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Diagnostic performance of plasma p-tau217 in a memory clinic cohort using the Lumipulse automated platform

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Abstract

Background Plasma biomarkers for Alzheimer's disease (AD) are a promising tool for accessible and accurate biological diagnostics. However, data in clinical practice are needed to better understand their diagnostic and prognostic ability in memory unit patients.

Methods We analyzed plasma phosphorylated tau at threonine 217 (p-tau217) and neuroflament light chain (NfL) levels and AD cerebrospinal fluid (CSF) biomarkers in a group of 493 subjects using the Lumipulse G600II platform. The sample includes 340 patients from our memory unit (142 dementia, 186 mild cognitive impairment, and 12 with subjective complaints) and 153 cognitively unimpaired volunteers. We have correlated plasma and CSF biomarkers; we have analyzed plasma biomarker levels as a function of clinical diagnosis, cognitive status and amyloid status. We have also studied the ability of p-tau217 to discriminate between amyloid-positive and -negative subjects according to CSF using receiver operating characteristic curves.

Results Plasma p-tau217 correlated significantly with CSF Aβ42/Aβ40 (Rho = -0.75; *p*-value < 0.001), p-tau181 ($r = 0.66$; *p*-value < 0.001), and t-tau ($r = 0.59$; *p*-value < 0.001). Plasma NfL correlated with CSF NfL ($r = 0.48$; *p*-value < 0.001). By clinical diagnosis, plasma p-tau217 levels showed to be higher in AD patients than in healthy controls (difference = 0.63 pg/ml; *p*-value < 0.001), FTD (difference = 0.60 pg/ml; *p*-value < 0.001), and nondegenerative dementias (difference = 0.61 pg/ml; *p*-value < 0.001). Plasma p-tau217 showed an area under the curve of 0.95 to discriminate between A + and A- subjects (95%CI 0.93–0.97).

Conclusion Plasma p-tau217 shows excellent results for detecting amyloid pathology at brain level in a clinical setting with an AUC of 0.95. It is a highly specific marker of AD and increases progressively along the disease *continuum*. Using plasma p-tau217 as an initial diagnostic tool with cut-offs at sensitivities and specificities of 95 or 97.5% could save between 57.4–84.8% of LP/PETs with diagnostic accuracies of 95–97%. Plasma NfL increases progressively at different cognitive stages.

Keywords Plasma p-tau217, Alzheimer's disease, Early diagnosis, Cross-sectional, Healthy controls, Biomarkers

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Introduction

Alzheimer's disease (AD) has gone in a few years from being a clinical-pathologic defined condition for which there were no disease-modifying treatments to being a biological-defined entity with available drugs targeting its pathophysiology [1–4].

The first change of concept arrived with the development of cerebrospinal fluid (CSF) and molecular imaging biomarkers, positron emission tomography (PET), that made it possible to detect cerebral deposition of amyloid beta protein (Aβ) and phosphorylated tau (p-tau) in vivo [1]. These "core" biomarkers helped to better understand the evolution of the disease, to avoid inaccurate clinical diagnoses and to develop disease-modifying drugs.

Nonetheless, obtaining CSF samples and performing PET scans is expensive, invasive and not available in all centres. To address these challenges, plasma biomarkers have emerged as a promising alternative, providing a simpler, less invasive, and more affordable option for biological diagnosis [5]. Furthermore, they open up the possibility of repeated measurements to monitor the evolution of the disease, response to treatment, and to establish population screening strategies.

Currently, different plasma biomarkers can be measured such as the Aβ42/Aβ40 ratio, neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP) or the different forms of p-tau (p-tau181, p-tau217, p-tau231) [6–10]. Each of these biomarkers provides complementary information with its advantages and limitations. However, p-tau217 has been established as the most promising plasma biomarker for several reasons [11, 12]. It is a very specific AD biomarker that allows differentiating with great precision this pathology from other dementias [13–15] and its levels are related to cerebral deposition of both amyloid and p-tau [16, 17]. Moreover, it begins to rise very early, almost at the same time as the amyloid ratio is altered in CSF and even before amyloid PET reaches pathological threshold [8]. When using the p-tau217/Aβ42 ratio [18] it has also demonstrated high accuracy in detecting amyloid pathology at brain level, both by PET and CSF, with areas under the curve (AUCs) reaching 0.97.

Plasma p-tau217 has also demonstrated prognostic value, since different studies have related its levels to future cognitive impairment and brain atrophy in AD patients [19–21]. Moreover, its high fold-change implies that it is less affected than other biomarkers such as p-tau181, NfL or Aβ42/Aβ40 ratio by analytical variability [14, 22]. In this regard, papers studying the diagnostic performance of p-tau217 measured by different immunoassays have found great and comparable results between different methods in patients with mild cognitive impairment (MCI) due to AD, and also in cognitively unimpaired (CU) subjects [23–25].

Indeed, a recent work has assessed the diagnostic ability of plasma p-tau217 measured by the Lumipulse platform and ALZpath single molecular array and compared head-to-head their results in a clinical cohort including cognitively impaired patients and healthy controls. The results of both techniques were comparable, with AUCs of 0.95 for discriminating between AD and other degenerative pathologies, and 0.93 for differentiating between AD and healthy subjects [26]. Other head-to-head comparisons have found similar performances between Fujirebio's platform and blood tests already available or under development for clinical use [27].

For its part, plasma NfL is a nonspecific biomarker of axonal damage that is altered in different neurological pathologies such as AD, frontotemporal dementia (FTD), multiple sclerosis or stroke, but also increases with age, even in healthy individuals [28]. It is useful for differentiating healthy controls from patients with neurodegenerative diseases and its levels appear to be higher in FTD spectrum than in AD [29]. It has also been shown to increase progressively along the AD *continuum* and to correlate with brain atrophy and future cognitive impairment so it may be useful for monitoring disease progression or response to treatments [30].

Thus, plasma biomarkers have been already included in recent recommendations from the Global CEO Initiative on AD, but they still need to be put into clinical practice to prove their usefulness [31]. Another challenge before plasma biomarkers can be used in clinical practice is to know how different physiological factors and comorbidities influence their levels. Chronic diseases are highly prevalent in people over 65 years of age, and some such as chronic kidney disease, hypertension or diabetes, seem to affect the concentrations and diagnostic accuracy of plasma p-tau217 and p-tau217/Aβ42 along the AD *continuum* [32–34].

With our work we aim to provide information on the clinical utility of plasma p-tau217, p-tau217/Aβ42, and NfL routinely measured in patients from our memory unit. For comparison, we also provided information on other plasma biomarkers and studied the influence that different physiological variables and comorbidities have on their concentrations.

Biomarkers in this work have been measured using Fujirebio's Lumipulse G600II platform. This chemiluminescence-based platform, besides being fully automated and widely available in hospitals worldwide, has demonstrated excellent results measuring plasma biomarkers in both symptomatic and preclinical stages of AD [25, 35–37].

Material and methods

Participants

Our study involves 340 patients assessed at the memory unit of our hospital. The research was approved by the ethics committee of our institution (Internal code: 2023.404).

For comparison, plasma p-tau217 data from 153 CU volunteers from the 'Valdecilla Cohort for the study of memory and brain ageing' of the Memory Unit of the Marqués de Valdecilla University Hospital (Santander, Spain) were used. Both groups (patients and volunteers) were fully composed by Caucasian individuals. Details of recruitment and characteristics of CU volunteers can be found elsewhere [38], but, briefly, this is a prospective cohort of subjects over 55 years of age with no memory complaints. Participants responded to an open call in the media in our region and underwent a first comprehensive assessment in which demographic data and basic physiological variables are measured. They also underwent a neuropsychological evaluation, a cranial magnetic resonance imaging (MRI), fluorodeoxyglucose positron emission tomography (FDG-PET), and both blood and CSF samples are drawn to measure AD biomarkers. In this cohort we have available CSF Aβ40, Aβ42, *p*-tau181, t-tau and NfL. In plasma we have available Aβ40, Aβ42, p-tau181, and p-tau217. Further neuropsychological studies and blood draws are performed at annual follow-ups. Exclusion criteria for this cohort are an age < 55 years; a Clinical Dementia Rating [39] (CDR) score > 0; any contraindication to perform the complementary studies (e.g. anticoagulation); major systemic or psychiatric pathology; or any sensory impairment that hinders the performance of the neuropsychological evaluation.

Patients were taken from our memory unit. We selected all those patients assessed from January 2013 to December 2023 who routinely underwent LP as part of the diagnostic process, ensuring we have CSF biomarkers as a reference. Thus, we have included patients with cognitive complaints who came to our unit referred from general neurologists and from primary care. Although we have not excluded patients with advanced dementia, most of the participants in this group were in the stage of MCI or mild dementia at first evaluation. The only exclusion criteria was any contraindication to perform the LP (e.g., anticoagulation).

