A plasma biomarker panel for detecting early amyloid-β accumulation and its changes in middle-aged cognitively unimpaired individuals at risk for Alzheimer's disease

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Summary

Background **Plasma biomarkers of Alzheimer's disease (AD) change during preclinical stages, indicating potential for detecting amyloid-β (Aβ) pathology in cognitively unimpaired (CU) individuals. Given the need for accurate, scalable biomarkers, we evaluated a fully automated plasma panel to detect and monitor longitudinal Aβ accumulation in CU individuals.**

Methods **In this longitudinal study, we examined a plasma panel (Aβ42/40, p-tau181, GFAP, NfL, p-tau217 and ApoE4) in CU participants at risk for AD. We assessed the biomarkers' performance to detect Aβ pathology and the cross-sectional and longitudinal relationships between the biomarkers and Aβ accumulation, neurodegeneration and cognition.**

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Findings We included 400 middle-aged CU participants, of whom 135 (33.8%) were CSF Aβ-positive. All plasma biomarkers differed between Aβ-positive and -negative individuals, with plasma Aβ42/40, p-tau217, p-tau181/Aβ42, and p-tau217/Aβ42 showing the best performance in detecting A+ CU individuals. However, plasma Aβ42/40 was sensitive to random variability. Plasma p-tau217/Aβ42 had the highest performance in detecting PET A+ individuals (AUC = 0.94). All baseline plasma biomarkers were associated with longitudinal increases in Aβ deposition (mean follow-up [SD]: 3.27 ± 0.5). Longitudinal changes in plasma p-tau217 and p-tau217/Aβ42 were associated with concurrent changes in Aβ (both CSF and PET) and soluble tau pathology.

Interpretation In CU individuals, several plasma biomarkers at baseline detect Aβ accumulation and are associated with its short-term change. Plasma p-tau217, and p-tau217/Aβ42 longitudinal changes reflect concurrent Aβ accumulation during this period. These findings help enrich studies in CU individuals at risk of progressing to AD.

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Keywords: Alzheimer; Biomarkers; Dementia; Diagnosis; Plasma; Modelling

Research in context

Evidence before this study

We conducted a comprehensive PubMed search and reviewed the literature on blood-based biomarkers in preclinical Alzheimer's disease (AD). The existing evidence indicates significant changes in these biomarkers during the preclinical stage of AD. However, previous studies including fully automated immunoassays often lack longitudinal data, especially those comparing baseline levels or changes in blood-based biomarkers with amyloid-β (Aβ) deposition. To address these knowledge gaps, our study focuses on investigating blood-based biomarkers using fully automated assays in cognitively unimpaired (CU) individuals at risk of AD and assessing the changes in these biomarkers in relation to longitudinal changes in Aβ pathology.

Added value of this study

Our study employs a fully automated panel of assays to measure blood-based biomarkers in CU individuals at risk of AD, revealing that these biomarkers provide distinct information in the preclinical stage of the disease. A key contribution of this study is the measurement of a broad range of plasma biomarkers (Aβ42, Aβ40, p-tau181, GFAP, NfL, p-tau217 and ApoE4), along with the ratios Aβ42/40, p-tau181/Aβ42, and p-tau217/Aβ42. These ratios performed best in distinguishing CU A+ from A− individuals, with plasma p-tau217/Aβ42 emerging as the most reliable biomarker for detecting Aβ pathology, particularly as defined by PET (AUC = 0.94). An additional strength is the examination of comorbidities (e.g., BMI and renal function) and the robustness of biomarker performance, incorporating simulated measurement variability to assess Aβ pathology discrimination performance. Plasma Aβ42/40 and p-tau217/Aβ42 were unaffected by renal function, and all biomarkers, except Aβ42/40, showed strong robustness under varying conditions. A novel aspect of our study is the association of all baseline plasma biomarkers with longitudinal increases in Aβ deposition over a relatively short timeframe of three years. Notably, longitudinal changes in plasma p-tau217 and p-tau217/Aβ42 were linked to concurrent changes in both Aβ (CSF and PET) and soluble tau pathology.

Implications of all the available evidence

Fully automated immunoassay-based blood biomarkers offer significant diagnostic, prognostic, and monitoring capabilities for CU individuals at risk of AD even in a relatively short time span. Their application can facilitate the early detection of Aβ-positive individuals and those at higher risk of accumulating Aβ pathology and experiencing subsequent neurodegeneration. This will aid in the execution of observational and interventional studies in the early preclinical population, contributing to better prevention and treatment strategies for AD.

Introduction

The preclinical stage of Alzheimer's disease (AD) is defined as the period when amyloid-β (Aβ) and phosphorylated tau accumulation begin to occur, but cognitive impairment is not yet evident.[1] Accurate detection of this stage *in vivo*, in the absence of post-mortem neuropathological examination, relies on core cerebrospinal fluid (CSF) and positron emission tomography (PET) biomarkers. However, the high costs, low accessibility and perceived invasiveness associated with these methods are significant obstacles, especially in the early phase, potentially hindering study

enrolment. Blood-based biomarkers present a minimally invasive and convenient alternative for detecting and monitoring the disease in its early stages.

The most promising blood-based biomarkers for AD are Aβ42/40 and phosphorylated tau (p-tau181, p-tau217, and p-tau231).[1–9] Glial fibrillary acidic protein (GFAP),[10] a biomarker indicating astrocytic reactivity, and neurofilament light (NfL),[11] a biomarker of neuroaxonal injury, are not AD-specific but they may also incorporate useful information.[1] In the preclinical stage of AD, all these blood-based biomarkers are significantly altered in response to increasing Aβ burden, but the sensitivity of each biomarker to detect these changes varies. Additionally, the ability of these biomarkers to detect Aβ pathology at the individual or group level differs and depends on the extent of the changes observed.[5,12]

Despite recent progress, there are still significant challenges in the use of blood-based biomarkers to detect the preclinical stage of AD. First, the performance of blood-based biomarkers immunoassays in the preclinical stage is not as high as compared to the clinical stage, with, perhaps, the exception of Immunoprecipitation-Mass Spectrometry (IP-MS) methods,[13–19] which are expensive and less scalable. Second, analytical variability, representing the variability of the measured levels inherent of any measurement method, is a crucial factor to consider with respect to between-group variability. For a biomarker to demonstrate robustness, the signal to noise ratio is important, and the percent fold change between groups compared should exceed the percent total analytical error.[20] Finally, in preclinical AD, limited data currently exists regarding the longitudinal changes of plasma biomarkers and their association with core AD pathology changes, downstream neurodegeneration, and cognitive decline using robust automated platforms. This underscores a key aspect in the design of observational and interventional studies during this stage. In this regard, the measurement of blood biomarkers using a fully automated method in a prospectively followed preclinical AD cohort could prove instrumental in overcoming these challenges.

The main aim of this work was to evaluate the performance and robustness of different plasma biomarkers to detect Aβ pathology in cognitively unimpaired (CU) individuals and assess whether these biomarkers or their changes are associated with Aβ longitudinal accumulation. For these purposes, we used a fully automated platform that includes the measurement of the main AD-related blood-based biomarkers, namely Aβ42/40, p-tau181, GFAP, NfL, p-tau217 and ApoE4. Additionally, we also investigated the performance of the p-tau181/Aβ42 and p-tau217/Aβ42 ratios.

Methods

Study design and participants

The ALFA + cohort is the nested longitudinal study from the ALFA (for ALzheimer's and FAmilies) study.[21] The ALFA study includes 2743 middle-aged, CU individuals (Clinical Dementia Rating = 0; Mini Mental State Examination [MMSE] ≥ 26; semantic fluency ≥ 12), with a high proportion of offspring of patients with AD and *APOE* ε4 carriers.[22] ALFA + includes 419 participants, who are followed longitudinally every 3 years and are comprehensively characterized with clinical and neuropsychological evaluations, CSF and blood biomarkers measurements, and neuroimaging biomarkers, including T1-weighted magnetic resonance imaging (MRI) and amyloid PET. As of January 2025, only 11 individuals in the ALFA + longitudinal cohort have progressed from CU state to mild cognitive impairment (MCI).

