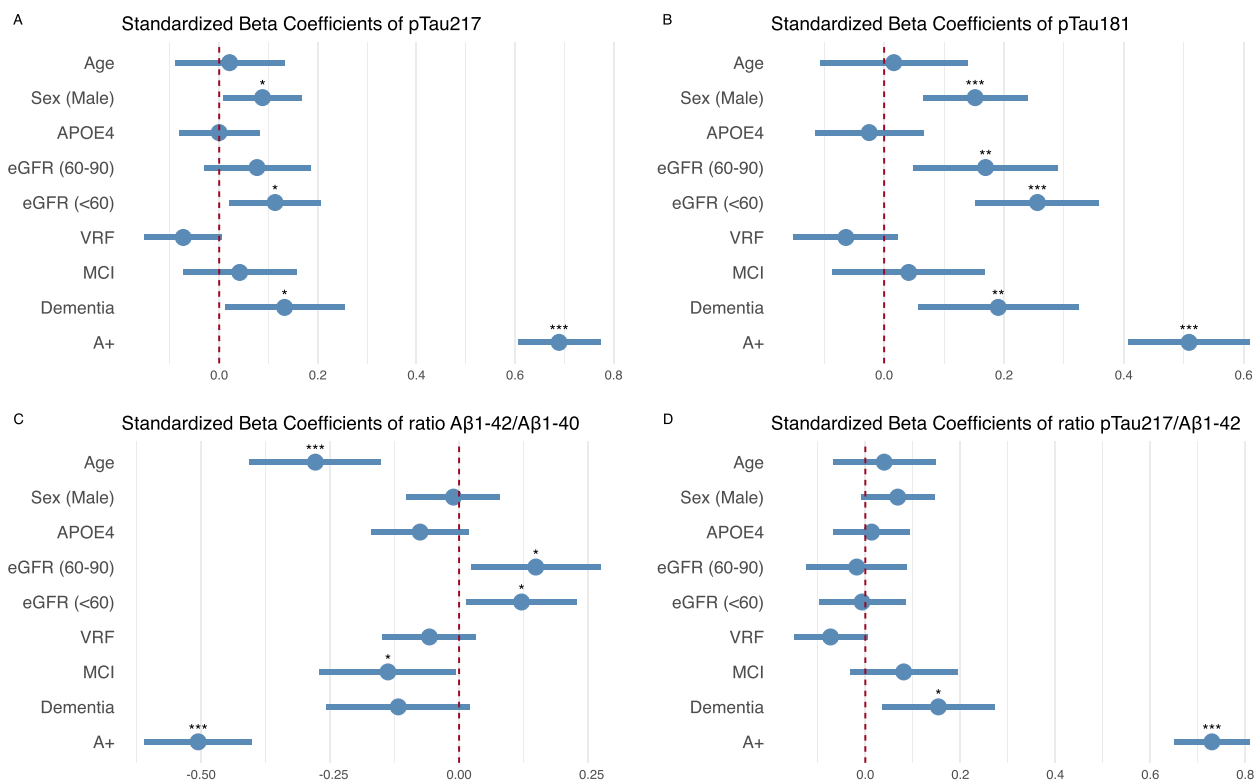


Fig. 2 Effect of different variables on plasma pTau₂₁₇, pTau₁₈₁, Aβ₁₋₄₂/Aβ₁₋₄₀ and pTau₂₁₇/Aβ₁₋₄₂. Dots and bars represent the standardized beta coefficients of each variable in a multivariate regression model. Lines represent the 95% confidence interval for each standardized beta coefficient. Red vertical dashed lines indicate a null effect. We can see the effect size of A positivity adjusted by other variables. pTau₂₁₇: phosphorylated tau 217. pTau₁₈₁: phosphorylated tau 181. Aβ₁₋₄₂: Amyloid β₁₋₄₂. Aβ₁₋₄₀: Amyloid β₁₋₄₀. MCI: mild cognitive impairment. VRF: vascular risk factors. eGFR: estimated glomerular filtration rate

(<60 mL/min/1.73m²) compared to those with normal renal function (>90 mL/min/1.73m², *p*=0.047). However, those differences were lost when using the Aβ₁₋₄₂/Aβ₁₋₄₀ or the pTau₂₁₇/Aβ₁₋₄₂ ratios.

Diagnostic accuracy of plasma biomarkers and their combinations for the discrimination of A+ from A-

In the whole sample, the AUC to discriminate A+ from A- participants were 0.94 (95% CI 0.92–0.97) for pTau₂₁₇, and 0.88 (95% CI 0.84–0.92) for both pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀ (Fig. 3). The diagnostic accuracy of pTau₂₁₇ to detect amyloid positivity was not outperformed by any other individual plasma biomarker, their ratios or their combinations. Aβ₁₋₄₂ and Aβ₁₋₄₀ individually had poor diagnostic accuracy, yielding AUCs below 0.70. Detailed two-by-two comparisons can be found as Supplementary Table 4. Sensitivity, specificity and Youden indices yielded by individual plasma markers are shown in Supplementary Fig. 7.