For patients, we have available CSF Aβ40, Aβ42, p-tau181, t-tau and NfL. In plasma Aβ40, Aβ42, p-tau181, p-tau217 and NfL were available in this group. As part of the routine evaluation, all patients were assessed by a neurologist with expertise in cognitive impairment. A complete anamnesis with demographic data collection, imaging tests (computed tomography or MRI as appropriate, and PET when necessary), and simultaneous CSF and plasma collection have been performed.

Sample collection and pre-analysis

We followed international recommendations for sample collection and storage [40, 41]. Plasma and CSF were obtained on the same day, with a time difference of less than 30 min and subjects fasting. CSF was collected in polypropylene tubes and centrifuged at room temperature (2000 g for 10 min). The resultant was aliquoted into 1 mL tubes (volumes of 500 µl) and frozen at −80 °C until its analysis in our hospital's immunology laboratory.

We followed the standardized operating procedure for plasma sample obtention [42]. Blood was stored in EDTA tubes and kept cold within the following three hours. Samples were then centrifuged (10 min at 1800 g). The supernatant was stored in polypropylene tubes and frozen at −80 °C until analyzed in our hospital's biochemistry laboratory.

Biomarker measurement

Our institution is part of the Alzheimer's Association Quality Control program, so we comply with the international recommendations for sample collection and storage [40, 41]. We have measured CSF biomarkers using the automated immunoassay analyzer Lumipulse G600II (Fujirebio Diagnostics, Malvern, PA, United States). We have used the following kits: Lumipulse G β-Amyloid 1–40 CSF; Lumipulse G β-Amyloid 1–42 CSF; Lumipulse G p-tau181 CSF; Lumipulse G t-tau CSF; and Lumipulse G NfL CSF [43].

To stablish CSF cut-offs, we have based our analysis on an unbiased Gaussian mixture modelling [44]. The model was built on a cohort of 578 subjects (258 CU from our volunteer cohort and 320 from our memory unit). With these cut-offs, participants were divided according to ATN classification [45]. They were considered as CSF Aβ-positive (A+) when Aβ42/Aβ40 ratio < 0.067; tau-positive (T+) when CSF p-tau181 > 55.0 pg/mL, and neurodegeneration-positive (N+) when CSF t-tau > 389 pg/mL. Throughout the paper, when Nx is stated, it includes both N- and N+ subjects.

Plasma Aβ40, Aβ42, p-tau181, p-tau217, and NfL values were also measured using Fujirebio's Lumipulse G600II with the following kits: Lumipulse G β-Amyloid 1–40 Plasma, Lumipulse G β-Amyloid 1–42 Plasma, Lumipulse G pTau 181 Plasma, Lumipulse G pTau 217 Plasma, and Lumipulse G NfL Blood. Lower limit of detection (LLD), intra- and inter-assay variations have been described previously [31, 36]. All our samples were above LLD.

ApoE status

Apolipoprotein E (*ApoE*) genotype was determined using TaqMan single nucleotide polymorphism genotyping assay (Applied Biosystems, Foster City, CA, United States). Subjects carrying ≥ 1 copy of the ε4 allele were considered ε4 + and the remaining ε4-.

Cognitive evaluation

Neuropsychological assessments are performed by two neuropsychologists specialized in cognitive disorders. They consist of a comprehensive battery of tests that address all cognitive domains. Mini-Mental State Examination (MMSE) [46] is used for assessing global cognitive functions, and global CDR score for evaluating cognition and functionality. We adjust scores of cognitive tests for age and educational level according to normative data from the NEURONORMA project since it provides normative data in Spanish population [47].

Detailed information on the cognitive domains studied and the tests used can be found in Supplementary Material 1.

Classification of participants

Subjects have been divided according to the ATN classification to have a biological reference criterion. We have also divided participants according to clinical diagnosis and cognitive status. To stratify the subjects according to their cognitive status we used the Global Deterioration Scale (GDS) [48] which consists of seven categories, with a score of 1 being the absence of cognitive impairment (normal individual) and 7 a very severe cognitive impairment. Subjects with GDS < 3 (no objective cognitive impairment) have been considered as CU; GDS = 3 (cognitive impairment with no impact on daily living activities) as MCI; and those with a GDS > 3 as patients with dementia.

Clinical diagnosis of each patient was made using established clinical criteria for AD, FTD, dementia with Lewy bodies (DLB), Creutzfeldt-Jakob disease (CJD), and vascular dementia [49–53] and using CSF biomarkers as support. Once the clinical diagnosis was established, for this study we considered AD and FTD as their own groups and, given the small sample size, we grouped patients with DLB and CJD under the name "Other degenerative". Patients with vascular cognitive impairment have been grouped with those with other type of non-degenerative pathology (e.g. psychiatric pathology, chronic adult hydrocephalus, epilepsy, chronic alcohol consumption, cognitive impairment following COVID-19 infection or polypharmacy) under the name "Non-degenerative".

Physiological variables and comorbidities

We have studied the influence of different variables on plasma biomarkers levels considering renal filtration rate and previous diagnosis of arterial hypertension (HT) and diabetes mellitus (DM). We have treated estimated glomerular filtration rate (eGFR) as a continuous variable. It was obtained through the Chronic Kidney Disease Epidemiology Collaboration formula [54] and expressed in mL/min/1.73m^2. Given that previous studies suggest that it is not punctual values of blood pressure or glycemia that influence plasma biomarker concentrations, but rather their chronic effect on the blood–brain barrier [55], participants were dichotomized according to their medical records into those who had previous history of HT and DM and those who had not. We considered a dichotomous variable called "Vascular Risk Factors" (VRF) which we considered positive if the patient had HT or DM and negative if he/she did not have either of the two risk factors. In patients we do not routinely collect weight and height, so body mass index was not available.

Statistical analysis

The distribution of variables was analyzed using Shapiro–Wilk test and then described by mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate. To comply with the assumption of normality, variables were log-10 transformed.

Correlations between both plasma p-tau217 and NfL and CSF biomarkers were performed using Pearson's correlation coefficient, except in the case of correlations involving CSF amyloid ratio, in which we used Spearman's Rho, since this is a state biomarker that has a non-linear relationship with the rest of the biomarkers. When outliers were detected on visual inspection, we have performed a sensitivity analysis removing values below Q1-1.5xIQR and above Q3 + 1.5xIQR to check the robustness of the tests.

To calculate differences between two groups (e.g. amyloid-positive vs. amyloid-negative) we used Student's t test. Differences in biomarker concentrations according to cognitive status, clinical diagnosis or ATN group were analyzed by ANOVA and when significant overall differences were found, Tukey's post-hoc test was performed. To study effect size, we have used Cohen's d; and when appropriate, we have used ANCOVA to adjust for other variables. $\chi 2$ test was used for categorical variables. Cases in with suspected comorbidity have been evaluated both from a clinical point of view considering their main diagnosis (clinical criteria supported by biomarkers) and from a purely biological point of view (CSF biomarkers).

Influence of physiological variables and comorbidities on plasma biomarkers was assessed through multiple linear regression models adjusted by age and sex that

included *ApoE* ε4 status, amyloid status, eGFR, and VRFs as predictors.

Ability of individual plasma biomarkers to detect CSF Alzheimer-type pathology (both A+and A+T+) was assessed through receiver operating characteristic (ROC) curves. We have calculated the areas under the curve (AUC) and computed the optimal cut-offs with their sensitivities and specificities by Youden index. Results of the best performing biomarkers have been compared through the DeLong test. To consider other variables we have also built ROC curves from logistic regression models in which we have taken amyloid status and AD status (considering AD+those subjects A+T+) as response and the different plasma biomarkers along with age, sex and *ApoE* ε4 status as predictors. Since plasma NfL is not considered a diagnostic biomarker, it has not been included in these analyses.

To test a two-cutoff approach suggested in other work [56], we selected logistic regression model taking plasma p-tau217 as predictor and CSF amyloid status as response. By bootstrapping we have chosen different cut-offs for sensitivities and specificities of 95 and 97.5% and then we stratified subjects into those with a high risk of being amyloid-positive, low risk and indeterminate groups. With these data we calculated proportion of false positive and false negative subjects, and overall accuracy.

Missing values have been handled by omission. All statistical analyses were performed with R studio software version 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria). *P*-values < 0.05 were considered as statistically significant.