Of the 419 ALFA + individuals, this study included those with available baseline CSF and at least one plasma biomarker measurement (N = 400), among whom 342 also had amyloid PET data. Among the 400 CU participants, *APOE* genotypes were as follows: 25 (6.25%) ε2/ε3, 9 (2.25%) ε2/ε4, 159 (39.75%) ε3/ε3, 174 (43.50%) ε3/ε4, and 33 (8.25%) ε4/ε4. ALFA + participants were categorized based on CSF Aβ cutoffs that reflect the transition from the absence of Aβ pathology to subtle pathology. Participants were considered CSF Aβ-positive (A+) if their CSF Aβ42/40 ratio was below 0.071.[23] [18F]flutemetamol PET acquisition and quantification was previously described.[24,25] We further classified participants according to Aβ PET status, using a previously derived cutoff of 12 Centiloid.[24] The MRI cortical AD signature was estimated for each participant from the thickness of the following areas: entorhinal, inferior temporal, middle temporal, and fusiform. The signature was calculated as the mean thickness across these regions weighted by their surface area, as previously proposed.[26,27]

We used a modified version of the Preclinical Alzheimer's Cognitive Composite (mPACC) as the main cognitive outcome, based on the original one proposed by Donohue et al.[28] and the later proposal by Papp et al.[29] and Jonaitis et al.[30] This mPACC composite score is an average of z-scores standardized using the baseline CSF-defined Aβ and tau-negative (A–T–) group as reference. It includes the Total Immediate Recall subtest of the Free and Cued Selective Reminding Test, the Total Delayed Recall subtest of the Logical Memory test from the Wechsler Memory Scale-IV, the Coding subtest from the Wechsler Adult Intelligence Scale-IV, and a Semantic Fluency test (animals within 1 min).

Further details on the cohort characteristics, inclusion and exclusion criteria, and Aβ status cutoff determination can be found in the Supplementary Data.

Sample processing and biomarker measurements

Blood samples collection and processing procedure was previously described.[5,12] In brief, blood samples were obtained on the same day of the lumbar puncture in fasting conditions. Whole blood was drawn with a 20G or 21G needle gauge into a 10 mL ethylenediaminetetraacetic acid tube (BD Hemogard 10 mL; K2EDTA; cat. no. 367525). Tubes were gently inverted 5–10 times and centrifuged at 2,000*g* for 10 min at 4 °C. The supernatant was aliquoted in volumes of 0.5 mL into sterile polypropylene tubes (Sarstedt Screw Cap Micro Tube; 0.5 mL; PP; ref. 72.730.105) and immediately frozen at –80 °C. The samples were processed at room temperature. The time between collection and freezing was less than 30 min. Plasma Aβ42, Aβ40, p-tau181, GFAP, NfL, p-tau217 and Apolipoprotein E4 isoform (ApoE4) concentrations were measured with the fully automated plasma NeuroToolKit (NTK), a panel of exploratory robust prototype assays (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) on a Cobas® e 411 analyser, Cobas e 601 or Cobas e 801 module (all Roche Diagnostics International Ltd, Rotkreuz, Switzerland).

CSF samples collection and processing followed standard procedures and were previously described.[23] CSF Aβ40 and Aβ42 were measured with NTK exploratory robust prototype assays on an e 411 analyser or e 601 module. CSF p-tau181 and t-tau (both corresponding to the mid-region domain of tau protein) were measured using the electrochemiluminescence Elecsys® Phospho-Tau (181P) CSF and Elecsys Total-Tau CSF immunoassays (both Roche Diagnostics International Ltd, Rotkreuz, Switzerland), on a fully automated e 601 module.[23]

All plasma and CSF biomarkers were analysed at the Clinical Neurochemistry Laboratory at the University of Gothenburg, Sweden.

Ethics

The ALFA + study (ALFA-FPM-0311) was approved by the Independent Ethics Committee "Parc de Salut Mar", Barcelona (reference number 2012/4583/I), and registered at Clinicaltrials.gov (Identifier: NCT02485730). All participants signed the study's informed consent form that had also been approved by the Independent Ethics Committee "Parc de Salut Mar", Barcelona.

Statistics

Correlation and regression analyses were conducted after excluding univariate outlier values. Outliers were defined as data points beyond the interval defined between the third quartile (Q3) and first quartile plus/minus three times the interquartile range (IQR) for each variable. Unless otherwise indicated, results presented refer to analysis performed after outliers' exclusion. Missing data was not imputed in any of the analysis described. Sensitivity analyses including outliers were also performed and are reported in the Supplementary Data.

To compare demographics differences between Aβ groups, we used the Wilcoxon rank-sum (Mann-Whitney U) test for two-groups comparisons. For categorical variables, we employed the chi-squared (χ^2) test.

For plasma biomarkers differences between Aβ groups, we performed a type III analysis of covariance (ANCOVA) and calculated partial eta-squared (η^2) as a measure of effect size. Partial eta-squared represents the proportion of variance in the dependent variable explained by each independent variable after accounting for the variance explained by other covariates. It was calculated using an ANCOVA to assess differences in plasma biomarkers between A+ groups (defined by either CSF or PET), adjusting for relevant confounders, including age, sex, body mass index (BMI) and renal function (estimated glomerular filtration rate [eGFR]). Effect size values can be interpreted as follows: 0–0.06 (small), 0.06–0.14 (medium), and >0.14 (large).

Multiple linear regressions were performed to assess the association of baseline plasma biomarkers with age, sex (self-reported by study participants), *APOE* ε4 carriership, BMI and renal function. Spearman rank correlation analysis was used to test the concordance between plasma biomarkers and their CSF counterparts.

The discriminative ability of each plasma biomarker to differentiate Aβ status (either defined by CSF or PET) was assessed using receiver operating characteristic (ROC) analyses. We calculated the area under the curve (AUC) values with their 95% confidence intervals (CIs). To evaluate the added value of plasma ApoE4 in discrimination performance, we followed a two-step approach. First, logistic regression models were fitted to predict Aβ status, incorporating plasma ApoE4 and each plasma biomarker, and predicted probabilities were extracted. Subsequently, the AUCs of these combined models were compared to those of models including the plasma biomarker alone using DeLong's test for two correlated ROC curves.

To evaluate the discrimination performance robustness of the plasma biomarkers, we simulated the addition of analytical variability by introducing random variability to the raw biomarker values, corresponding to increasing coefficients of variation (CV), ranging from 0% to 25% with step increments of 0.5%. Using these modified values, we then reassessed the prediction of Aβ status with ROC analyses. We performed this process 1000 times for each CV and used a non-parametric regression approach to fit the trajectories of the resulting AUC values across increasing CVs. Extended details on the discrimination performance robustness analyses can be found in the Supplementary Data.

We assessed the change of baseline plasma biomarkers across Aβ pathology (either CSF Aβ42/40 or Aβ PET), and soluble tau pathology (CSF p-tau181) "AT" groups and across genetic *APOE* ε4 allele dosage. To do

so, we first performed an ANCOVA to adjust the group means for the effect of age, sex, BMI and renal function (eGFR). With the estimated marginal means we then performed post-hoc pairwise comparisons using Tukey Honest Significant Difference tests.

We also used multiple linear regression models to study the associations between baseline plasma biomarkers, and both baseline and three-year longitudinal changes (difference between baseline and follow-up measurements) in primary pathology (Aβ and tau), neurodegeneration markers (CSF NfL and MRI cortical AD signature), and cognitive function (mPACC). In addition, we used multiple linear regression models to examine the relationship between longitudinal plasma biomarkers changes and concurrent longitudinal changes in Aβ and soluble tau pathology, neurodegeneration markers, or cognitive function.

All linear regression models were adjusted for age, sex, BMI and renal function. Models including cognitive tests were additionally adjusted for years of education. These variables were selected based on clinical relevance, prior evidence, and results from univariate analyses. To account for multiple comparisons increase in type I error, p values were adjusted by controlling the False Discovery Rate (FDR) at level $\alpha = 0.05$ by applying the Benjamini-Hochberg procedure.[31] Throughout the text, we refer to results as 'nominally significant' if they meet the threshold of $p < 0.05$ prior to FDR correction.

We conducted regression diagnostics to ensure the validity of our models. For linear models this included assessments for non-normal residual distribution, homogeneity of residual variances, autocorrelation of residuals, and multicollinearity among predictors. If residuals were not normally distributed, we applied Box-Cox family of data transformations on the outcome. When necessary, we also used heteroskedasticity-consistent covariance matrices (i.e., HC3) to account for heteroskedasticity, ensuring robust results. Further details on model assumptions diagnosis can be found in the Supplementary Data.

All analyses were performed on the R programming language (v. 4.4.1).