The diagnostic performance of pTau₂₁₇ was also high across different clinical categories. In the MCI

group, the accuracy of pTau₂₁₇ (AUC=0.92, 95% CI 0.87–0.98) was not significantly different than that pTau₁₈₁ (AUC=0.86, 95% CI 0.79–0.93) or that of the basic model with Age, Sex and APOE ε4 (AUC=0.81, 95% CI 0.74–0.88). However, the addition of pTau₂₁₇ to Age, Sex and APOE ε4 increased the accuracy of the model significantly from 0.81 (95% CI 0.74–0.88) to 0.94 (95% CI 0.9–0.99). pTau₂₁₇ also showed very high accuracies (AUC=0.97; 95% CI 0.94–1.00) to discriminate A+ patients with a diagnosis of AD from different A- clinical groups (CN, other dementias and other not degenerative, Supplementary Fig. 8). The performance of plasma biomarkers to discriminate A+T+ from A-T- participants yielded similar results (Supplementary Fig. 9).

Cutoffs application

Table 2 shows the accuracy of different thresholds for pTau₂₁₇, pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀ to detect amyloid positivity with a sensitivity and specificity of 97.5%, 95% and 90%. As pTau₂₁₇ was the individual biomarker with

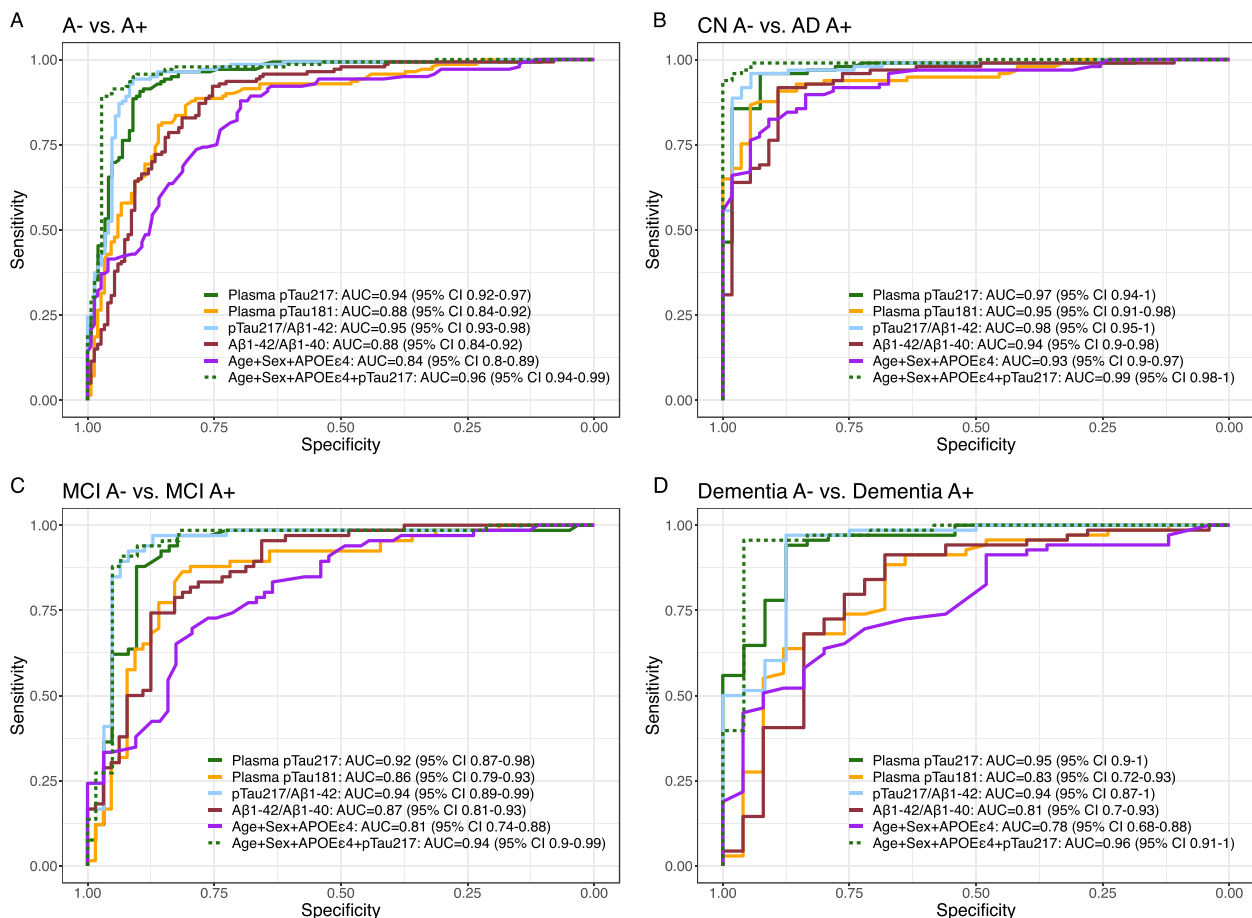


Fig. 3 Diagnostic accuracy of plasma biomarkers for the discrimination of A+ from A- categories. pTau₂₁₇: phosphorylated tau 217, pTau₁₈₁: phosphorylated tau 181, Aβ₁₋₄₂: Amyloid β₁₋₄₂, Aβ₁₋₄₀: Amyloid β₁₋₄₀, CN, cognitively unimpaired, MCI, mild cognitive impairment

Table 2 Thresholds for plasma pTau₂₁₇, pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀ to detect A+ participants