Results

Sample description

The sample consists of 493 subjects with a mean age of 67.6 years (± 7.9). Women accounted for 50.1%; and 31.5% of the subjects were carriers of at least one *ApoE* ε4 allele. Our population included both CU subjects (33.5%) and those with cognitive impairment of different aetiologies, being AD the most frequent (41.1%), followed by non-degenerative pathology (13.8%), FTD (9.3%), and other degenerative dementias (2.2%). Of patients clinically diagnosed with AD ($n=203$), 118 were MCI and 85 were in the dementia phase. Of 46 patients diagnosed with FTD, 16 were MCI and 30 were in the dementia stage. 49 of 68 patients diagnosed with nondegenerative pathology were MCI and 19 were in the dementia phase. Finally, 11 patients were diagnosed with other degenerative pathologies, 3 were MCI and 8 were in dementia stage.

In the overall sample, 229 subjects (46.5%) were amyloid-positive according to CSF. Of the amyloid-positive subjects, 126 (55.0%) had MCI and 97 (42.4%) were in the dementia phase. The remaining 6 subjects (2.3%) were CU.

CU volunteers were significantly younger than patients (difference = 5.5 years; *p*-value < 0.001) and there was a higher proportion of women in volunteer group than in patient group (X-squared = 25.3; *p*-value < 0.001).

We have classified subjects according to their main clinical diagnosis, but it should be noted that some patients are likely to have copathology. Of subjects not diagnosed with AD, 25 were amyloid positive and, of those, 5 were also tau positive. Of these amyloid-positive patients 12 met FTD criteria (3 were A+T+), 8 with non-degenerative pathology (2 were A+T+), and 5 with other degenerative dementias. All other features are shown in Table 1.

Correlation between plasma p-tau217 and CSF biomarkers

When correlating plasma p-tau217 levels with CSF Aβ42/Aβ40 ratio in the overall sample we found a strong and significant correlation (Rho = −0.75 ; *p*-value < 0.001) (Fig. 1A). The correlation was also significant in both A- (Rho = −0.18 *p*-value = 0.003) and A+subjects (Rho = −0.31; *p*-value < 0.001). When we made this correlation in subjects clinically diagnosed with AD, the results were also significant (Rho = −0.22; *p*-value = 0.004) and remained significant in AD-MCI (Rho = −0.30; *p*-value = 0.002), but not in AD-dementia group (Rho = −0.04; *p*-value = 0.75). Correlation between plasma p-tau217 and CSF Aβ42/Aβ40 in subjects with dementias other than AD was also significant (Rho = −0.29; *p*-value < 0.001).

In the overall sample, plasma p-tau217 correlated significantly with CSF p-tau181 ($r=0.66$; *p*-value < 0.001) (Fig. 1B) and this correlation was also significant in AD patients ($r=0.39$; *p*-value < 0.001). In AD-MCI patients they correlated significantly ($r=0.41$; *p*-value < 0.001) and also in AD-dementia ($r=0.34$; *p*-value = 0.004). When stratified by amyloid status, the correlation was only significant in A+subjects ($r=0.43$; *p*-value < 0.001).

Plasma p-tau217 in the overall sample correlated significantly with CSF t-tau ($r=0.59$; *p*-value < 0.001) (Fig. 1C). They also correlated in A+subjects ($r=0.40$; *p*-value < 0.001), and in AD patients ($r=0.42$; *p*-value < 0.001). The correlation in AD patients remained significant after stratifying by AD-MCI ($r=0.44$; *p*-value < 0.001) and AD-dementia ($r=0.34$; *p*-value = 0.004).

Plasma p-tau217 was not significantly correlated with CSF NfL in either the overall sample ($r=-0.03$; *p*-value = 0.61) (Fig. 1D) neither in non-AD subjects ($r=-0.03$; *p*-value = 0.61). However, the correlation was weak but significant in AD patients ($r=0.25$; *p*-value < 0.001) and remained significant only in AD-MCI patients

Table 1 Sample description

Characteristic	Overall	CU volunteers	Patients
	***n*=493**	***n*=153**	***n*=340**
Females, n. (%)	247 (50.1%)	111 (72.5%)	136 (40.0%)
Age, mean (SD)	67.6 (7.9)	64.0 (6.3)	69.4 (8.2)
ApoE ε4 carrier, n. (%)	***n*=359** 113 (31.5%)	***n*=151** 29 (19.2%)	***n*=208** 84 (40.4%)
Cognitive Status, n. (%)			
Cognitively unimpaired	165 (33.5%)	153 (100%)	12 (3.5%)
Mild cognitive impairment	186 (37.7%)	-	186 (54.7%)
Dementia	142 (28.8%)	-	142 (41.8%)
GDS=4	88 (17.8%)	-	88 (25.9%)
GDS>4	54 (11.0%)	-	54 (15.9%)
Clinical Diagnosis, n. (%)			
Alzheimer's Disease	203 (41.1%)	-	203 (59.7%)
Frontotemporal dementia	46 (9.3%)	-	46 (13.5%)
Dementia with Lewy bodies	6 (1.2%)	-	6 (1.7%)
Creutzfeldt-Jakob disease	5 (1.0%)	-	5 (1.5%)
Non-degenerative pathology	68 (13.8%)	-	68 (20.0%)
Psychiatric	23 (4.7%)	-	23 (6.7%)
Vascular	12 (2.4%)	-	12 (3.5%)
Others	33 (6.7%)	-	33 (9.7%)
Alcohol-related	7 (1.4%)	-	7 (2.0%)
Post-COVID syndrome	5 (1.0%)	-	5 (1.5%)
Adult chronic hydrocephalus	2 (0.4%)	-	2 (0.6%)
Epilepsy	9 (1.8%)	-	9 (2.6%)
Polypharmacy	10 (2.0%)	-	10 (2.9%)
Cognitive complaints	12 (2.4%)	-	12 (3.5%)
Control	153 (31.0%)	153 (100%)	-
Physiological variables and comorbidities			
eGFR, median (IQR), mL/min/1.73m^2	92.3 (70.9-100.4)	93.7 (86.4-98.4)	90.0 (65.3-101.1)
HT, n. (yes %)	204 (41.4%)	49 (30.0%)	155 (45.6%)
DM, n. (yes %)	75 (15.2%)	12 (7.8%)	63 (19.2%)
CSF Biomarkers			
Ratio Aβ42/40, median (IQR)	0.076 (0.04-0.09)	0.089 (0.083-0.095)	0.050 (0.040-0.080)
T-tau, median (IQR), pg/mL	371.5 (264.2-590.7)	289.0 (228.0-356.0)	491.5 (305.7-764.3)
P-tau181, median (IQR), pg/mL	46.6 (32.4-93.1)	34.6 (28.2-40.3)	72.5 (39.0-121.0)
NfL, median (IQR), pg/mL	898.0 (649.5-1384.5)	-	898.0 (649.5-1384.5)
Plasma Biomarkers			
P-tau217, median (IQR), pg/mL	0.15 (0.09-0.51)	0.10 (0.08-0.12)	0.36 (0.12-0.78)
P-tau181, median (IQR), pg/mL	1.4 (1.01-2.20)	1.1 (0.87-1.23)	1.9 (1.18-2.79)
Aβ40, median (IQR), pg/mL	283.1 (250.2-314.9)	291.9 (260.7-317.2)	277.1 (244.5-313.4)
Aβ42, median (IQR), pg/mL	24.6 (21.3-27.8)	25.1 (22.1-27.3)	24.5 (20.7-28.3)
Ratio Aβ42/Aβ40, median (IQR)	0.085 (0.080-0.092)	0.085 (0.080-0.090)	0.086 (0.080-0.094)
NfL, median (IQR), pg/mL	23.6 (17.9-31.8)	-	23.4 (17.8-31.8)
ATN group, n. (%)			
A-T-N-	249 (50.5%)	142 (92.8%)	107 (31.4%)
A+T-N-	38 (7.7%)	0	38 (11.2%)
A+T+N-	8 (1.6%)	0	8 (2.4%)
A+T+N+	183 (37.1%)	0	183 (53.8%)
A-T+Nx	15 (3.1%)	11 (7.2%)	4 (1.2%)

Abbreviations: *n* number of subjects, *SD* standard deviation, *CSF* cerebrospinal fluid, *Aβ* amyloid beta, *IQR* interquartile range, *T-tau* total tau, *P-tau* phosphorylated tau, *NfL* neurofilament light chain, *A* amyloid, *T* tau, *N* neurodegeneration (when Nx is stated, it includes both N- and N+ subjects), *eGFR* estimated glomerular filtration rate, *HT* hypertension, *DM* diabetes mellitus, *GDS* global dementia scale rating, *CU* cognitively unimpaired