Role of funders

The funding sources were not involved in the study design, the analysis and interpretation of the data, the writing of this manuscript, or in the decision to submit this manuscript for publication. Roche Diagnostics provided NTK reagents in-kind to perform biomarkers measurements. A few Roche Diagnostics employees, listed as co-authors, made direct contributions to this research (see the Contributors section). In brief, CQR, GK helped in acquiring the biomarker data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Baseline characteristics of study participants

We included 400 CU participants of the ALFA + cohort (Table 1). Among them, 135 (33.8%) were Aβ-positive (A+), as defined by the CSF Aβ42/40 ratio, and 265 (66.2%) were Aβ-negative (A–). 342 of those participants also had amyloid PET available, with 54 (15.8%) of them being A+, as defined by amyloid PET Centiloid (Supplementary Table S1). A+ participants were older and had a higher prevalence of *APOE* ε4 carriership. No significant differences were observed in sex distribution, baseline mPACC, or MMSE scores between the two groups. All plasma biomarkers were significantly different in the A+ group compared to the A– one (Table 1, Supplementary Table S1) and plasma p-tau217/Aβ42 and p-tau217 had the largest effect sizes ($\eta^2 = 0.28$ [0.22–1.00] and 0.23 [0.17–1.00], respectively), followed by plasma Aβ42/40 ($\eta^2 = 0.17$ [0.12–1.00]).

Plasma GFAP, NfL, p-tau217 and p-tau217/Aβ42 increased with age, while plasma Aβ42/40 decreased. Plasma GFAP was higher in females (Supplementary Fig. S1 and Table S2). In *APOE* ε4 carriers, plasma Aβ42/40 and NfL were lower, while plasma p-tau217, p-tau181/Aβ42, and p-tau217/Aβ42 were higher. A stepwise change in plasma Aβ42/40, p-tau181/Aβ42, and p-tau217/Aβ42 was observed with increasing ε4 allele dosage (Supplementary Figs. S1 and S2 and Table S2). Renal function influenced all plasma biomarkers except for the ratios Aβ42/40 and p-tau217/Aβ42, while BMI was significantly associated with plasma p-tau181 and the p-tau181/Aβ42 ratio (Supplementary Fig. S1 and Table S2).

There was a significant positive correlation between plasma and CSF for Aβ42/40 (r = 0.59, 95% CI [0.53–0.65], $p < 0.0001$), p-tau181 (r = 0.26, 95% CI [0.17–0.36], $p < 0.0001$), GFAP (r = 0.54, 95% CI [0.47–0.61], $p < 0.0001$), and NfL (r = 0.33, 95% CI [0.23–0.41], $p < 0.0001$) (Supplementary Fig. S3). p-tau217 was not available in CSF.

Discrimination performance of plasma biomarkers for Aβ status

We evaluated the performance of the plasma biomarkers to discriminate A+ from A– CU individuals, using CSF Aβ42/40 or amyloid PET as standards of truth. The highest discrimination performance was achieved by the following plasma biomarkers: plasma Aβ42/40 (AUC = 0.86 for CSF Aβ status; AUC = 0.88 for PET Aβ status), p-tau217 (AUC = 0.80 for CSF Aβ status; AUC = 0.91 for PET Aβ status), p-tau181/Aβ42 (AUC = 0.82 for CSF Aβ status; AUC = 0.90 for PET Aβ status), and p-tau217/Aβ42 (AUC = 0.85 for CSF Aβ status; AUC = 0.94 for PET Aβ status) (Fig. 1, Table 2, Supplementary Table S3). AUCs for plasma Aβ42/40, p-tau181/Aβ42 and p-tau217/Aβ42 did not differ significantly in detecting CSF Aβ status, while plasma p-tau217/Aβ42 outperformed all other biomarkers for PET Aβ status (DeLong's test $p < 0.05$; Supplementary

Characteristic	N	CSF A–, N = 265	CSF A+, N = 135	p	η^2
Age (years), Median (IQR)	400	60.5 (57.3, 63.9)	63.0 (58.5, 65.9)	0.0001[a]	
Women, n (%)	400	165 (62%)	81 (60%)	0.66[b]	
Education (years), Median (IQR)	400	12.0 (11.0, 17.0)	12.0 (11.0, 17.0)	0.37[a]	
APOE ε4 carriership, n (%)	400			<0.0001[b]	
Non-carrier		152 (57%)	32 (24%)		
Heterozygous		100 (38%)	83 (61%)		
Homozygous		13 (4.9%)	20 (15%)		
Amyloid PET (Centiloid), Median (IQR)	342	−4 (−9, 0)	10 (1, 26)	<0.0001[a]	
T positive (CSF p-tau181), n (%)	400	13 (4.9%)	31 (23%)	<0.0001[b]	
mPACC, Median (IQR)	395	0.07 (−0.40, 0.46)	0.02 (−0.52, 0.54)	0.85[a]	
BMI, Median (IQR)	400	26.5 (24.5, 29.9)	26.3 (24.1, 28.8)	0.35[a]	
MMSE, Median (IQR)	400	29.00 (29.00, 30.00)	29.00 (28.00, 30.00)	0.84[a]	
eGFR (mL/min/1.73 m^2), Median (IQR)	397	93 (79, 111)	94 (75, 106)	0.28[a]	
Plasma biomarkers					
Plasma Aβ42/40, Median (IQR)	399	0.138 (0.132, 0.144)	0.119 (0.112, 0.127)	<0.0001[c]	0.17 (0.12, 1.00)
Plasma p-tau181 (pg/mL), Median (IQR)	398	0.79 (0.69, 0.93)	1.00 (0.78, 1.26)	0.00045[c]	0.03 (0.01, 1.00)
Plasma GFAP (pg/mL), Median (IQR)	398	69 (53, 87)	90 (66, 124)	<0.0001[c]	0.07 (0.03, 1.00)
Plasma NfL (pg/mL), Median (IQR)	398	1.61 (1.16, 2.12)	1.94 (1.42, 2.58)	0.00012[c]	0.03 (0.01, 1.00)
Plasma p-tau217 (pg/mL), Median (IQR)	389	0.15 (0.12, 0.18)	0.24 (0.17, 0.40)	<0.0001[c]	0.23 (0.17, 1.00)
Plasma p-tau181/Aβ42, Median (IQR)	398	0.022 (0.019, 0.025)	0.031 (0.024, 0.039)	<0.0001[c]	0.07 (0.03, 1.00)
Plasma p-tau217/Aβ42, Median (IQR)	389	0.004 (0.003, 0.005)	0.007 (0.005, 0.012)	<0.0001[c]	0.28 (0.22, 1.00)

Data are expressed as median and IQR (Q1, Q3), for numerical variables or n and percentage (%) of one of the levels, for categorical ones. The Wilcoxon rank sum test (Mann Whitney U test) was used to compare numerical variables between CSF-defined Aβ groups for numerical variables except for plasma biomarkers, which were compared using a one-way ANCOVA. For the categorical variables, Pearson $\chi 2$ test was used to detect count differences among CSF Aβ groups. Of the 342 individuals with both CSF and amyloid PET measurements, 278 were in concordance (81.3%), 63 were CSF A+ but PET A− (18.4%), and only one individual (0.3%) was CSF A− and PET A+. Baseline characteristics of the study cohort stratified by PET Aβ status, are provided in Supplementary Table S1. Abbreviations: Aβ40, amyloid-β 40; Aβ42, amyloid-β 42; ANCOVA, analysis of covariance; APOE, apolipoprotein E; BMI, body mass index; CSF, cerebrospinal fluid; eGFR, estimated glomerular filtration rate; GFAP, glial fibrillary acidic protein; MMSE, Mini-Mental State Examination; NfL, neurofilament light; mPACC, modified Preclinical Alzheimer's Cognitive Composite; p-tau, phosphorylated tau. [a]Wilcoxon rank sum test. [b]Pearson's Chi-squared test. [c]One-way ANCOVA adjusted by sex, age, BMI and eGFR.

Table 1: **Baseline characteristics of the study cohort stratified by CSF Aβ status.**

Table S3). Plasma p-tau181, GFAP, and NfL had lower overall performance, but their accuracy improved with the addition of plasma ApoE4. Specifically, adding plasma ApoE4 significantly improved the discrimination of CSF Aβ status by plasma p-tau181 (AUC = 0.80), and the discrimination of both CSF and PET Aβ status by plasma GFAP and NfL (AUC: 0.69–0.79) (Table 2).

Plasma ApoE4 shows a stepwise increase with *APOE* ε4 genetic allele dosage (Supplementary Fig. S4a) and discriminates *APOE* ε4 carriers from non-carriers with a high accuracy (AUC = 0.98), and between *APOE* ε4 homozygotes and heterozygotes (AUC = 0.95) (Supplementary Fig. S4b). Plasma ApoE4 was not associated with any other plasma biomarkers in participants carrying an *APOE* ε4 allele, whether heterozygous or homozygous (Supplementary Fig. S4c).