Threshold	Sensitivity	Specificity	NPV	PPV	Youden	Accuracy
Accuracy of plasma pTau ₂₁₇						
0.130	97.8%	65.5%	96.9%	73.1%	0.634	81.3%
0.186	95.0%	82.1%	94.4%	83.5%	0.770	88.4%
0.247	90.6%	89.7%	90.9%	89.4%	0.803	90.1%
0.249	89.9%	89.7%	90.3%	89.3%	0.796	89.8%
0.388	69.8%	95.2%	76.7%	93.3%	0.650	82.7%
0.552	45.3%	97.2%	65.0%	94.0%	0.426	71.8%
Accuracy of plasma pTau ₁₈₁						
1.573	97.9%	32.0%	94.1%	57.3%	0.299	63.8%
1.740	95.0%	44.7%	90.5%	61.6%	0.397	69.0%
2.125	90.0%	71.3%	88.4%	74.6%	0.613	80.3%
2.815	64.3%	90.0%	73.0%	85.7%	0.543	77.6%
3.345	47.9%	94.7%	66.0%	89.3%	0.425	72.1%
3.840	31.4%	97.3%	60.3%	91.7%	0.288	65.5%
Accuracy of Aβ ₁₋₄₂ /Aβ ₁₋₄₀						
0.086	97.9%	41.3%	95.4%	60.9%	0.392	68.6%
0.080	95.0%	65.3%	93.3%	71.9%	0.603	79.7%
0.078	90.0%	75.3%	89.0%	77.3%	0.653	82.4%
0.073	65.0%	90.0%	73.4%	85.8%	0.550	77.9%
0.070	37.9%	94.7%	62.0%	86.9%	0.325	67.2%
0.067	20.7%	97.3%	56.8%	87.9%	0.180	60.3%

pTau₂₁₇: phosphorylated tau 217, pTau₁₈₁: phosphorylated tau 181. Aβ₁₋₄₂: Amyloid β₁₋₄₂, Aβ₁₋₄₀: Amyloid β₁₋₄₀. NPV, negative predictive value. PPV, positive predictive value. Threshold units for pTau are in pg/mL

highest diagnostic accuracy, we assessed the performance of selected cutoffs for this marker in different clinical groups, stratifying by decade. We found that the cutoff that had a global sensitivity of 95% (0.186 pg/mL) yielded accuracies above 84% across all decades and clinical groups when the amyloidosis prevalence was above 20%. In groups with lower amyloidosis prevalence (i.e. CN) the cutoff with specificity of 95% (0.388 pg/mL) showed higher accuracies. We observed that the cutoff that had a specificity of 95% showed a progressive decrease in accuracy with age in the MCI group (accuracy 84% in 60–69, 75% in 70–79 and 62% over 80 years). The negative predictive value of this cutoff was low over 70 years (58%). Detailed information on the accuracy of cutoffs in distinct clinical groups and decades can be found in Supplementary Material (tables 5, 6, 7 & 8).

We conducted a supervised decision tree analysis to determine the potential of various biomarkers and demographic factors in correctly identifying individuals with amyloidosis. This analysis incorporated plasma pTau₂₁₇, pTau₁₈₁, ratio Aβ₁₋₄₂/Aβ₁₋₄₀, Age, Sex, APOE ε4 allele presence, and clinical diagnosis group (cognitively unimpaired, mild cognitive impairment, and dementia). The most effective discriminators for amyloidosis were plasma pTau₂₁₇ followed by the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio when

pTau₂₁₇ was high (Supplementary Fig. 10). Other variables were deemed less critical for amyloidosis detection and thus excluded from the decision tree. This algorithm exhibited a misclassification rate of 7.4%, with a sensitivity of 91%, specificity of 94%, overall accuracy of 93%, positive predictive value (PPV) of 94%, and negative predictive value (NPV) of 91%, accompanied by a false-negative rate (FNR) of 9.4% and a false-positive rate (FPR) of 5.6%.

Potential of plasma pTau₂₁₇ to predict Alzheimer disease pathophysiology

We assessed the predictive capability of pTau₂₁₇ to properly classify participants with amyloidosis in our dataset, defined as CSF Aβ₁₋₄₂/Aβ₁₋₄₀ < 0.062. As the model that combined pTau₂₁₇ with Age, Sex and APOEε4 did not perform better than pTau₂₁₇ alone in any comparison, we chose the simplest predictive model with pTau₂₁₇. We bootstrapped to obtain robust predictive cutoffs. We found no differences between the initial prediction and the mean of the 1000 predictive iterations, with a perfect correlation between them (Rho = 1, p < 0.001) thus reinforcing the robustness of our initial predictions.

To facilitate the clinical implementation of plasma biomarkers while ensuring accuracy, we applied a

two-threshold approach to classify participants into three groups, those with high, medium, and low likelihood of being CSF amyloid positive. Following this approach, those with a medium risk would benefit from a confirmation with gold standard tests like CSF or Amyloid PET. Figure 4 shows the thresholds in two different scenarios based on two levels of restrictiveness in sensitivity and specificity (97.5% and 95%). Using a highly accurate combination of cutoffs (one for 97.5% Sens and another for 97.5% Spec), only 41.9% of patients in the whole sample would require an additional test, with a global misclassification rate of 4.2%. Using less restrictive cutoffs (95% Sens/Spec), the proportion could be reduced to 19% with a global misclassification rate of 6%. Similar results were found in the subgroup of patients with CU, MCI and Dementia.