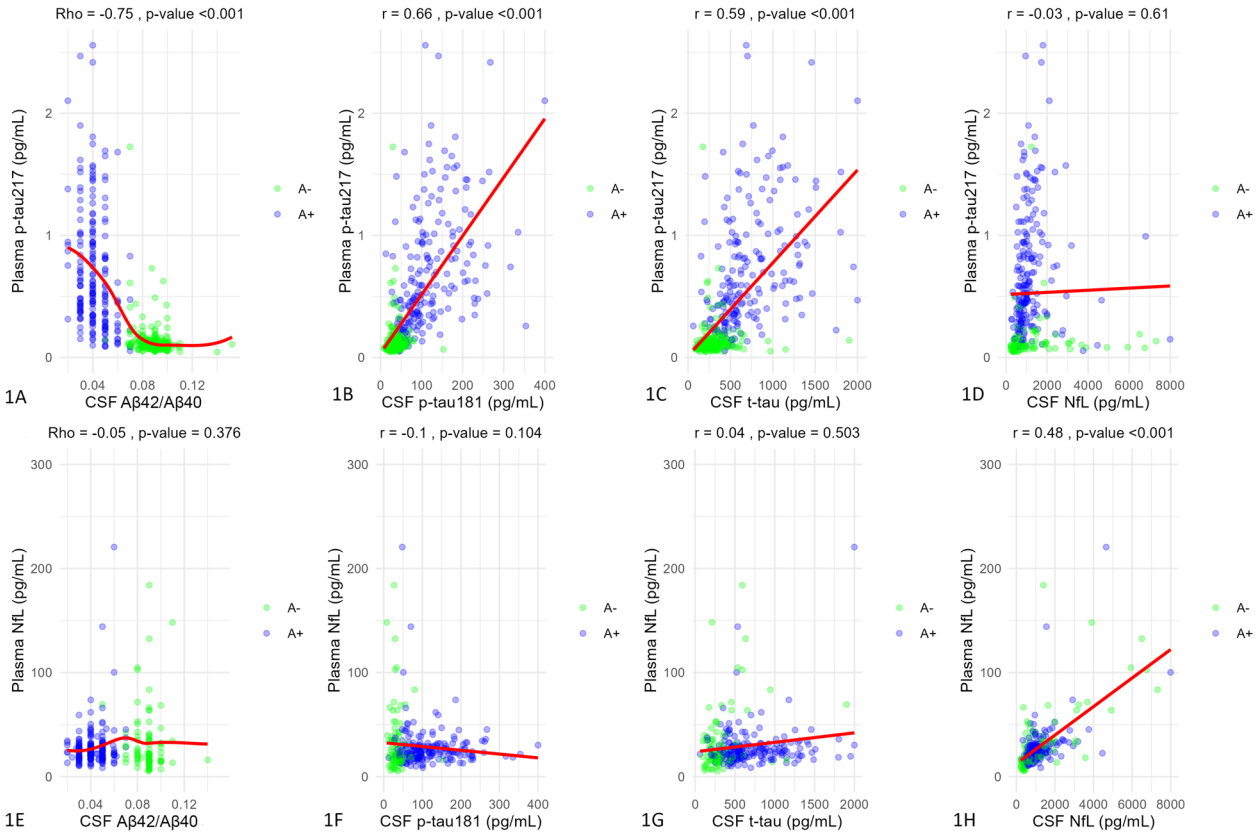

Fig. 1 Correlations between both plasma p-tau217 and NfL and CSF biomarkers. The plots show correlations between plasma p-tau217 (**A**-**D**) and NfL (**E**-**H**) and CSF biomarkers. CSF amyloid ratio correlations were measured by Spearman's Rho and the rest by Pearson's correlation coefficient. X-axes corresponds to CSF values (all except amyloid ratio expressed in pg/mL) and Y-axes to plasma values (expressed in pg/ml). Dots represent a pair of values of both variables for each observation. The green ones are those corresponding to amyloid-negative subjects and the blue ones represent amyloid-positive subjects. Red lines are regression lines. Above each plot are the correlation values and their statistical significance. Abbreviations: CSF, cerebrospinal fluid. P-tau, phosphorylated tau. T-tau, total tau. NfL, neurofilament light chain. R, Pearson's correlation coefficient. *p*-value, statistical significance. A, amyloid status

($r = 0.23$; p-value $= 0.002$). Correlations according to different clinical groups as well as correlations between plasma p-tau217/Aβ42 and CSF biomarkers can be found in Supplementary Material 2.

Correlation between plasma NfL and CSF biomarkers

Plasma NfL concentrations did not correlate significantly with CSF Aβ42/Aβ40 ratio in the overall sample (Rho $= -0.05$; p-value $= 0.37$) (Fig. 1E). We only found a significant correlation in non-AD subjects (Rho $= -0.22$; p-value $= 0.02$). The correlation between plasma NfL and CSF p-tau181 was not significant in the overall sample ($r = -0.09$; p-value $= 0.10$) (Fig. 1F); nor it was in AD ($r = 0.04$; p-value $= 0.63$) or in patients without AD ($r = -0.1$; p-value $= 0.1$).

CSF t-tau did not correlate significantly with plasma NfL in the overall sample ($r = 0.04$; p-value $= 0.50$) (Fig. 1G). However, it was significant in subjects without AD diagnosis ($r = 0.25$; p-value $= 0.01$). When splitting the sample by amyloid status we found a correlation in A + subjects ($r = 0.18$; p-value $= 0.01$). In the overall sample, the correlation between plasma and CSF NfL was significant ($r = 0.48$; p-value < 0.001) (Fig. 1H). In patients diagnosed with AD, the correlation was also significant ($r = 0.38$; p-value < 0.001), even after stratifying by AD-MCI ($r = 0.44$; p-value < 0.001) and AD-dementia ($r = 0.28$; p-value $= 0.03$). The same happened in non-AD patients ($r = 0.47$; p-value < 0.001). The correlation was significant in both A- ($r = 0.47$; p-value < 0.001) and A + ($r = 0.55$; p-value < 0.001).

Full correlations between plasma NfL and CSF biomarkers (both in the overall sample and in A- and A + subjects) before and after removing plasma NfL outliers, and description of the method used can be found in Supplementary Material 2.

Plasma p-tau217, p-tau217/Aβ42, and NfL values by clinical diagnosis

When comparing plasma p-tau217 levels according to clinical diagnosis, we found significant overall differences (p-value < 0.001) (Fig. 2A). Therefore, we performed a post-hoc analysis that showed significant differences between AD-MCI and AD-dementia patients (difference = 0.24 pg/ml; p-value < 0.001); AD-MCI and healthy controls (difference = 0.55 pg/ml; p-value < 0.001); AD-dementia and controls (difference = 0.79 pg/ml; p-value < 0.001); AD-MCI and FTD patients (difference = 0.51 pg/ml; p-value < 0.001); AD-dementia and FTD (difference = 0.76 pg/ml; p-value < 0.001); AD-MCI and non-degenerative pathology (difference = 0.52 pg/ml; p-value < 0.001); AD-dementia and non-degenerative pathology group (difference = 0.76 pg/ml; p-value < 0.001); and AD-dementia and other degenerative pathologies (difference = 0.41 pg/ml; p-value = 0.04).

Plasma p-tau217/Aβ42 ratio showed overall differences (p-value < 0.001) so we performed a post-hoc analysis. Main differences were found between both AD-MCI and AD-dementia patients and the rest of groups. Thus, AD-MCI patients showed significant differences with FTD patients (difference = 0.023; p-value < 0.001), non-degenerative group (difference = 0.024; p-value < 0.001), other degenerative dementias (difference = 0.019; p-value = 0.001), and with controls (difference = 0.024; p-value < 0.001) (Fig. 2B). AD-dementia patients showed significantly higher values of plasma p-tau217/Aβ42 than FTD patients (difference = 0.037; p-value < 0.001), non-degenerative group (difference = 0.038; p-value < 0.001), other degenerative dementias (difference = 0.028; p-value = 0.001), and with controls (difference = 0.038; p-value < 0.001) (Fig. 2B). The remaining comparisons of plasma p-tau217 and p-tau217/Aβ42 values, including those with overall AD group and after stratifying for AD-MCI and AD-dementia, with effect sizes and its confidence intervals can be found Table 2.

We analyzed differences in plasma NfL concentrations according to clinical diagnosis and found no overall differences (p-value = 0.051) so no post-hoc analysis was performed (Fig. 2C).