We then evaluated the clinical robustness of the plasma biomarkers by simulating the impact of random variability, which is inherent in the routine use of any assay, on the biomarker values. Plasma Aβ42/40 was the most sensitive biomarker to the addition of random variability, while the others remained reasonably stable (Fig. 2, Supplementary Fig. S5). In fact, plasma Aβ42/40 discriminative ability was only performing better than plasma p-tau181 for a CV lower than 16% for (CSF Aβ status) or 9% (PET Aβ status). In addition, when determining the percent of difference between CSF-defined A+ and A−, plasma Aβ42/40 was the one with the lowest absolute percent difference from A− (Aβ42/40: −12.1%; NfL: +21.9%; p-tau181: +25.2%; GFAP: +31.9%; p-tau181/Aβ42: +40.2%; p-tau217: +86.1; p-tau217/Aβ42: +109.6%; Fig. 2, Supplementary Fig. S5).

Cross-sectional associations of plasma biomarkers with AD biomarkers and cognition

We first assessed differences across AT groups, with 'A' defined by CSF Aβ42/40 and 'T' defined by CSF p-tau181 (Fig. 3). A stepwise increase was observed for plasma p-tau181 and p-tau181/Aβ42 across AT stages, while the rest of plasma biomarkers showed differences between A−T− and A+T− but no differences between A+T− and A + T+. The same analysis using amyloid PET to define 'A' status is shown in Supplementary Fig. S6.

We next studied the cross-sectional associations between baseline plasma biomarkers and baseline markers of Aβ pathology (CSF Aβ42/40 and amyloid PET) or soluble tau pathology (CSF p-tau181). We observed that all plasma biomarkers are significantly altered ($p_{adj} < 0.05$) as a function of higher Aβ pathology

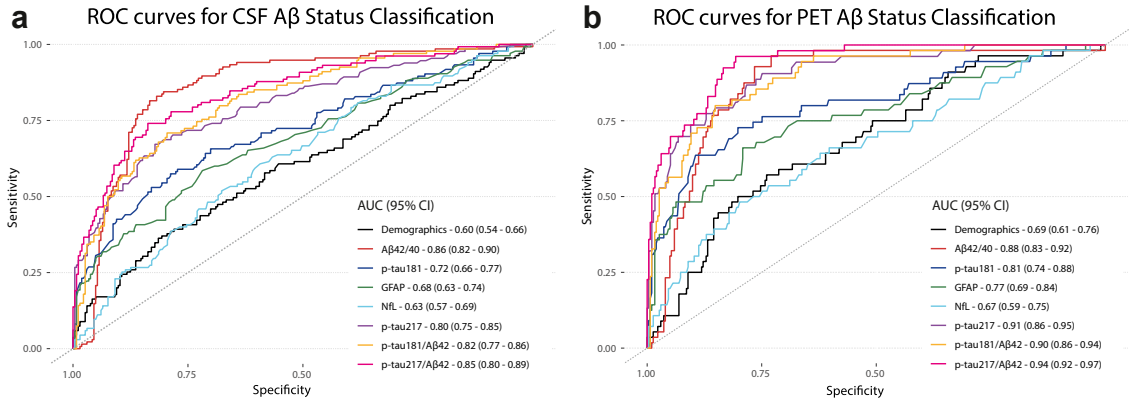

Fig. 1: **ROC analyses of plasma biomarkers for discriminating between CU A+ from A–.** Areas under the curve (AUC) with their corresponding 95% confidence intervals (CIs) for each plasma biomarker in discriminating Aβ status based on CSF (Aβ-positive defined as CSF Aβ42/40 < 0.071) (a); and PET Aβ status (Aβ-positive defined as >12 Centiloid) (b). For comparison, a demographic model (age + sex) was also included. Abbreviations: Aβ40, amyloid-β 40; Aβ42, amyloid-β 42; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; PET, positron emission tomography; p-tauX, phosphorylated tau at position X.

(that is, lower CSF Aβ42/40 or higher amyloid PET Centiloid; Table 3). After stratifying by CSF Aβ status, associations were statistically significant for all plasma biomarkers only in the CSF A+ group (Table 3, Supplementary Fig. S7a). Notably, all associations were modified by PET-based Aβ status, as indicated by the significant interaction term. After stratifying by PET Aβ status, plasma Aβ42/40, p-tau181/Aβ42, and p-tau217/Aβ42 were associated with amyloid PET in the PET A– group, suggesting an early rise (or decrease for Aβ42/40) in these plasma biomarkers before the onset of abnormal Aβ accumulation (Table 3, Supplementary Fig. S7b). Plasma p-tau181 and p-tau181/Aβ42 were only significant after removing outliers (Table 3, Supplementary Table S4). All plasma biomarkers were associated with CSF p-tau181, but similarly, plasma p-tau181 and p-tau181/Aβ42 only after outlier removal (Supplementary Tables S5 and S6).

We next assessed the association of the plasma biomarkers with downstream markers of neurodegeneration, namely biomarkers of axonal damage (CSF NfL) and cortical thickness (MRI cortical AD signature), as well as with cognitive performance (mPACC). Plasma GFAP, NfL, p-tau181, and p-tau181/Aβ42 were associated with CSF NfL, with the latter two showing associations only after excluding outliers (Supplementary Tables S7 and S8). None of the plasma biomarkers were associated with cortical thickness nor

Model	CSF Aβ status						PET Aβ status					
	Cutoff	PPA	NPA	AUC	AUC (+ApoE4)	Improves with plasma ApoE4	Cutoff	PPA	NPA	AUC	AUC (+ApoE4)	Improves with plasma ApoE4
Demographics (Age + Sex)	–	0.36	0.81	0.60 (0.54–0.66)	–	–	–	0.57	0.74	0.69 (0.61–0.76)	–	–
Plasma Aβ42/40	0.13	0.81	0.83	0.86 (0.82–0.90)*	0.86 (0.82–0.90)*	No	0.13	0.93	0.77	0.88 (0.83–0.92)*	0.88 (0.83–0.92)*	No
Plasma p-tau181 (pg/mL)	0.96	0.57	0.79	0.72 (0.66–0.77)*	0.80 (0.75–0.84)*	Yes	1.12	0.64	0.89	0.81 (0.74–0.88)*	0.82 (0.76–0.89)*	No
Plasma GFAP (pg/mL)	83.50	0.59	0.72	0.68 (0.63–0.74)*	0.79 (0.74–0.84)*	Yes	94.50	0.66	0.79	0.77 (0.69–0.84)	0.78 (0.71–0.85)*	Yes
Plasma NfL (pg/mL)	1.74	0.61	0.59	0.63 (0.57–0.69)	0.74 (0.69–0.79)*	Yes	2.24	0.48	0.80	0.67 (0.59–0.75)	0.69 (0.62–0.77)	Yes
Plasma p-tau217 (pg/mL)	0.20	0.67	0.82	0.80 (0.75–0.85)*	0.84 (0.80–0.88)*	Yes	0.20	0.91	0.75	0.91 (0.86–0.95)*	0.91 (0.87–0.95)*	No
Plasma p-tau181/Aβ42	0.026	0.71	0.80	0.82 (0.77–0.86)*	0.84 (0.80–0.88)*	No	0.029	0.80	0.85	0.90 (0.86–0.94)*	0.90 (0.86–0.94)*	No
Plasma p-tau217/Aβ42	0.0056	0.74	0.84	0.85 (0.80–0.89)*	0.87 (0.83–0.91)*	No	0.0062	0.96	0.81	0.94 (0.92–0.97)*	0.94 (0.92–0.97)*	No

ROC analyses were conducted to assess whether each plasma biomarker discriminates between Aβ-positive (A+) and Aβ-negative individuals (A–), as defined by CSF Aβ42/40 or amyloid PET. The model was assessed for each plasma biomarker alone and then compared to a model with the addition of plasma ApoE4 using a DeLong's test (p value < 0.05). An asterisk next to the area under the curve (AUC) denotes a significantly higher AUC (p value < 0.05) compared to a basic demographics model (age + sex). Cutoffs values were calculated and the resulting positive percent agreement (PPA) and negative percent agreement (NPA), which are used as proxies for sensitivity and specificity in the absence of a gold standard (neuropathological examination), were reported. Optimal cutoffs for each plasma biomarker were determined using the highest Youden's index. Aβ40, amyloid-β 40; Aβ42, amyloid-β 42; ApoE, Apolipoprotein E; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tauX, phosphorylated tau at position X.