We finally assessed the robustness of those cutoffs in the whole sample, considering the increase of prevalence of amyloidosis with age in our population. In Fig. 5, we show the NPV, PPV, and global accuracy of pTau₂₁₇ to detect A positivity by decades using cutoff combinations with 95% and 97.5% sensitivity and specificity in different clinical groups. We found high global accuracy (75%-100%) of the two-threshold application in all the scenarios, with variations of PPV and NPV according to CSF amyloid positive prevalence in our sample.

Discussion

In this study, we found that the concentration of plasma pTau₂₁₇, plasma pTau₁₈₁, and the ratio Aβ₁₋₄₂/Aβ₁₋₄₀ measured in a fully automated platform, yielded excellent accuracy to detect the AD pathophysiology in the setting of the routine clinical practice of a memory clinic. Of all plasma markers, the recently developed assay that measures pTau₂₁₇ was the most accurate followed by the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio and pTau₁₈₁. We also found that different comorbidities had a mild significant effect on plasma markers of AD, but the amyloid status was the single variable with the largest effect on their concentration. In patients with advanced chronic kidney disease, the use of ratios could reduce the impact of having higher plasma concentrations associated to low

renal function. Furthermore, we applied predictive models to obtain stratification profiles that showed the potential to reduce the need of more costly or invasive procedures by approximately 60 to 80%.

The performance of plasma markers to detect the AD pathophysiology has been assessed in previous studies using different analytical platforms, with AUCs ranging from 0.70 to 0.96 for pTau₁₈₁ [7, 23, 39–43], and from 0.64 to 0.86 for Aβ₁₋₄₂/Aβ₁₋₄₀ [5, 8, 10]. Plasma pTau₂₃₁ and pTau₂₁₇ have shown to better capture the earliest cerebral Aβ changes in CU, before overt Aβ plaque pathology is present [26]. For plasma pTau₂₁₇, accuracies have varied depending on the platform, yet the performance has consistently been high in discriminating amyloidosis in MCI and predicting progression, typically outperforming that of other plasma pTau isoforms [7, 23, 33, 44]. Most research studies reported better accuracies with the use of composite measures that combined two or more markers and/or clinical or genetic information [45–47]. In our study, plasma pTau₂₁₇, the Aβ₁₋₄₂/Aβ₁₋₄₀ ratio and pTau₁₈₁ measured with a fully automated platform showed high diagnostic performance to detect amyloid positivity. Of these, pTau₂₁₇ was the marker that showed higher accuracy and, importantly, it was not outperformed by composite measures indicating that it is a good candidate for its implementation as a single biomarker.

Automated platforms have revolutionized CSF analysis by resolving critical analytical factors and, similarly, they hold promise for transforming plasma testing, enhancing both precision and efficiency in biomarker quantification. Recent studies have assessed the diagnostic performance of pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀ in the Lumipulse platform. Janelidze et al. reported an AUC of 0.7 for pTau₁₈₁ for the identification of CSF amyloidosis in MCI [23] and of 0.74 to detect progression to dementia. However, Wilson et al. reported a higher accuracy of 0.96 [40] for the discrimination between Aβ⁻ CU and Aβ⁺ AD patients. Another recent study using the Lumipulse platform analyzed the accuracy to detect AD of plasma pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀ ratio in cognitively unimpaired participants and found that Aβ₁₋₄₂/Aβ₁₋₄₀ ratio was the most cost-effective (AUC

(See figure on next page.)

Fig. 4 Plasma pTau₂₁₇ predictive models. Strict and lenient cutoffs in the whole sample and in the distinct clinical groups. We illustrate the implementation of various cutoff thresholds within our sample, denoted by dashed lines in red and green at distinct Y-axis levels, each line representing the associated sensitivity and specificity values. Displayed in red dots are individuals with amyloid positivity in cerebrospinal fluid (CSF) and in green those amyloid negatives. Those participants with pTau₂₁₇ concentrations above dashed red lines are classified as high risk of amyloid CSF positivity. The medium risk category is between dashed red and green lines. Below dashed green line are the participants classified as low risk. Observations that have been incorrectly categorized into high or low risk groups are represented by distinct sizes and colors. To the right, the corresponding percentages of the sample assigned to each risk category are presented. pTau₂₁₇: phosphorylated tau 217, pTau₁₈₁: phosphorylated tau 181. Aβ₁₋₄₂: Amyloid β₁₋₄₂. Aβ₁₋₄₀: Amyloid β₁₋₄₀. Model 1 included only plasma pTau₂₁₇ for predictions. S, sensitivity. Sp, specificity