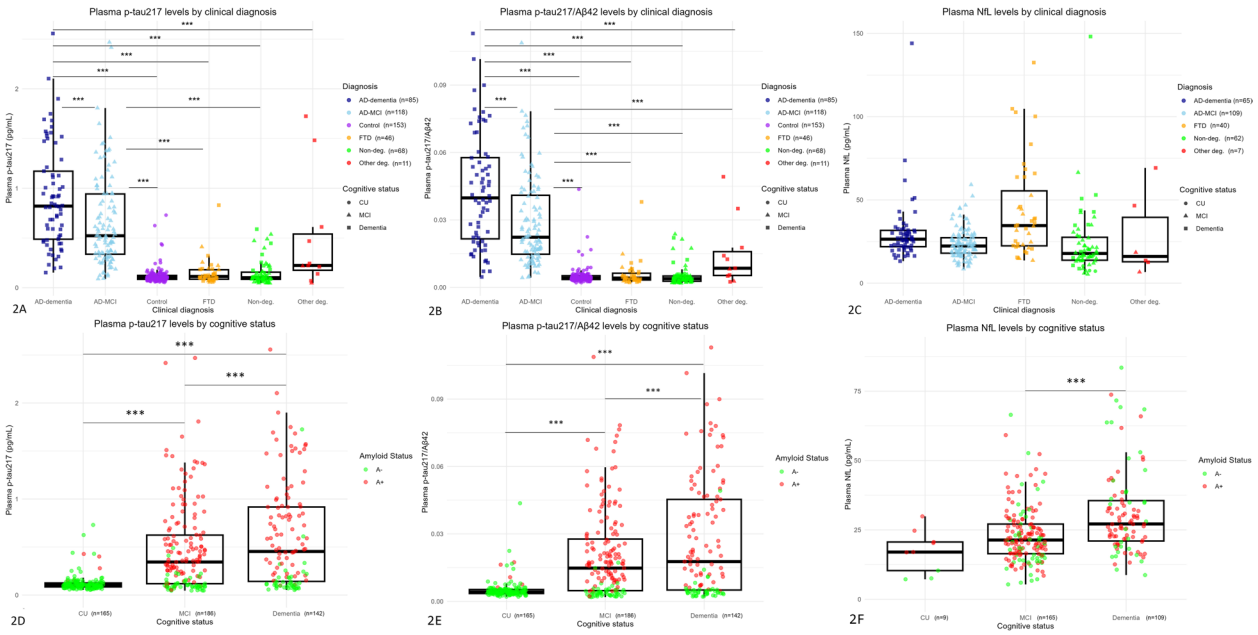

Fig. 2 Plasma p-tau217, p-tau217/Aβ42, and NfL values by clinical diagnosis and cognitive status. Figures show box and whiskers plots of plasma biomarkers by groups. In Figures **A**, **B**, and **C**, X axis represents the different clinical diagnostic groups (AD-MCI, AD-dementia, FTD, other degenerative pathology, non-degenerative pathology, and controls). In Figures **C**, **D**, and **E**, X axis represents different cognitive status (cognitively unimpaired, MCI and dementia). Y axis corresponds to plasma concentrations expressed in pg/ml. Boxes show the interquartile range (the upper boundary is the Q3, and the lower boundary is the Q1). The line inside the box corresponds to the median of the sample and the whiskers represent the maximum (upper) and minimum (lower) values. In first row individual values are shown in different colors and shapes according to clinical diagnosis and cognitive status. Dots represent CU subjects, triangles are MCI patients, and squares show patients with dementia. Dark blue color corresponds to patients with AD-dementia, light blue to AD-MCI patients, purple to controls, green to non-degenerative pathology, and red to other degenerative pathology. In second row, green dots correspond to amyloid-negative subjects and red ones to amyloid-positive subjects. Significant differences are represented with three asterisks between boxes. Healthy volunteers are not included in NfL analysis because plasma NfL was not available for this group. Abbreviations: AD, Alzheimer's Disease. FTD, frontotemporal dementia. P-tau, phosphorylated tau. NfL, neurofilament light chain. CU, cognitively unimpaired. MCI, mild cognitive impairment. A, amyloid group. n, number of participants

Plasma p-tau217, p-tau217/Aβ42, and NfL values by cognitive status

We initially correlated plasma p-tau217 levels with GDS scale, and the age-adjusted results were statistically significant ($r = 0.41$; p-value < 0.001).

When analyzing plasma p-tau217 levels as a function of cognitive status independent of clinical diagnosis, in the overall sample we found significant differences between CU and MCI subjects (difference = 0.35 pg/mL; p-value < 0.001), between CU and dementia subjects (difference = 0.49 pg/mL; p-value < 0.001); and between MCI and dementia patients (difference = 0.13 pg/mL; p-value = 0.01) (Fig. 2D). We also analyzed differences in plasma p-tau217 concentrations between patients with MCI and dementia due to clinically diagnosed AD, and they were significant (difference = 0.24 pg/mL; p-value = 0.002; Cohen's d = 0.4).

Plasma p-tau217/Aβ42 ratio also correlated significantly with GDS scale ($r = 0.39$; p-value < 0.001). In the overall sample, CU and MCI subjects showed significant differences (0.015; p-value < 0.001), and so did CU and dementia subjects (difference = 0.022; p-value < 0.001), and MCI and dementia groups (difference = 0.007; p-value = 0.003) (Fig. 2E). When analyzing differences in plasma p-tau217/Aβ42 ratio between MCI and patients with dementia due to AD, we found a significant difference of 0.014 (p-value < 0.001; Cohen's d = 0.63).

Plasma NfL levels correlated significantly with GDS scale values in the overall sample ($r = 0.26$; p-value < 0.001). When we analyzed differences by cognitive status, CU subjects did not show significantly different levels from those with MCI (difference = 6.04 pg/mL; p-value = 0.95). The difference was also no significant between CU and dementia groups (difference = 33.6 pg/mL; p-value = 0.21). However, the difference in plasma NfL concentrations was significant between MCI and dementia patients (difference = 27.5 pg/mL; p-value < 0.001) (Fig. 2F), even after adjusting for age (difference = 26.43 pg/mL; p-value < 0.001). We have also assessed differences in plasma NfL between subjects with MCI and dementia clinically diagnosed with AD and the results were significant (difference = 6.7 pg/mL; p-value = 0.002) even after adjusting for age (difference = 6.01 pg/mL; p-value = 0.01).

Plasma p-tau217, p-tau217/Aβ42 ratio, and NfL values by amyloid status

In the overall sample we analyzed the differences in plasma biomarker concentrations according to amyloid status as defined by CSF Aβ42/Aβ40. P-tau217

Table 2 Plasma p-tau217 and p-tau217/Aβ42 differences by clinical diagnosis

Comparison groups	Plasma p-tau217			Plasma p-tau217/Aβ42		
	Difference (pg/mL)	Adjusted *p*-value	Cohen's d, 95%CI	Difference	Adjusted *p*-value	Cohen's d, 95%CI
AD-MCI – AD-Dementia	0.24	**<0.001**	0.49 (0.18-0.80)	0.015	**<0.001**	0.63 (0.31-0.94)
AD - FTD	0.60	**<0.001**	1.32 (0.95-1.69)	0.028	**<0.001**	1.34 (0.97-1.71)
AD-MCI	0.51	**<0.001**	1.26 (0.87-1.66)	0.023	**<0.001**	1.33 (0.94-1.73)
AD-Dementia	0.76	**<0.001**	1.81 (1.35-2.27)	0.037	**<0.001**	1.82 (1.36-2.28)
AD - Non degenerative	0.61	**<0.001**	1.41 (1.09-1.71)	0.029	**<0.001**	1.45 (1.14-1.76)
AD-MCI	0.52	**<0.001**	1.38 (1.04-1.72)	0.024	**<0.001**	1.49 (1.15-1.84)
AD-Dementia	0.76	**<0.001**	2.02 (1.61-2.44)	0.038	**<0.001**	2.07 (1.65-2.49)
AD – Other degenerative	0.26	0.09	-	0.019	**0.001**	0.86 (0.24-1.47)
AD-MCI	0.17	0.35	-	0.014	**0.01**	0.73 (0.10-1.36)
AD-Dementia	0.41	**0.04**	0.79 (0.14-1.44)	0.028	**<0.001**	1.17 (0.50-1.84)
AD – Control	0.63	**<0.001**	1.71 (1.45-1.96)	0.029	**<0.001**	1.69 (1.44-1.95)
AD-MCI	0.55	**<0.001**	1.78 (1.48-2.10)	0.024	**<0.001**	1.83 (1.53-2.12)
AD-Dementia	0.79	**<0.001**	2.68 (2.30-3.05)	0.038	**<0.001**	2.63 (2.25-3.01)
FTD - Non degenerative	0.004	0.99	-	<0.001	0.99	-
FTD - Other degenerative	0.34	**0.02**	1.20 (0.48-1.93)	0.008	0.43	-
FTD - Control	0.03	0.98	-	0.001	0.99	-
Non degenerative - Other degenerative	0.34	**0.01**	1.46 (0.77-2.14)	0.009	0.32	-
Non degenerative - Control	0.02	0.97	-	<0.001	0.99	-
Other degenerative - Control	0.37	**0.003**	2.27 (1.61-2.94)	0.009	0.24	-