Table 2: **ROC analyses to assess the performance of plasma biomarkers for Aβ status discrimination.**

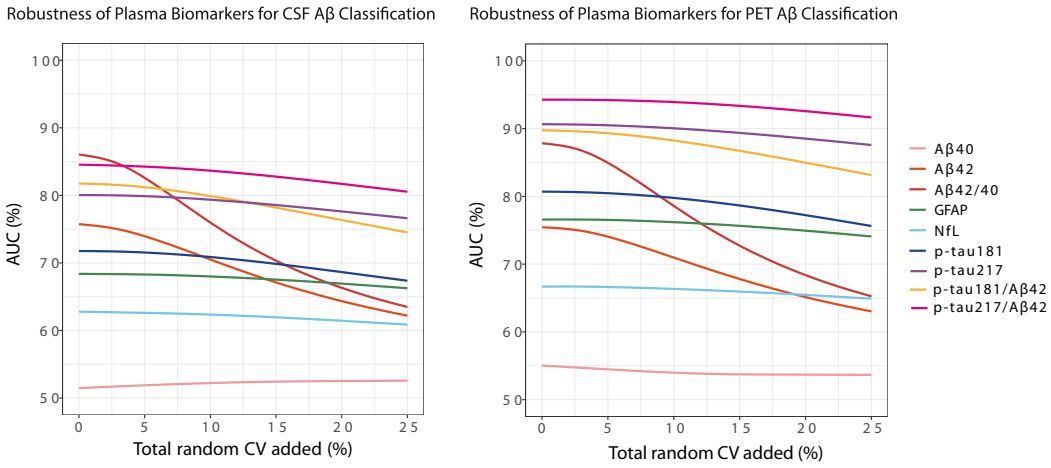

***Fig. 2:* Impact of introducing random variability on the ability of plasma biomarker to discriminate Aβ positivity.** The horizontal axis shows the amount of random variability added to the original data, expressed as CV increments ranging from 0 to 25% in 0.5% steps. On the vertical axis is represented the AUC of such simulated biomarker values to discriminate the corresponding Aβ standard of truth for each amount random variability added. Plasma Aβ42 and Aβ40 were also studied separately to disentangle the individual contribution of each biomarker to the robustness of the ratios. Abbreviations: Aβ40, amyloid-β 40; Aβ42, amyloid-β 42; CSF, cerebrospinal fluid; CV, coefficient of variation; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; PET, positron emission tomography; p-tauX, phosphorylated tau at position X.

cognition at baseline, irrespective of Aβ status or outlier inclusion (Supplementary Tables S9–S12).

Longitudinal associations of plasma biomarkers with AD biomarkers and cognition

We studied the association between baseline plasma biomarkers and longitudinal changes in Aβ and soluble tau pathology, downstream neurodegeneration and cognition (Mean follow-up [SD]: 3.27 ± 0.5) in a subset of the sample with available longitudinal data (N = 275). All plasma biomarkers at baseline were associated with longitudinal increase in amyloid PET Centiloid (Supplementary Fig. S8 and Tables S13 and S14), while plasma Aβ42/40, p-tau181/Aβ42 and p-tau217/Aβ42 ratios were associated to longitudinal decreases in CSF Aβ42/40, although the latter only after removing outliers (Supplementary Fig. S8 and Tables S13 and S14). All plasma biomarkers at baseline, except GFAP, were also

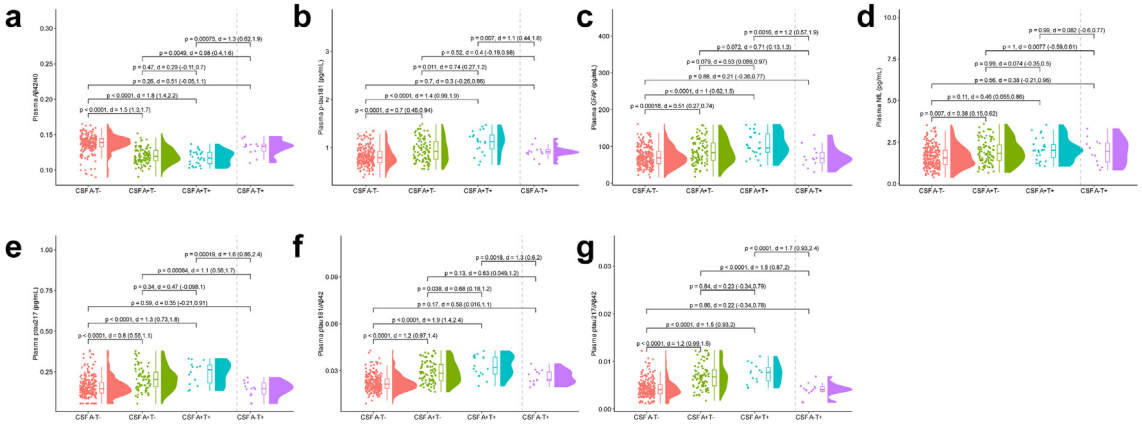

***Fig. 3:* Plasma biomarkers across AT groups.** Raincloud plots depicting differences in plasma biomarkers across AT groups: (a) Aβ42/40, (b) p-tau181, (c) GFAP, (d) NfL, (e) p-tau217, (f) p-tau181/Aβ42, and (g) p-tau217/Aβ42. 'A' was defined by CSF Aβ42/40 and 'T' by CSF p-tau181. The same analysis using amyloid PET to define 'A' status is shown in Supplementary Fig. S6. The box plot represents the median (horizontal line), interquartile range (box), and 1.5× interquartile range (whiskers), with individual biomarker values displayed. Group differences were assessed using ANCOVA, adjusting for age, sex, BMI, and renal function (estimated glomerular filtration rate [eGFR]), followed by post-hoc pairwise comparisons using Tukey's Honest Significant Difference test. *p* values and corresponding Cohen's d values are reported in the graph. Abbreviations: Aβ40, amyloid-β 40; Aβ42, amyloid-β 42; BMI, body mass index; eGFR, estimated glomerular filtration rate; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tauX, phosphorylated tau at position X.

	CSF Aβ												PET Aβ											
	Global CSF			Interaction CSF			CSF A+			CSF A-			Global PET			Interaction PET			PET A+			PET A-		
Plasma biomarkers	B	β	p_{adj}	B	β	p_{adj}	B	β	p_{adj}	B	β	p_{adj}	B	β	p_{adj}	B	β	p_{adj}	B	β	p_{adj}	B	β	p_{adj}
Aβ42/40	0.82 (0.71, 0.93)	0.60 (0.50, 0.70)	<0.0001	0.47 (0.29, 0.65)	0.34 (0.21, 0.48)	<0.0001	0.61 (0.44, 0.77)	0.56 (0.41, 0.71)	<0.0001	11 (0.15, 21)	0.13 (0.0018, 0.25)	0.16	-11 (-13, -8.7)	-0.44 (-0.55, -0.32)	<0.0001	11 (9.5, 12)	-1.3 (-1.5, -1.2)	<0.0001	-25 (-46, -4.7)	-0.37 (-0.66, -0.068)	0.12	-74 (-130, -14)	-0.14 (-0.26, -0.028)	0.039
p-tau181	-0.0034 (-0.0043, -0.0024)	-0.34 (-0.44, -0.25)	<0.0001	-0.0097 (-0.019, -0.00019)	-0.12 (-0.23, 0.00057)	0.089	-0.011 (-0.019, -0.0021)	-0.24 (-0.43, -0.047)	0.021	-0.071 (-0.46, 0.32)	-0.024 (-0.16, 0.11)	0.84	0.49 (0.33, 0.64)	0.33 (0.21, 0.45)	<0.0001	0.91 (0.81, 1)	0.87 (0.65, 1.1)	<0.0001	0.18 (-0.11, 0.48)	0.23 (-0.15, 0.60)	0.33	3.6 (-0.50, 7.7)	0.11 (-0.015, 0.23)	0.15
GFAP	-0.000028 (-0.000036, -0.000020)	-0.36 (-0.48, -0.25)	<0.0001	-0.000060 (-0.00013, 0.000010)	-0.092 (-0.20, 0.017)	0.12	-0.00010 (-0.00017, -0.0000035)	-0.30 (-0.49, -0.10)	0.0074	-0.0043 (-0.0085, -0.00019)	-0.14 (-0.28, -0.0062)	0.16	0.0028 (0.0016, 0.0040)	0.27 (0.14, 0.40)	<0.0001	0.0087 (0.0079, 0.0094)	0.96 (0.75, 1.2)	<0.0001	0.15 (-0.0074, 0.30)	0.30 (-0.015, 0.61)	0.21	0.0026 (-0.030, 0.035)	0.010 (-0.12, 0.14)	0.88
NfL	-0.00062 (-0.0010, -0.00022)	-0.16 (-0.27, -0.056)	0.0031	-0.0022 (-0.0038, -0.00061)	-0.16 (-0.27, -0.043)	0.016	-0.0033 (-0.0063, -0.00027)	-0.20 (-0.38, -0.016)	0.033	0.11 (-0.11, 0.33)	0.071 (-0.066, 0.21)	0.43	0.072 (0.014, 0.13)	0.14 (0.019, 0.26)	0.023	0.42 (0.38, 0.46)	0.74 (0.5, 0.99)	<0.0001	0.13 (-0.034, 0.3)	0.23 (-0.059, 0.52)	0.27	0.45 (-0.80, 1.7)	0.045 (-0.079, 0.17)	0.56
p-tau217	-0.0082 (-0.010, -0.0060)	-0.37 (-0.48, -0.27)	<0.0001	-0.022 (-0.054, 0.011)	-0.088 (-0.23, 0.054)	0.22	-0.036 (-0.067, -0.0054)	-0.24 (-0.45, -0.036)	0.025	-1.6 (-3.5, 0.26)	-0.11 (-0.24, 0.018)	0.21	1.2 (0.83, 1.6)	0.33 (0.19, 0.46)	<0.0001	3.1 (2.7, 3.6)	1.2 (0.94, 1.4)	<0.0001	0.19 (-0.048, 0.42)	0.32 (-0.14, 0.78)	0.29	10 (-2.5, 23)	0.097 (-0.023, 0.22)	0.16
p-tau181/Aβ42	-0.21 (-0.25, -0.17)	-0.47 (-0.55, -0.38)	<0.0001	-0.54 (-0.88, -0.20)	-0.20 (-0.32, -0.075)	0.0063	-0.52 (-0.81, -0.23)	-0.33 (-0.51, -0.14)	0.0021	-1.1 (-16, 14)	-0.0096 (-0.14, 0.12)	0.88	19 (14, 24)	0.39 (0.28, 0.51)	<0.0001	28 (24, 31)	1.0 (0.81, 1.2)	<0.0001	1.4 (-4.6, 7.4)	0.078 (-0.36, 0.51)	0.72	170 (31, 300)	0.14 (0.026, 0.26)	0.039
p-tau217/Aβ42	-0.51 (-0.61, -0.42)	-0.48 (-0.58, -0.39)	<0.0001	-0.83 (-1.8, 0.16)	-0.11 (-0.25, 0.022)	0.12	-1.2 (-2.0, -0.37)	-0.29 (-0.49, -0.089)	0.0089	-56 (-130, 18)	-0.098 (-0.23, 0.031)	0.24	52 (40, 64)	0.44 (0.32, 0.55)	<0.0001	96 (82, 110)	1.0 (0.84, 1.2)	<0.0001	4.8 (-3.5, 13)	0.22 (-0.26, 0.71)	0.41	520 (120, 920)	0.15 (0.036, 0.27)	0.039