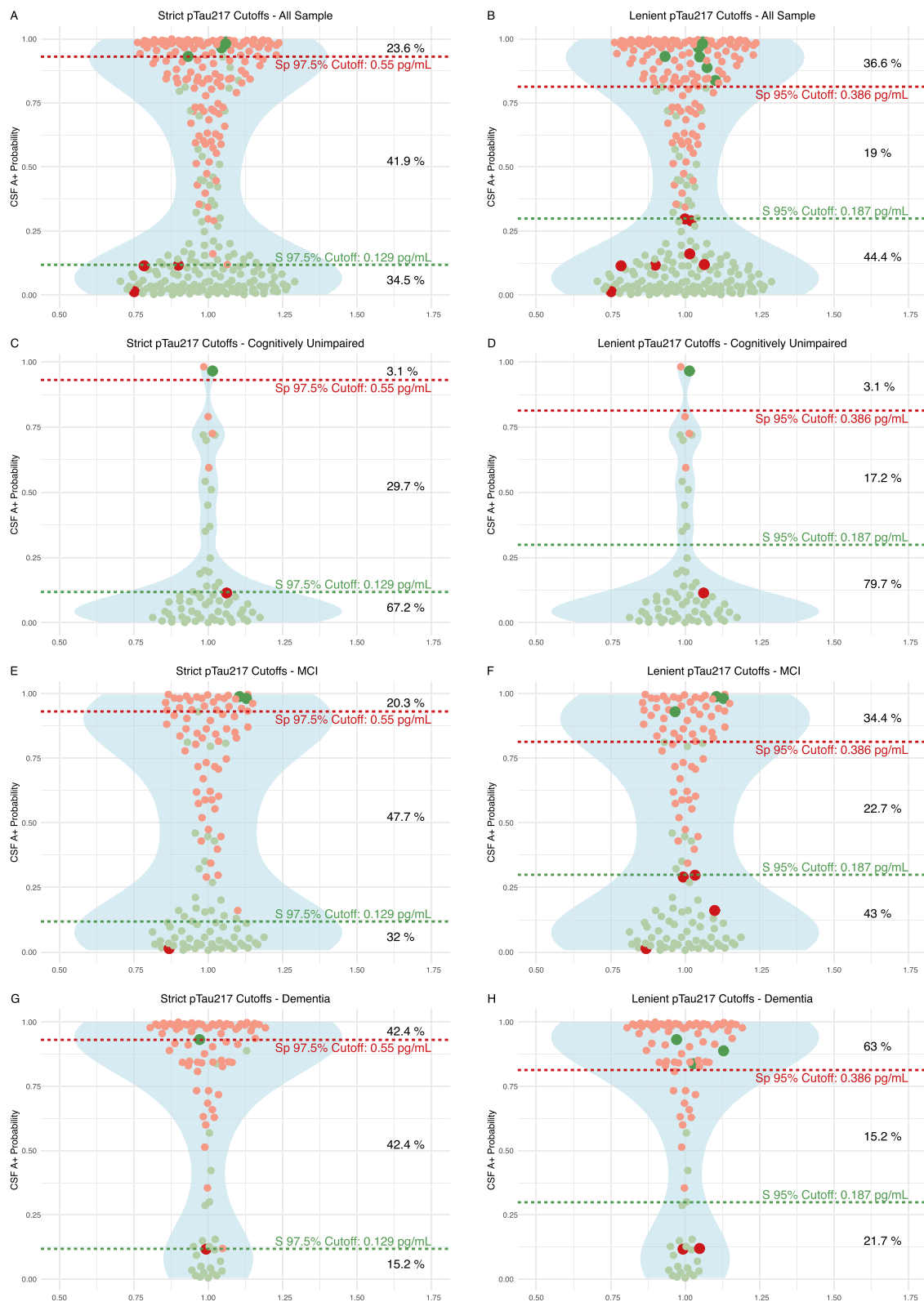


Fig. 4 (See legend on previous page.)