The table shows mean differences of plasma p-tau217 and p-tau217/Aβ42 between different diagnostic groups, statistical significance and size of effect. Significant results are highlighted in bold

Abbreviations: *AD* Alzheimer's Disease, *MCI* mild cognitive impairment, *FTD* frontotemporal dementia. *P*-value statistical significance, *CI* confidence interval

showed significantly higher levels in A + subjects than in A- (difference = 0.58 pg/mL; *p*-value < 0.001) and the same was true for p-tau217/Aβ42 (difference = 0.027; *p*-value < 0.001). However, we found no significant difference in plasma NfL levels (difference = 17.7 pg/mL; *p*-value = 0.09). Next, we analyzed differences in plasma biomarker concentrations according to amyloid status within each cognitive group. Thus, we compared the concentrations of these biomarkers in subjects with MCI presumably due to AD-type pathology (A +) versus those with MCI of other origin (A-) and did the same in subjects with dementia. Plasma p-tau217 values were significantly different between A- and A + groups in MCI patients (difference = 0.53 pg/mL; *p*-value < 0.001; Cohen's d = 1.31). The same happened when comparing p-tau217 values between subjects with dementia due to AD compared to those with dementia due to other pathologies (difference = 0.65 pg/mL; *p*-value < 0.001; Cohen's d = 1.41) (Fig. 2D).

Plasma p-tau217/Aβ42 values were significantly different between A- and A + groups in MCI patients (difference = 0.023; *p*-value < 0.001; Cohen's d = 1.40) and in patients with dementia (difference = 0.033; *p*-value < 0.001; Cohen's d = 1.57) (Fig. 2E).

Regarding plasma NfL concentrations, in subjects with MCI there were no significant differences between A- and A + groups (difference = 2.7 pg/mL; *p*-value = 0.13). However, in dementia patients we found significant differences between A- and A + groups (difference = 45.7 pg/mL; *p*-value = 0.01) (Fig. 2F).

Influence of physiological variables and comorbidities on plasma p-tau217 and p-tau217/ Aβ42 ratio

We have performed multiple regression models to study how eGFR, HT and DM (VRF) affect plasma p-tau217 values. We have adjusted by other relevant variables such as *ApoE* ε4 status, amyloid status, age and sex. In the overall sample, p-tau217 showed to be influenced by eGFR (standarized β = −0.08; *p*-value = 0.005), female sex (standarized β = −0.16; *p*-value = 0.03), and amyloid positivity (standarized β = 0.58; *p*-value < 0.001). This model had an adjusted R-squared of 0.50.

Plasma p-tau217/Aβ42 ratio was only affected by amyloid positivity with a standarized β of 0.02 (*p*-value < 0.001). This model had an Adjusted R-squared of 0.51.

Regarding p-tau181, its plasma levels were influenced by eGFR (standarized β = −0.27; *p*-value < 0.001), female sex (standarized β = −0.21; *p*-value < 0.001) and amyloid positivity (standarized β = 0.56; *p*-value < 0.001). The main factors influencing plasma Aβ42/Aβ40 levels were CSF amyloid positivity (standarized β = −0.63; *p*-value < 0.001) and ApoE4 status (standarized β = −0.12; *p*-value = 0.01).

Plasma NfL showed to be significantly influenced by eGFR (standarized β = −0.33; *p*-value = 0.01) and female sex (standarized β = −0.36; *p*-value = 0.003). Influence of the remaining factors studied on plasma biomarkers can be found in Supplementary Material 3.

Ability of plasma biomarkers to detect amyloid and AD (A + T +) pathology

Then we tested the ability of single plasma biomarkers to detect the biological signature of AD in CSF. Thus, we have studied their ability to detect amyloid pathology (differentiate between A- and A + subjects) and biologically defined Alzheimer pathology (here we differentiate between AD- and AD + subjects being the AD + those A + T +) including no covariates. In the overall sample, plasma p-tau217 showed an AUC of 0.95 to discriminate between A + and A- subjects (95%CI 0.93–0.97) with an optimal cut-off of 0.18 pg/ml (sensitivity = 0.92; specificity = 0.91). To discriminate between AD + and AD- subjects the AUC was 0.95 (95%CI 0.93–0.97) with an optimal cutoff point of 0.25 pg/ml (sensitivity = 0.90; specificity = 0.92).

For comparison, we have also evaluated the diagnostic performance of other plasma biomarkers such as p-tau181, Aβ42/Aβ40 ratio and p-tau217/Aβ42 ratio. P-tau181 discriminated between A + and A- subjects with an AUC of 0.90 (95%CI 0.87–0.93) with an optimal cutoff point of 1.37 pg/ml (sensitivity = 0.87; specificity = 0.80). To differentiate between AD + and AD- subjects the AUC was 0.90 (95%CI 0.87–0.93) with an optimal cutoff point at 1.54 pg/ml (sensitivity = 0.87; specificity = 0.83).

Aβ42/Aβ40 ratio showed an AUC of 0.72 (95%CI 0.67–0.77) to discriminate between A + and A- subjects with an optimal cut-off of 0.08 (sensitivity = 0.86; specificity = 0.56). To differentiate between AD + and AD- subjects, the AUC was 0.71 (95%CI 0.67–0.76) with an optimal cut-off placed in 0.08 (sensitivity = 0.88; specificity = 0.53).

Finally, plasma p-tau217/Aβ42 ratio differentiated between A + and A- subjects with an AUC of 0.97 (95%CI 0.95–0.98) with an optimal cut-off in 0.008 (sensitivity = 0.91; specificity = 0.94). For discriminating between AD + and AD- subjects, AUC was 0.96 (95%CI 0.95–0.98) with an optimal cut-off of 0.009 (sensitivity = 0.94; specificity = 0.92). AUC of p-tau217/Aβ42 ratio was significantly higher than that of p-tau217 alone (Z = −3.46; *p*-value < 0.001).

All ROC curves for both CSF amyloid and AD pathology can be seen in Fig. 3A and B, respectively. Results of logistic regression models including age, sex, and *ApoE*

ε4 status as predictors, and full information on single biomarkers can be found in Supplementary Material 4. In addition, in an exploratory manner, we have analyzed the diagnostic ability of other combinations of biomarkers. The complete information can be found in Supplementary Table 5.

Two-cutoff approach in our population

To test the practical application of plasma p-tau217 in our population to detect CSF amyloid pathology we used a two-cutoff approach. We performed a logistic regression model taking plasma p-tau217 values as predictor and CSF amyloid status as response and then, we built a ROC curve. We have adjusted sensitivities and specificities to 95% and 97.5% and analyzed how many subjects were above ("high risk"), below ("low risk") and between the thresholds. Subjects classified as high and low risk would be considered as A + and A- respectively, based on plasma p-tau values alone. Subjects in the intermediate zone would be candidates for confirmatory testing (LP or PET) or a new determination during follow-up.

With sensitivity and specificity at 95% (p-tau217 cut-offs 0.133 and 0.252 pg/mL, respectively), global accuracy was 95% and 15.2% of subjects fell into the intermediate-risk group. With these thresholds, 3.4% of the total sample were false positive (FP) and 1.4% false negative (FN) (Fig. 4A). Placing sensitivity and specificity at 97.5% (p-tau217 cut-offs 0.099 and 0.433 pg/mL, respectively) global accuracy reached 97%, with more participants in the intermediate-risk group (42.6%). In this case, 1.2% were FP and 1.0% FN (Fig. 4B).

Although our objective was to study the diagnostic ability of plasma p-tau217 alone, since p-tau217/Aβ42 ratio showed a significantly higher AUC than p-tau217 to detect CSF amyloid pathology, we have also tested this two-cutoff approach with p-tau217/Aβ42 ratio. With sensitivity and specificity at 95% (cut-offs 0.006 and 0.008, respectively), global accuracy was 91%, and only 4.7% of subjects were classified as intermediate risk. With this approach there was a 3.4% FP rate and 2.0% were FN (Fig. 4C). In comparison, with sensitivity and specificity at 97.5% (cut-offs 0.004 and 0.014, respectively) global accuracy was 97%, but intermediate group included 27.1% of participants. FP and FN accounted for 1.2% and 1.0%, respectively (Fig. 4D). Full information on these two-cutoff approach is shown in Supplementary Table 6.