For each plasma biomarker, a linear model was used to assess its association with an Aβ pathology biomarker (CSF Aβ42/40 or amyloid PET Centiloid), adjusting for age, sex, BMI and renal function. Analyses were stratified by Aβ status, namely Aβ-negative (A−) or Aβ-positive (A+) groups, defined either by CSF Aβ42/40 ratio (CSF Aβ status; N = 400) or amyloid PET Centiloid (PET Aβ status; N = 342). The table reports unstandardized (B) and standardized (β) regression coefficients, and significant *p* values. Interaction *p* values for 'plasma biomarker × Aβ status' are also provided. Statistical significance was set at $p < 0.05$. Analyses excluded univariate outliers; results including outliers are available in Supplementary Table S4. Abbreviations: Aβ40, amyloid-β 40; Aβ42, amyloid-β 42; BMI, body mass index; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; PET, positron emission tomography; p-tauX, phosphorylated tau at position X.

***Table 3*: Cross-sectional associations between plasma biomarkers and Aβ pathology biomarkers.**

associated with longitudinal changes of CSF p-tau181 (Supplementary Fig. S9 and Tables S15 and S16). When looking at downstream neurodegeneration, all plasma biomarkers were associated with longitudinal increases in CSF NfL, with plasma Aβ42/40, p-tau181 and p-tau181/Aβ42 showing associations only after excluding outliers (Supplementary Tables S17 and S18). None of the baseline plasma biomarkers were associated with longitudinal increase in cortical thickness nor cognitive change in the three years timespan (Supplementary Tables S19–S22).

Finally, we assessed the association between concurrent longitudinal changes in plasma biomarkers and longitudinal changes in Aβ and soluble tau pathology (N = 275), downstream neurodegeneration or cognition. Plasma p-tau217 and p-tau217/Aβ42 increases were significantly associated with a longitudinal increase of Aβ burden (i.e., decrease of CSF Aβ42/40 and increases in amyloid PET Centiloid) (Fig. 4); and this association was not modified by Aβ baseline status (Supplementary Tables S23 and S24). Increases in plasma GFAP over time were associated with decreases in CSF Aβ42/40, and with Aβ PET accumulation, the latter only observed in individuals who were initially Aβ PET-negative. Plasma NfL increases were also associated with decreasing CSF Aβ42/40. Moreover, plasma p-tau217 and p-tau217/Aβ42 increases were associated with longitudinal increases in CSF p-tau181, and plasma GFAP and p-tau217 with longitudinal CSF NfL increases (Supplementary Tables S25–S28). Plasma p-tau217/Aβ42 longitudinal increases were associated with concurrent decreases in cortical thickness but only after removing outliers (Supplementary Tables S29 and S30). None of the plasma biomarkers changes were associated with longitudinal increase in cognitive change after removing outliers (Supplementary Tables S31 and S32).

Discussion

In this study, we investigated the performance of a fully automated immunoassay platform for detecting Aβ accumulation in CU individuals and assessed its prognostic and monitoring capacities. We included the most relevant AD-related blood-based biomarkers (Aβ42/40, p-tau181, GFAP, NfL, p-tau217), along with the p-tau181/Aβ42 and p-tau217/Aβ42 ratios. Our findings indicate that plasma Aβ42/40, p-tau217, p-tau181/Aβ42, and p-tau217/Aβ42 performed best in distinguishing A+ from A− CU individuals (AUC: 0.80–0.94, based on CSF or PET Aβ status). However, the robustness of plasma Aβ42/40 was limited, potentially restricting its clinical utility, especially in settings with high analytical variability. Importantly, the plasma Aβ42/40 and p-tau217/Aβ42 ratios were not affected by renal function, and its influence was reduced for p-tau181/Aβ42 compared to p-tau181 alone, which showed the highest susceptibility of any plasma biomarker to renal function. Notably, plasma p-tau217/Aβ42 showed the strongest performance in detecting PET A+ individuals (AUC = 0.94; PPA and NPA: 0.96 and 0.81). In contrast, plasma p-tau181, and GFAP had lower performance individually but improved when combined with ApoE4 measurement (AUC: 0.78–0.82). All baseline plasma biomarkers were associated with longitudinal increases in amyloid PET Centiloid. Furthermore, longitudinal changes in plasma p-tau217 and p-tau217/Aβ42 tracked with concurrent changes in Aβ (both CSF and PET) and soluble tau pathology, underscoring their potential as monitoring biomarkers. Overall, our study highlights that different blood-based biomarkers provide unique insights into preclinical AD, each of them offering valuable information.

Evidence of Aβ pathology in the brain is the earliest sign marker of AD and occurs decades before symptom