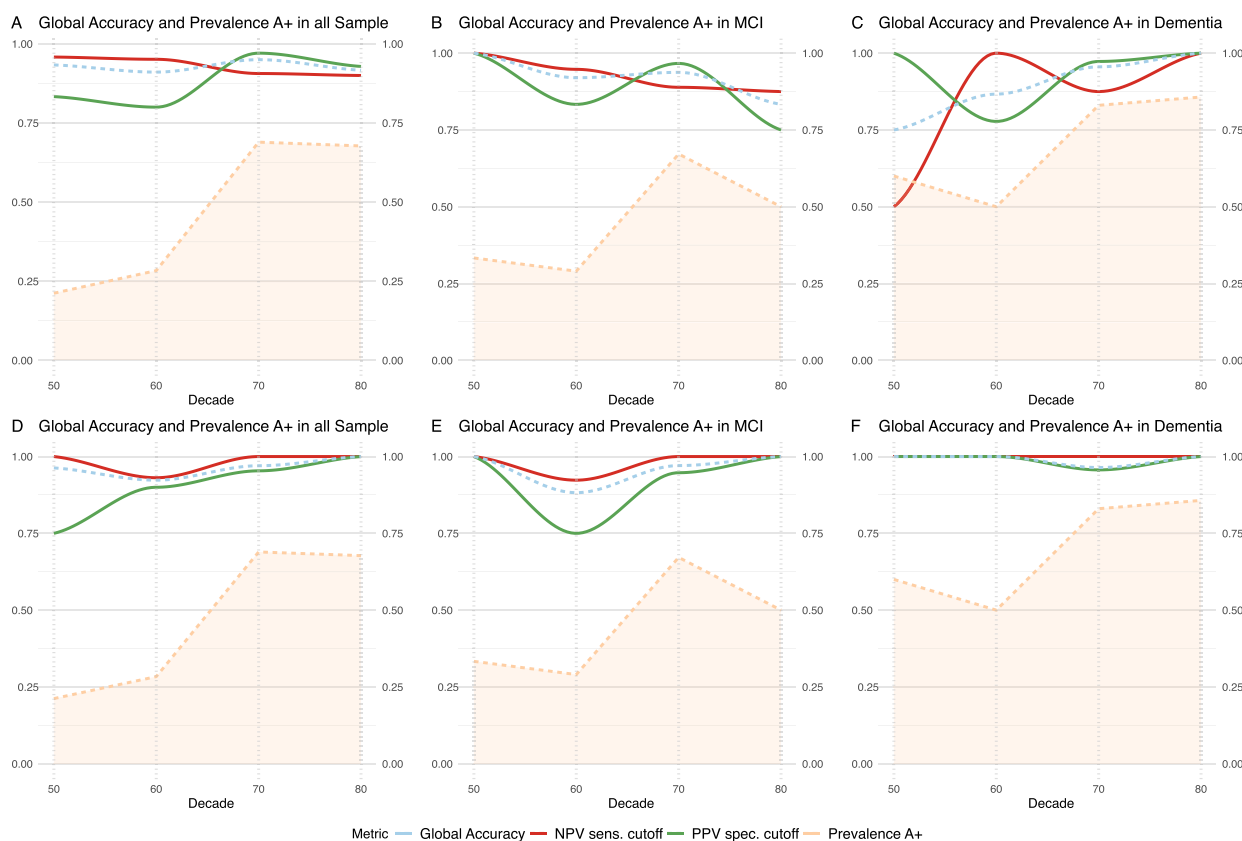


Fig. 5 Negative predictive value, positive predictive value and global accuracy of pTau₂₁₇ for the combination of cutoffs with 95% (A, B, C) and 97.5% (D, E, F) sensitivity and specificity and their relationship with age and prevalence of CSF Amyloidosis. The figure illustrates the association between the prevalence of cerebrospinal fluid (CSF) Amyloidosis (depicted by a dashed orange line), Positive Predictive Value (PPV, red line), Negative Predictive Value (NPV, green line), and global accuracy (dashed blue line) across different decades of life (X-axis) within our study cohort. In panels A-C, the PPV was determined using a cutoff for 95% specificity set at 0.386 pg/mL, while the NPV was ascertained using a 95% sensitivity cutoff at 0.187 pg/mL. In panels D-F, the PPV was determined using a cutoff for 97.5% specificity set at 0.55 pg/mL, while the NPV was ascertained using a 97.5% sensitivity cutoff at 0.129 pg/mL. The term 'global accuracy' in this context refers to the proportion of participants accurately classified as either positive or negative, based on the application of these two cutoffs. It should be noted that this calculation of global accuracy excludes participants categorized as 'indeterminate' (falling within the grey zone), for whom a confirmatory test is recommended. pTau₂₁₇: phosphorylated tau 217, Sens, sensitivity. Spec, specificity. NPV, negative predictive value. PPV, positive predictive value. MCI, mild cognitive impairment

0.9 for A+ and 0.89 for A+ T+), followed by pTau₁₈₁ that showed an AUC of 0.76 for A+ and 0.86 for A+ T+ [48]. Our results are in line with previous studies, and show a global accuracy of 0.88 for plasma pTau₁₈₁ and for the plasma Aβ₁₋₄₂/Aβ₁₋₄₀ ratio. A variety of reasons could explain the minor discrepancies between studies, including differences in preanalytical conditions [49], in the kits that were used, characteristics of the sample and cohorts, and the design of the studies. Our design including consecutive patients that underwent a lumbar puncture for routine diagnostic work-up in a memory clinic setting, provides information about the potential implementation of plasma markers in this context.

Together with the commercially available pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀, we evaluated the performance of plasma

pTau₂₁₇, recently developed for the same automated platform, and found that it outperformed pTau₁₈₁ and Aβ₁₋₄₂/Aβ₁₋₄₀ in the detection of amyloid positivity. In previous research studies, plasma pTau₂₁₇ has consistently shown exceptionally high accuracy across different platforms and has demonstrated strong correlations with other markers of AD (CSF biomarkers, amyloid PET and Tau PET) and with neuropathology [30]. The fact that it has shown greatest fold changes and effect sizes compared to other pTau isoforms, makes it a perfect candidate for its implementation in clinical settings, as small analytical variations (5–10%) would not substantially affect its diagnostic performance. Its implementation on a fully automated platform would not only simplify the process but also enhance accessibility for clinical laboratories.

In our study using a fully automated platform, we found that A+ patients had 4.42 times higher concentrations of plasma pTau₂₁₇. This magnitude of effect, combined with the advantages of automation, makes this assay particularly promising for integration into standard clinical practices.