Discussion

Currently, plasma biomarkers are only allowed in research settings [30, 57]. However, it is essential to obtain data on their usefulness in memory clinics to scale their use to this context or even to primary care [58]. In daily practice, patients present with a wide range of phenotypes and tools are required to make a biological

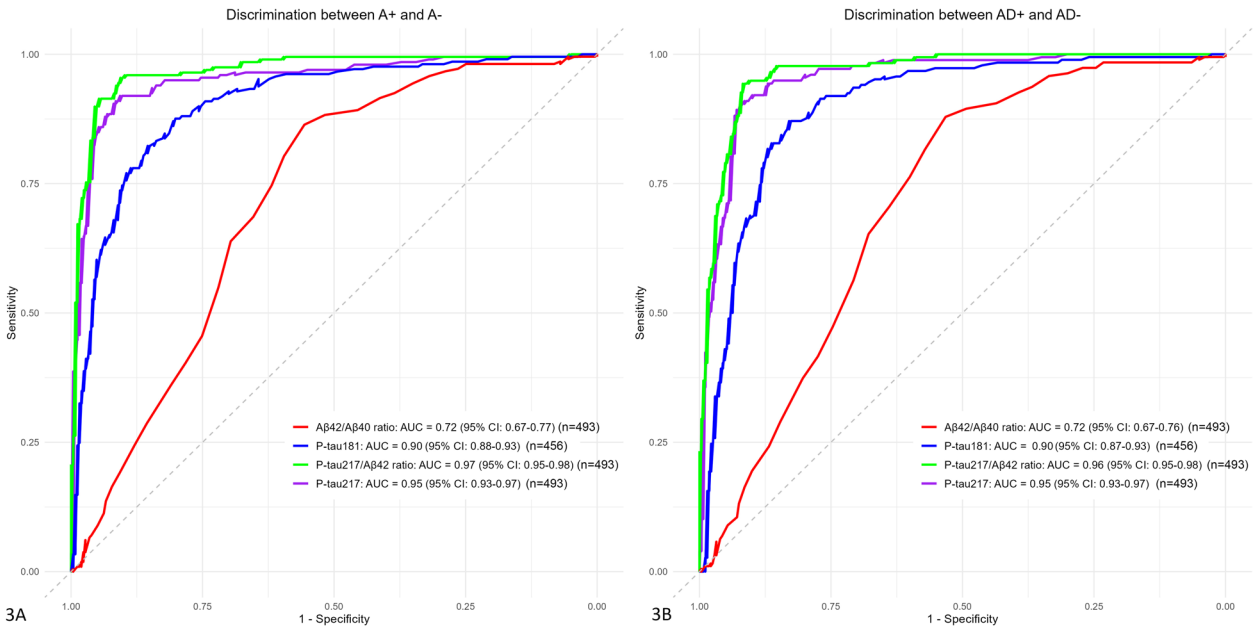

Fig. 3 Ability of single plasma biomarkers to detect CSF amyloid pathology. ROC curves showing the ability of single plasma biomarkers to detect CSF pathology. **A** corresponds to amyloid pathology and **B** to **A,D** pathology (A+ plus T+). X axis shows 1-specificity, and Y axis corresponds to sensitivity. Red curve corresponds to Aβ42/Aβ40 ratio; the blue one corresponds to p-tau181; green one to p-tau217/Aβ42 ratio; and purple one to p-tau217. Abbreviations: ROC, receiver operating characteristic. A, amyloid. AUC, area under the curve. P-tau, phosphorylated tau. CI, confidence interval. n, number of participants

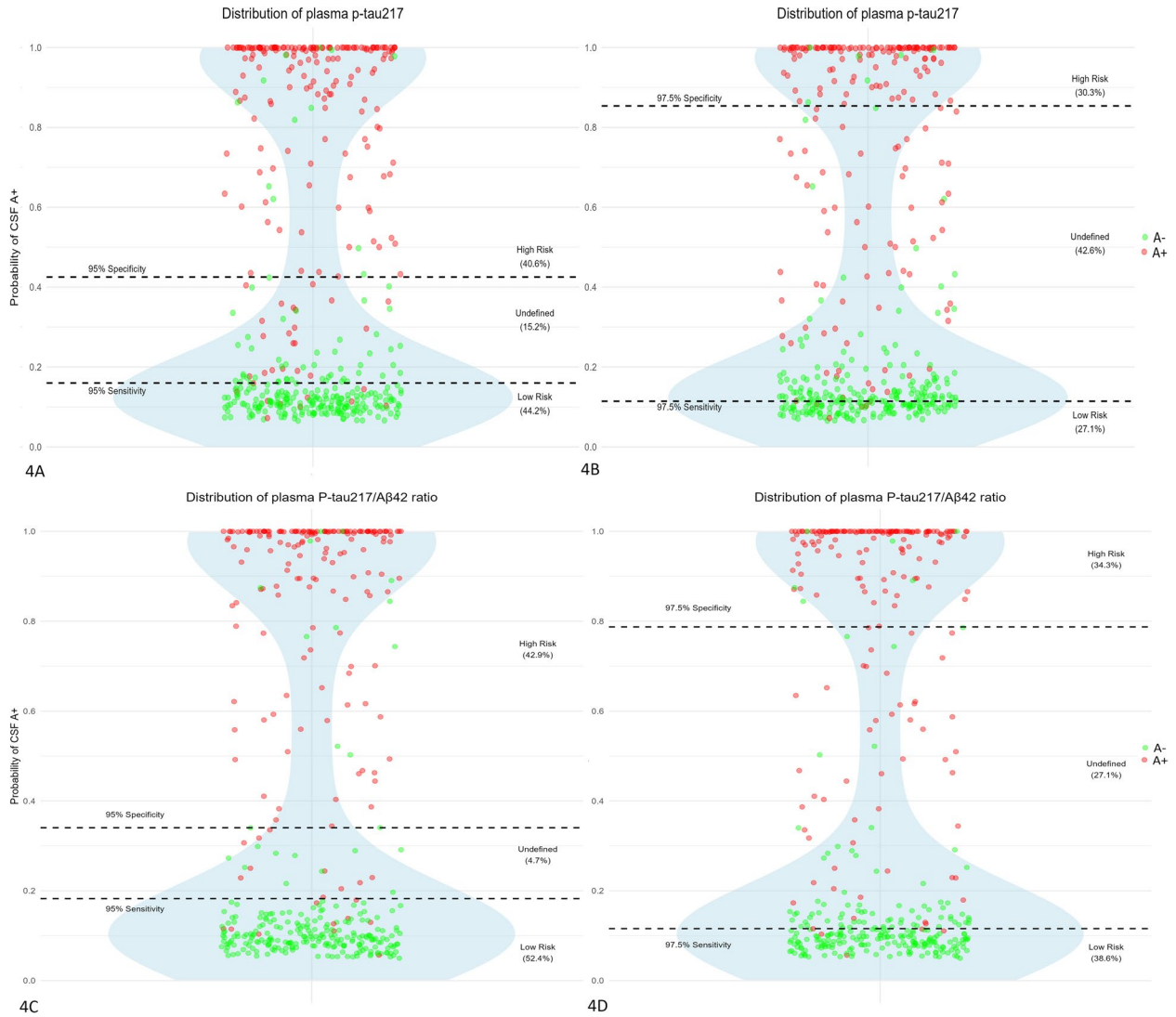

Fig. 4 Two-cutoff approach in our sample. The results are shown in violin plots. Y axis represents the probability of being considered amyloid positive according to a logistic regression model in which amyloid status was taken as the response and plasma p-tau217 (Figures **A** and **B**) or plasma p-tau217/Aβ42 ratio (Figures **C** and **D**) values as predictive variable. Blue shaded area represents the concentration of observations at each probability. Dots are the individual values of plasma p-tau217 and p-tau217/Aβ42 ratio. Those subjects considered as amyloid-positive according to CSF are red dots and the amyloid-negative are the green ones. Black dashed lines represent the cut-off of the different specificities and sensitivities (95% in Figures **A** and **C**, 97.5% in Figure **B** and **D**). Values above the specificity line are considered "high risk", those below the sensitivity line are "low risk" and values between the lines are subjects classified as undefined. Abbreviations: p-tau, phosphorylated tau. Aβ, amyloid beta. CSF, cerebrospinal fluid. A, amyloid status

diagnosis [59]. This is of particular importance now that the first AD-modifying drugs are being approved [60].