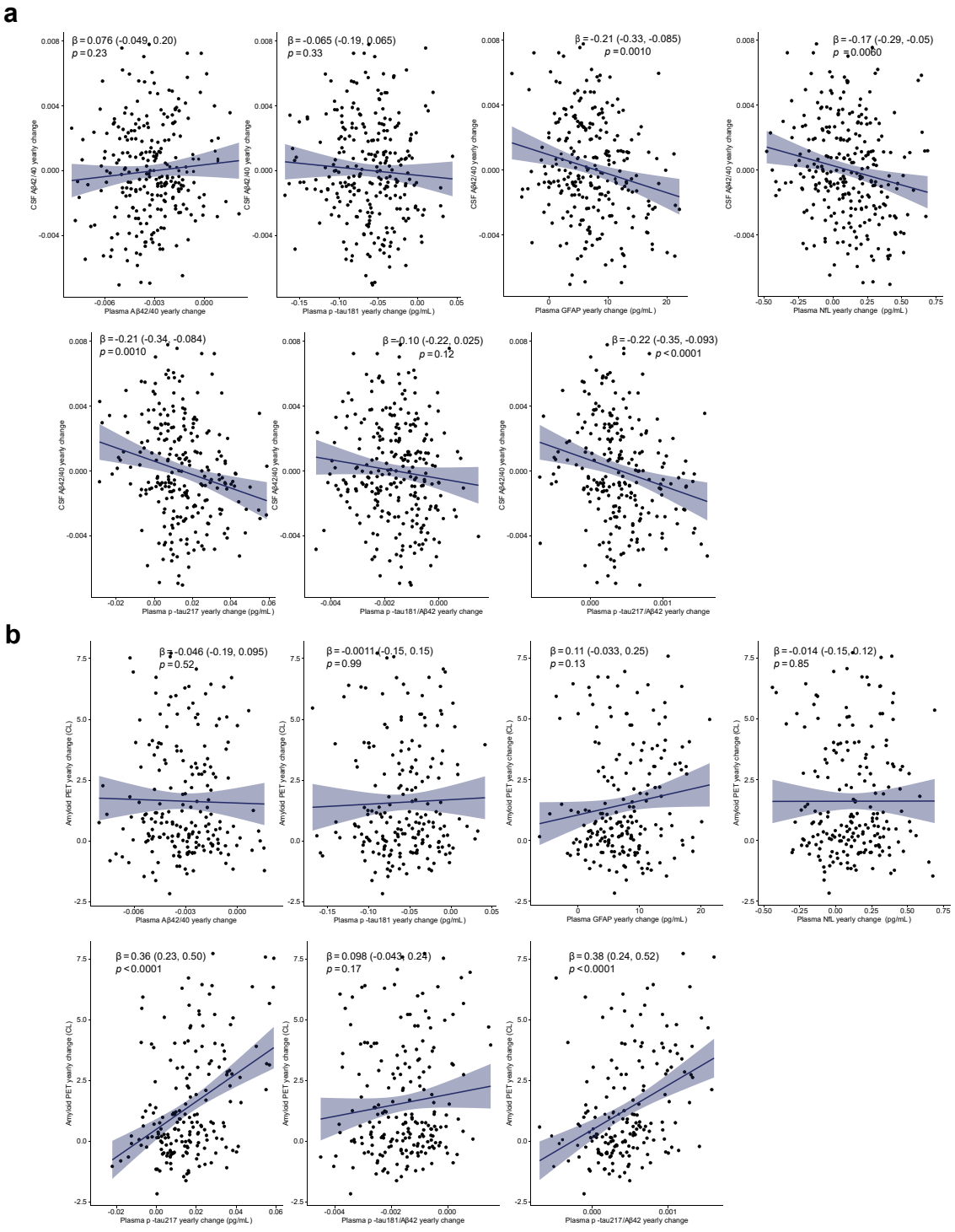

Fig. 4: **Association between annual rate-of-change in plasma biomarkers and concurrent changes in amyloid biomarkers (CSF and PET) over three years.** We used the difference in CSF Aβ42/40 from baseline to follow-up (a) or the change in amyloid PET (b). Standardized regression coefficients (β) and *p* values (unadjusted; FDR-corrected values are provided in Supplementary Tables S23 and S24) were calculated using a linear model adjusted for age, sex, BMI, and renal function (eGFR). Abbreviations: Aβ40, amyloid-β 40; Aβ42, amyloid-β 42; CSF, cerebrospinal fluid; FDR, false discovery rate; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; PET, positron emission tomography, p-tauX, phosphorylated tau at position X.

onset.[32–34] Pharmacological and non-pharmacological interventional studies targeting this early stage require biomarkers that indicate AD pathology. Core CSF biomarkers and amyloid PET are the standards of truth. CSF Aβ42/40 may be a more sensitive marker for detecting early Aβ pathology, with amyloid PET providing a more quantitative measure, correlating more strongly with longitudinal changes in other biomarkers.[35,36] This implies that while CSF analysis may be better suited for early detection, amyloid PET could be more useful for tracking disease progression over time. However, novel blood-based biomarkers have the potential to further facilitate the detection of individuals at higher risk of AD.[12,37–44] Interventional studies can be offered to these individuals, and, in the future, they may be eligible for disease-modifying drugs. Trials focused on the preclinical stages of AD face challenges due to the prolonged duration required to observe clinical benefits. Blood-based biomarkers should not only accurately and reliably detect Aβ pathology but also predict the likelihood of further Aβ accumulation and neurodegeneration, while offering the ability to monitor disease progression over time. This could be achieved through fully automated panels capable of simultaneously measuring multiple plasma biomarkers, facilitating repeated measurements.

Previous studies have shown that blood-based biomarkers are able to accurately detect those CU individuals who are A+,[5,8,10,12,15,45–49] but the discrimination accuracies are not as high as those found in CI individuals. In contrast, IP-MS techniques outperform immunoassays also in CU individuals,[13,14,19] but they are less accessible and scalable. Fully automated platforms specifically investigating a CU group include Lumipulse,[50–52] Sysmex high sensitivity chemiluminescence enzyme[53] and Elecsys.[17,54,55] Here, we investigated a fully automated Elecsys-based assay and we found that plasma Aβ42/40, p-tau217, p-tau181/Aβ42 and p-tau217/Aβ42 had the better discrimination performances. Plasma Aβ42/40 reached an AUC between 0.86 and 0.88, which is consistent with previous reports using the same fully automated platform[17,54,55] or different platforms.[5,8,49,52,53,56,57] However, the dynamic range of plasma Aβ42/40 was narrow and simulated between-assay variations had an important influence on plasma Aβ42/40 performance to detect A+ CU individuals, particularly by amyloid PET. A random variability beyond a CV of 9% in plasma Aβ42/40 lowered the AUC below that of plasma p-tau181. Other studies have reported robustness issues with plasma Aβ42/40, also using Elecsys[58] or with other platforms, like single molecule array (Simoa) and IP-MS.[59] These results altogether seem to suggest that these robustness issues are not specific for a particular platform but rather inherent to the biomarker. Additionally, a study evaluating the biological variation of AD blood biomarkers found that physiological fluctuations for Aβ42/40 were very low, requiring an unattainable, nearly perfect analytical performance to distinguish signal from noise in longitudinal monitoring.[60] A common treatment for heart disease, which frequently co-occurs with cognitive symptoms, has also been shown to affect this biomarker with a magnitude higher than that reported for AD pathology.[61] Therefore, this unsatisfactory robustness may preclude using plasma Aβ42/40 immunoassays as a screening tool in clinical trials or observational studies in preclinical stages, despite its good discrimination accuracy, while it can still be used in single cohorts' and retrospective studies where the analytical performance requirements could be guaranteed, and no predefined cutoffs need to be used. In contrast, p-tau217, p-tau181/Aβ42, and p-tau217/Aβ42 not only performed well in detecting Aβ pathology but were also less influenced by random variability. Notably, plasma p-tau217/Aβ42 showed the strongest ability to identify PET A+ CU individuals, surpassing all other plasma biomarkers. Moreover, unlike some plasma biomarkers, its levels were not affected by renal function, a known confounding factor.[62,63] Taken together, these findings position p-tau217/Aβ42 as a promising candidate for detecting Aβ pathology in CU individuals.

Apart from the superior discrimination performance showcased by plasma p-tau217/Aβ42, p-tau181/Aβ42 and p-tau217, another p-tau biomarker, plasma p-tau181 reached an AUC of 0.81 to discriminate PET Aβ status, and an AUC of 0.80, if combined with ApoE4, to discriminate CSF Aβ status. In contrast to plasma Aβ42/40, its performance remained stable even after introducing a random variability higher than a CV of 9%. Plasma GFAP had also a fair performance if combined with ApoE4 (AUC: 0.78–0.79) and was also less sensitive to random variability. These results align with those from Simoa-measured p-tau181 and GFAP[59] or Lumipulse p-tau181,[51,52] suggesting that these blood-based biomarkers show consistency across different platforms.

Several evidence indicate that the changes of some of the plasma biomarkers occur in early stages. All plasma biomarkers were significantly changed in the A+T– compared to the A–T– group. Moreover, plasma Aβ42/40, p-tau181/Aβ42 and p-tau217/Aβ42 are associated with higher amyloid PET Centiloid in individuals classified as PET A–, which may imply that these biomarkers rise even before CU individuals progress to PET Aβ-positivity.

We also investigated the predictive value to detect longitudinal Aβ deposition of these blood-based biomarkers, and we found that all plasma biomarkers were associated with longitudinal amyloid PET deposition. Furthermore, baseline plasma Aβ42/40, p-tau181/Aβ42 and p-tau217/Aβ42 were also associated with longitudinal decrease in CSF Aβ42/40, which is known to change before amyloid PET abnormalities arise. It is important to emphasize that these associations occur in CU individuals at higher risk for AD within a relatively

short time frame of only three years. This holds significance in the planning of both observational and interventional studies at this stage, where enriching cohorts with individuals progressing to established AD pathology in a relatively short timeframe is crucial. Similarly, the concept of enriching studies also applies to individuals who are more likely to undergo downstream neurodegeneration. In this context, we found that all baseline plasma biomarkers are linked to later increases in CSF NfL.