To facilitate a more rational utilization of plasma biomarkers, we followed the methodology delineated by Brum et al. stratifying the risk of having AD pathophysiology [38]. By implementing this strategy, it is feasible to define threshold values that are highly sensitive and specific to either detect or rule out CSF amyloidosis minimizing the probability of misclassification. This would also allow for selecting those patients that fall within an intermediate likelihood category or 'grey zone' and that would benefit of further etiological investigations (CSF biomarkers, amyloid or Tau PET). This stratification framework represents a pragmatic and flexible approach for the incorporation of plasma biomarkers in clinical settings. An open point of discussion is the optimal context of use—primary care, general neurology, or specialized memory clinics—and the management strategies for participants identified as high or low risk. Our study showed that following this approach, pTau₂₁₇ had an excellent performance in the context of a specialized memory clinic, but these classifications will need to be contextualized within other clinical settings [4, 34]. The effect of comorbidities as CKD on plasma biomarker concentrations points in the same direction as recently published studies [15, 50], in which the use of ratios could attenuate the effect of CKD. Moreover, we found that the effect of renal dysfunction on plasma pTau₂₁₇ concentrations was significantly less than that of the amyloid positivity status, suggesting that the actual impact of CKD on the diagnostic performance of this marker would be minimal. When we assessed the impact of renal dysfunction on different plasma pTau biomarker concentrations, we found that the effect size of eGFR < 60 mL/min/1.73m³ in pTau₁₈₁ was double that observed in pTau₂₁₇, with standardized beta coefficients of 0.25 and 0.11, respectively (Fig. 2), both statistically significant. When we compared the AUC of plasma pTau₂₁₇ and pTau₁₈₁ in the subset of patients with eGFR < 60 mL/min/1.73m³, although higher in pTau₂₁₇ (AUC 0.83, CI 95% 0.64–1) than in pTau₁₈₁ (AUC 0.75, CI 95% 0.53–0.97), accuracy did not differ between both biomarkers, probably because of low statistical power to capture differences in this subset in our cohort, as only 11 patients were A- and 12 patients A+.

One of the strengths of our study is that we included all consecutive participants from routine clinical practice that underwent lumbar puncture throughout one year in our memory clinic including a variety of diagnoses. This

approach reduces the risk of selection biases and ensures a reliable representation of the population assessed in the setting of a specialized memory clinic, also providing relevant information on their potential implementation in the routine diagnostic work-up in this context. Other strengths in our study are the fact that all markers were measured using the same batch of reagents and that the clinical information available allowed us to analyze the potential impact of comorbidities and perform sub analyses within distinct clinical stages.

Limitations

Our study also has some limitations. First, as the inclusion criteria required that participants had received a lumbar puncture for CSF biomarkers, the extrapolation to other contexts of use different than specialized memory units, such as primary care or population screening programs, should be made cautiously. Second, we could not compare plasma pTau₂₁₇ with its counterpart in CSF as the assay was specifically developed for plasma. Another limitation is the lack of Amyloid/Tau PET or neuropathological confirmation in our participants, and although the CSF biomarker cutoffs in our center were validated against amyloid PET [37], we cannot be certain about how using a different gold-standard might affect our results. Finally, this is a single-center study, and even though this increases uniformity and we performed a bootstrapped cross-validation to ensure the predictive robustness of our models, the accuracy and true predictive power of the cutoffs derived from our results need to be verified in diverse datasets with comparable characteristics.

Conclusions

Our study provides evidence that plasma markers can reliably be measured in an automated platform, and highlights plasma pTau₂₁₇ as the most promising plasma biomarker, showing great potential for the detection of AD pathophysiology in the context of a memory clinic. With the arrival of disease-modifying treatments into clinical practice, it is urgent to have easily accessible and efficient diagnostic methods to identify patients that could benefit from these therapies. The implementation of plasma biomarkers in readily accessible fully automated platforms will streamline the diagnosis and enhance the accessibility of disease modifying therapies.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13195-024-01513-9>.

Supplementary Material 1.

Acknowledgements

We are grateful to all participants in the study and their families. We also thank all the clinical team members that were involved in the selection and assessment of participants in the SPIN cohort, and the laboratory teams for sample handling, biomarker analyses, and structural support. We also thank Fujirebio for providing technical support and the reagents necessary to complete the study.

Authors' contribution

DA and AL designed the study. JA, NZ, SR-G, IR-B, RF, MC-I, IB, II-G, MS-S, AL, JF, MT, DA acquired data relevant for the study. MC-I, II-G, JF, AL, MT, DA contributed vital reagents/tools/patents. MC-I, II-G, MS-S, JF, AL, DA obtained funding for the study. DA and JA performed statistical analysis. DA, JA contributed in analysis and interpretation of data. DA, AL, JF participated in study supervision or coordination. JA and DA drafted the first version of the manuscript.

Funding

This study was supported by the Fondo de Investigaciones Sanitarias (FIS), Instituto de Salud Carlos III (PI21/00791 to IIG, PI14/01126, PI17/01019 and PI20/01473 to JF, PI13/01532 and PI16/01825 to RB, PI18/00335 to MCI, PI19/00882 to MS-S, PI18/00435, PI22/00611 and INT19/00016 to DA, PI17/01896 and AC19/00103 to AL) and the CIBERNED program (Program 1, Alzheimer Disease to AL), jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, "Una manera de hacer Europa". This work was also supported by the National Institutes of Health (NIA grants 1R01AG056850-01A1; R21AG056974; and R01AG061566 to JF), by Generalitat de Catalunya (2017-SGR-547, SLT006/17/125 to DA, SLT006/17/119 to JF, SLT002/16/408 to AL) and "Marató TV3" foundation grants 20141210 to JF, 044412 to RB and 20142610 to AL. This work was also supported by a grant from the Fundació Bancaria La Caixa to RB (DABNI project). Fundació Catalana Síndrome de Down and Fundació Víctor Grifols i Lucas partly supported this work. Horizon 21 Consortium is partly funded by Jérôme Lejeune Foundation (Clinical and trial outcome measures for dementia in individuals with Down syndrome).

The reagents necessary to complete the study were funded by Fujirebio-Europe.