In our cross-sectional study focused on amyloid pathology we have seen that plasma p-tau217 correlates consistently with CSF AD biomarkers. Moreover, its levels are significantly higher in AD patients than in healthy controls and patients with other diseases. Plasma p-tau217 is, therefore, a highly specific marker of AD, as previous works have described [14, 61]. It should be noted that, although no significant differences were found between AD patients and the group of other neurodegenerative pathologies, this may be due to the small sample size of the latter group and because some of the patients with other neurodegenerative diseases showed CSF AD copathology. Such presence of copathology should lead us to cautiously interpret the phenotypes of patients, since co-pathology, far from being anecdotical, is quite common [62]. In cases where copathology is

suspected, "Biomarkers of non-AD copathology" recently proposed in the revised diagnostic criteria for AD can be useful for understanding patients as a whole [59].

Plasma NfL, however, did not show a significant correlation with CSF Aβ, p-tau181 or t-tau levels, but did correlate moderately with CSF NfL, suggesting that, although there is a peripheral component, brain-derived NfL influences plasma levels [63]. Plasma NfL did not show different concentrations by clinical diagnosis but presented progressively higher values in the different cognitive stages as reported previously, reinforcing the idea that its main usefulness lies in its prognostic value [29].

When comparing plasma p-tau217 and NfL according to amyloid status, as expected, p-tau217 showed higher levels in both MCI and dementia in A + patients compared to A- patients. However, NfL presented significantly higher levels in A- dementia patients. The most likely explanation is that the higher levels correspond to FTD patients, who have so far shown overall higher concentrations of plasma NfL.

Plasma p-tau217 has shown excellent results in detecting CSF amyloid and AD (A + T +) pathology with AUCs of 0.97 and 0.94, respectively. These results are similar to those previously reported [37, 64], and place p-tau217 as the most accurate single marker for detecting brain amyloid pathology as it has consistently shown better results than the other plasma biomarkers [11, 36], and comparable results than those of CSF biomarkers for detecting tau and amyloid PET positivity [15, 65]. In addition, it has also shown high diagnostic accuracy in preclinical cohorts for detecting CSF A + subjects with an AUC of 0.85 [25].

By testing the previously proposed two-cutoff approach [56] we have seen that, depending on the selected thresholds, between 57.4–84.8% of subjects for plasma p-tau217, and 72.9–95.3% for plasma p-tau217/Aβ42 ratio fell outside the indeterminate zone, with overall accuracies > 90%, similar to those described in literature [15, 56]. However, in clinical practice, patients in the intermediate zone would still generate diagnostic doubts and would be candidates for confirmatory tests such as LP or PET or a new determination during follow-up. When sensibility and specificity thresholds were increased, more subjects fell into the indeterminate category, thus reducing the number of misclassifications. This may prevent classification errors but could not be useful from a clinical point of view if too many subjects are not classified. Therefore, the thresholds should be adapted to the scenario in which they are going to be used [31]. We have made the approximation with cutoff points at 95% and 97.5% because they have shown the best overall accuracies [15, 56]. However, in memory units it may be necessary to adopt stricter approaches with sensitivities and specificities of 97.5% as it reduces the number of false positives (1.2 vs. 3.4%) and false negatives (1.0 vs. 1.4%), thus avoiding misdiagnosis and mistreatment.

Plasma p-tau217/Aβ42 ratio showed advantages over p-tau217 in terms of potential clinical application. Overall accuracy values were not better, but it did show a lower percentage of unclassified subjects, although this numerical difference was not tested for significance. We have also seen that the ratio is less influenced by physiological factors such as glomerular filtration rate. In our case, eGFR showed a significant influence on plasma values of p-tau217, but not on those of p-tau217/Aβ42. However, as in other studies, its influence was much less than that of CSF amyloid status [15]. This is consistent with previous work, which has shown that the diagnostic accuracy of p-tau217/Aβ42 is less altered than that of p-tau217 by factors such as eGFR or DM [66].

Some limitations of our study should be pointed out. First, this is a cross-sectional study that would require longitudinal follow-up and repeated measurements plasma biomarkers to better understand their dynamics. This longitudinal approach with repeated measures can provide information on the evolution of plasma biomarkers in subjects who go from being A- to A + and their relationship with cognitive impairment or functional scales (e.g. GDS scale). This longitudinal approach is also of special interest in the group of A + CU subjects who are at higher risk of progressing to MCI than general population. In this way, conclusions could be drawn at the individual level and not only at the group level. On the other hand, although we have tried to approximate daily clinical practice, all analyses have been performed with the same kits, thus reducing the analytical variability that will be present in real life when samples are analyzed with different kits as they are collected [67, 68]. When plasma biomarkers can be used in clinical practice, strategies that reduce analytical variability, such as standardization of sample collection, handling and storage, should be employed [42, 69]. In this sense, since Lumipulse platform is fully automated and available in many hospitals worldwide, it allows a homogeneous integration into daily practice with minimal human intervention. However, platforms such as these need to be available in low-income countries where AD is an under-diagnosed disease, and its prevalence is expected to increase [70].

It should also be noted that our clinical population is fully composed by Caucasian subjects, and it is biased towards cases where CSF biomarkers are more useful such as atypical cases, young patients, or patients with MCI, thus limiting the generalizability of the results. Moreover, there are subgroups with very small sample sizes such as other neurodegenerative dementias or FTD that do not allow firm conclusions to be drawn. The

low proportion of patients with DLB is surprising. This is likely because, clinically, they offer fewer diagnostic doubts, they are usually older and other tests such as DaTSCAN are performed, so fewer lumbar punctures are employed. To adequately address these pathologies, strategies involving collaborative multicenter studies or targeted recruitments would be of interest. It is also noteworthy the overrepresentation of less common pathologies such as CJD, because they undergo routine LP. Finally, to better assess plasma NfL, series of patients with a greater representation of advanced stages and more FTD patients, which is where it is most altered, are needed.

In conclusion, in our memory clinic-based cohort, plasma p-tau217 shows excellent results for detecting amyloid pathology at brain level and its ratio with Aβ42 reduced the misclassification rate. Moreover, it is a highly specific marker of AD and increases progressively along the disease *continuum*. Plasma NfL is a marker of neurodegeneration that increases progressively at different cognitive stages. Our work reinforces the growing evidence that plasma biomarkers are a useful tool for biological diagnosis. Their easy accessibility will reduce costs in the diagnostic process, and they can help to select candidates for disease-modifying treatments, to confirm diagnostic doubts in memory units, or even to serve as a screening tool. However, they must be interpreted according to the clinical context [71].

Abbreviations

A+/-	Amyloid positive/negative
Aβ	Amyloid-β
AD	Alzheimer's disease
ApoE	Apolipoprotein E
AUC	Area under the curve
CDR	Clinical dementia rating
CJD	Creutzfeldt-Jakob disease
CSF	Cerebrospinal fluid
CU	Cognitively unimpaired
DLB	Dementia with Lewy bodies
DM	Diabetes mellitus
eGFR	Estimated glomerular filtration rate
FDG-PET	Fluorodeoxyglucose positron emission tomography
FN	False negative
FP	False positive
FTD	Frontotemporal dementia
GDS	Global deterioration scale
GFAP	Glial fibrillary acidic protein
HT	Hypertension
IQR	Interquartile range
LLD	Lower limit of detection
LP	Lumbar puncture
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
N+/-	Neurodegeneration positive/negative
NfL	Neurofilament light chain
NPS	Neuropsychology
P-tau	Phosphorylated tau
PET	Positron emission tomography
ROC	Receiver operating characteristic
SD	Standard deviation
T+/-	Tau positive/negative
T-tau	Total tau
VRF	Vascular Risk Factors

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-025-01719-5.

Supplementary Material 1.

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Authors' contributions
FMD and ERR designed the study, analyzed the data, and drafted the manuscript. SLG, CLM, MFM, ANC, AVB, MRS, APC, MGM, ACP, MB, EML, MDP and PSJ performed clinical and neuropsychological assessment, obtained biological samples, and reviewed the manuscript. MLH and JIV performed CSF analysis and reviewed the manuscript. AGR, NHCS and MTGU performed the analysis of plasma markers, drafted the manuscript, and reviewed the final paper.

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

Declarations

Ethics approval and consent to participate
This research has been conducted in accordance with the Declaration of Helsinki and has been approved by the ethics committee of the Hospital Universitario Marqués de Valdecilla. Title: Valdecilla Cohort for the study of memory and brain aging. Internal code: 2018.111.
All subjects have given their signed consent to participate.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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