A key feature of our study is that we investigated longitudinal changes in plasma biomarkers and assessed whether changes over a short timeframe (three years) in the preclinical stage of AD can offer insights beyond cross-sectional baseline measurements. Notably, plasma p-tau217 and p-tau217/Aβ42 emerged as particularly significant in this regard. Longitudinal increases in these biomarkers occurred concurrently with rises in Aβ burden, and longitudinal increases in CSF p-tau181. Additionally, GFAP increases over time were associated with greater longitudinal Aβ PET accumulation, but only in individuals initially classified as PET A– negative. Furthermore, the longitudinal increases in plasma p-tau217 and GFAP were associated with concurrent increases in CSF NfL. The consistent association between plasma p-tau217 and GFAP and Aβ deposition was observed in the BLSA cohort[56] and the TRAILBLAZER-ALZ trial,[64] indicating the potential of plasma p-tau217 and GFAP as a marker for monitoring Aβ changes. Remarkably, plasma p-tau217 and GFAP responded to anti-Aβ drugs in both the TRAILBLAZER-ALZ trial,[64] and in the Clarity AD trial[65] (together with plasma Aβ42/40 and p-tau181).

In contrast to the associations with AD pathology, we found little to no significant association between baseline or longitudinal change of blood-based biomarkers and longitudinal change in cognition or MRI structural imaging. Recently, Ashton et al.[49] demonstrated that plasma p-tau217 increased across the AD *continuum* and was associated with clinical decline and brain atrophy in preclinical AD, suggesting that this biomarker may serve as a surrogate marker for disease progression. Using the same fully automated assay, Palmqvist et al. showed that baseline plasma p-tau181 was the best single biomarker to predict AD dementia within 6 years in CU individuals, and its combination with plasma p-tau217 and ApoE4 improved this prediction.[55] The relatively short follow-up of our study (around three years) and the early stage of the cohort may explain the lack of significant changes in structural MRI and cognition. On the contrary, it is noteworthy that we can observe an association between changes in plasma p-tau217 and p-tau217/Aβ42 and changes in Aβ pathology in this short time frame. Yet, it remains to be determined whether this association also happens within an 18-month period, which is the approximate timeframe of all published Aβ-antibodies clinical trials.[65–67]

This study has limitations. First, despite adjusting for key confounders such as age, sex, BMI and renal function, unmeasured factors—such as lifestyle factors, occupational exposures, or genetic variants—may still influence plasma biomarker levels and Aβ accumulation. Second, our cohort consists of middle-aged, CU individuals enriched for AD risk factors, which may limit the generalizability of the results. For example, despite being an at risk cohort, the proportion of individuals progressing to MCI is relatively low, potentially limiting the applicability of cognitive findings to older preclinical cohorts. Third, this is a single-centre study, and the results need to be replicated in independent cohorts. Fourth, the number of participants was determined by the availability at that time, without performing a previous power analysis. While some analyses yielded narrow confidence intervals, others—particularly the longitudinal analyses with fewer participants—showed wider intervals, indicating limited precision and warranting cautious interpretation. Fifth, the longitudinal follow-up was relatively short, which may limit the ability to capture long-term longitudinal changes in biomarkers or Aβ accumulation. This means our results need to be confirmed with serially measured biomarkers. However, the availability of longitudinal measurements and the finding of significant associations even in this short period in preclinical stages represents also a strength of this study. Finally, the robustness analyses were conducted in retrospective measurements and through *in-silico* simulation; these findings require validation to ascertain that they represent a real scenario by using prospective measurements encompassing all possible sources of error.

In conclusion, blood-based biomarkers measured with a fully automated immunoassay provide diagnostic, prognostic and monitoring information in CU individuals at risk of AD. Plasma biomarkers ratios showed the strongest performance for detecting Aβ pathology and were associated with longitudinal Aβ deposition. However, plasma Aβ42/40 may have limitations in robustness, and these findings require confirmation in prospective studies at the individual level. Future studies with longer follow-up, larger sample sizes, and inclusion of additional confounders will be critical to further confirm and extend these findings.

Contributors

AGE, KB and MSC conceived and designed the study. NJA, CQR, GK, HZ and KB acquired the biomarker data. AGE, MMA, WSB and MSC analysed the data. POR, CM, MS, MdC, FA, GSB, OGR, JDG, NVT and MSC gathered participants' data and provided interpretation. AGE and MSC did the literature search and wrote the manuscript. All authors helped review and/or revise the manuscript. All authors have read and approved the final version of the manuscript. AGE and MSC have directly assessed and verified the underlying data reported in the manuscript.

Data sharing statement

All requests for raw and analysed data and materials will be promptly reviewed by the corresponding authors and the Barcelonaβeta Brain

Research Center to verify whether the request is subject to any intellectual property or confidentiality obligations. Bulk Anonymized data can be shared by request from any qualified investigator for the sole purpose of replicating procedures and results presented in the article, providing data transfer is in agreement with EU legislation.

Declaration of interests

NJA received consulting fees from Athria, ImaginationLand LLC, MapLight Therapeutics, SpeaBio, Neurogen Biomarking, Quanterix and TauRx during the past 36 months; has served as consultant for Quanterix and has given lectures in symposia sponsored by Alamar Biosciences, Biogen, Eli-Lilly, Quanterix and VJDementia. He has also been granted a patent application (No.: PCT/US2024/037,834 (WSGR Docket No. 58484–709.601 ('Methods for Remote Blood Collection, Extraction and Analysis of Neuro Biomarkers'). MdC received funding from Alzheimer's research and therapy. Her institution received funding from Attraction Talent Comunidad de Madrid, PROYECTOS I + D + I – 2020″- Retos de investigación from the Ministerio Español de Ciencia e innovación, a Ramon y Cajal Fellowship, PROYECTOS I + D + I – 2024″- Retos de investigación from the Ministerio Español de Ciencia e innovación and La Caixa Innovation Hub. She also received payment for lectures from Novonordisk and Springer Healthcare. She is a BBB-PIA chair and a Scientic advisor in ADPD. CQR is a full-time employee of Roche Diagnostics International Ltd, Rotkreuz, Switzerland. GK is a full-time employee of Roche Diagnostics GmbH, Penzberg, Germany. CM's institution received funding from EuFingers JPND research grant and ADDF digital biomarkers research grant. GSB has been a speaker in internal meetings at Roche Pharma in Spain. OGR received research funding from Roche Pharma. He has given lectures in symposia sponsored by Roche Diagnostics. JDG received research support funding from Hoffmann—La Roche, GE HealthCare and Roche Diagnostics; and funding from the grants EIT Digital, Spanish Research Agency and La Marató de TV3. He received consulting fees from Roche Diagnostics. He is also the inventor, co-founder, and owner of Betascreen. He received payment as speaker from Biogen, Philips Nederlands, Esteve and Life Molecular Imaging. He also received inscriptions to team members from GE Healthcare and participated in the Molecular Imaging Advisory Board at Prothena Biosciences. He is now a full-time employee of AstraZeneca. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZpath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, Roche, and WebMD, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). He is chair of the Alzheimer's Association Global Biomarker Standardization Consortium and chair of the IFCC WG-BND. NVT received funding from the environMENTALment project (funded by the Ajuntament de Barcelona and "la Caixa" Foundation (project code: 23S06083-001), funding from the Alzheimer's Disease Data Initiative (ADDI) through the William H. Gates Sr. Fellowship program; and funding from receives funding from the Alzheimer's Disease Data Initiative (ADDI) through the William H. Gates Sr. Fellowship program. She is a Board member Spanish Statistical Society and a Board member Catalan Statistical Society. KB has served as a consultant and at advisory boards for Abbvie, AC Immune, ALZpath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Neurimmune, Novartis, Ono Pharma, Prothena, Roche Diagnostics, Sanofi and Siemens Healthineers; has served at data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. MSC has received in the past 36mo consultancy/speaker fees (paid to the institution) from Almirall, Eli Lilly, Quanterix, Novo Nordisk, and Roche Diagnostics. He has received consultancy fees or served on advisory boards (paid to the institution) of Eli Lilly, Grifols, Novo Nordisk, and Roche Diagnostics. He was granted a project and is a site investigator of a clinical trial (funded to the institution) by Roche Diagnostics. In-kind support for research (to the institution) was received from ADx Neurosciences, Alamar Biosciences, ALZPath, Avid Radiopharmaceuticals, Eli Lilly, Fujirebio, Janssen Research & Development, Meso Scale Discovery, and Roche Diagnostics; MSC did not receive any personal compensation from these organizations or any other for-profit organization.

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During the preparation of this work the authors used generative AI tools in order to improve readability and language of the work. The authors have reviewed and confirmed the validity of the text and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105741.

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