The sponsors of the study did not take part in the design and conduct of the study; collection, management, analysis, and interpretation of the data; writing and review of the report; or the decision to submit the article for publication.

Availability of data and materials

Raw anonymized data and code for statistical analysis are available upon reasonable request. All requests should be sent to the corresponding author detailing the study hypothesis and statistical analysis plan. The steering committee of this study will decide whether data/code sharing is appropriate based on the novelty and scientific rigor of the proposal. All applicants will be asked to sign a data access agreement.

Declarations

Ethics approval and consent to participate

The ethics committee of Hospital Sant Pau approved all procedures included in this study following the standards for medical research in humans recommended by the Declaration of Helsinki. All participants or their legally authorised representative gave written informed consent before enrolment in the study.

Consent for publication

All authors revised the manuscript for content and provided critical feedback.

Competing interests

Daniel Alcolea is employed by Hospital de la Santa Creu i Sant Pau and received research grants from Pla Estratègic de Recerca i Innovació en Salut (PERIS SLT006/17/125), and from Instituto de Salud Carlos III (PI18/00435 and INT19/00016). He participated in advisory boards from Fujirebio-Europe and Roche Diagnostics and received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Zambon S.A.U., Esteve, and from Krka Farmacèutica S.L. Javier Arranz is employed by Biomedical Research Institute Sant Pau. He is funded by a "Rio Hortega" research grant from the Institute of Health Carlos III. Declarations of interest: none.

Nuole Zhu is employed by Biomedical Research Institute Sant Pau. He is funded by a "Rio Hortega" research grant from the Institute of Health Carlos III. Declarations of interest: none.

Sara Rubio-Guerra is employed by Hospital de la Santa Creu i Sant Pau. Declarations of interest: none.

Iñigo Rodríguez-Baz is employed by Biomedical Research Institute Sant Pau. He is funded by a "Rio Hortega" research grant from the Institute of Health Carlos III. Declarations of interest: none.

Rosa Ferrer is employed by Hospital de la Santa Creu i Sant Pau. Declarations of interest: none.

María Carmona-Iragui is employed by Hospital de la Santa Creu i Sant Pau. Declarations of interest: none.

Isabel Barroeta is employed by Hospital de la Santa Creu i Sant Pau. Declarations of interest: none.

Dr. Illán-Gala is a senior Atlantic Fellows for Equity in Brain Health at the Global Brain Health Institute (GBHI), and is supported with funding from GBHI, Alzheimer's Association, and Alzheimer's Society (GBHI ALZ UK-21-720973 and AACSF-21-850193). Dr Illán-Gala was also supported by the Juan Rodés Contract (JR20/0018) and Fondo de Investigaciones Sanitarias, (PI21/00791) from Instituto de Salud Carlos III.

Ignacio Illán-Gala reported receiving personal fees from Nutricia, Esteve, UCB, and Neuraxpharm Spain outside the submitted work.

Miguel Santos-Santos is employed by Hospital de la Santa Creu i Sant Pau. His research is supported by funding from the Spanish Institute of Health Carlos III (Juan Rodés contract JR18-00018; Fondo de investigación sanitaria grant PI19/00882), the Alzheimer's Association clinician scientist fellowship (AACSF-22-972945), and the National Institutes of Health (R01AG080470).

Juan Fortea is employed by Hospital de la Santa Creu i Sant Pau and received research grants from Instituto de Health Carlos III, National Institutes of Health, Fundació La Marató de TV3, and Pla Estratègic de Recerca i Innovació en Salut (PERIS). Dr. Fortea has served as a consultant for Novartis and Lundbeck, has received honoraria for lectures from Roche, NovoNordisk, Esteve and Biogen and served at advisory boards for AC Immune, Zambon and Lundbeck.

Alberto Lleó is employed by Hospital de la Santa Creu i Sant Pau and received research grants from CIBERNED, Institute of Health Carlos III, Generalitat de Catalunya (PERIS and AGAUR) and Fundació Tatiana and BBVA. He participated in advisory boards from Biogen, Eisai, Fujirebio-Europe, Novartis, NovoNordisk, Nutricia, Otsuka Pharmaceutical, and Zambón, and received speaker honoraria from Lilly, Biogen, KRKA and Zambon.

Mireia Tondo is employed by Hospital de la Santa Creu i Sant Pau and has received research grants from Instituto de Salud Carlos III (PI18/00164; PI21/00140) and has served as consultant in Araclon.

Author details

¹Sant Pau Memory Unit, Department of Neurology, IR SANT PAU, Hospital de La Santa Creu i Sant Pau, C/Sant Quintí 89, 08041 Barcelona, Spain.

²Department of Neurology, Unidad Alzheimer-Down, IR SANT PAU, Hospital de La Santa Creu i Sant Pau; Barcelona Down Medical Center, Fundació Catalana Síndrome de Down, Barcelona, Spain.

³Universitat Autònoma de Barcelona, Barcelona, Spain.

⁴Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas, CIBERNED, Madrid, Spain.

⁵Servei de Bioquímica i Biologia Molecular, IR SANT PAU, Hospital de La Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, C/Sant Quintí 89, 08041 Barcelona, Spain.

⁶Centro de Investigación Biomédica en Red en Diabetes y Enfermedades Metabólicas, CIBERDEM, Madrid, Spain.

Received: 8 December 2023 Accepted: 20 June 2024

Published: 26 June 2024

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