



Plasma markers predict changes in amyloid, tau, atrophy and cognition in non-demented subjects

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It is currently unclear whether plasma biomarkers can be used as independent prognostic tools to predict changes associated with early Alzheimer's disease.

In this study, we sought to address this question by assessing whether plasma biomarkers can predict changes in amyloid load, tau accumulation, brain atrophy and cognition in non-demented individuals. To achieve this, plasma amyloid- β 42/40 (A β 42/40), phosphorylated-tau181, phosphorylated-tau217 and neurofilament light were determined in 159 non-demented individuals, 123 patients with Alzheimer's disease dementia and 35 patients with a non-Alzheimer's dementia from the Swedish BioFINDER-2 study, who underwent longitudinal amyloid (¹⁸F-flutemetamol) and tau (¹⁸F-RO948) PET, structural MRI (T₁-weighted) and cognitive testing.

Our univariate linear mixed effect models showed there were several significant associations between the plasma biomarkers with imaging and cognitive measures. However, when all biomarkers were included in the same multivariate linear mixed effect models, we found that increased longitudinal amyloid-PET signals were independently predicted by low baseline plasma A β 42/40 ($P = 0.012$), whereas increased tau-PET signals, brain atrophy and worse cognition were independently predicted by high plasma phosphorylated-tau217 ($P < 0.004$). These biomarkers performed equally well or better than the corresponding biomarkers measured in the CSF. In addition, they showed a similar performance to binary plasma biomarker values defined using the Youden index, which can be more easily implemented in the clinic. In addition, plasma A β 42/40 and phosphorylated-tau217 did not predict longitudinal changes in patients with a non-Alzheimer's neurodegenerative disorder.

In conclusion, our findings indicate that plasma A β 42/40 and phosphorylated-tau217 could be useful in clinical practice, research and drug development as prognostic markers of future Alzheimer's disease pathology.

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Abbreviations: AIC = Akaike information criterion; A β 42/40 = amyloid- β 42/40; MMSE = Mini-Mental State Examination; NfL = neurofilament light; P-tau = phosphorylated tau; SUVR = standardized uptake value ratio

Introduction

There is growing recognition that the pathophysiology of Alzheimer's disease is a highly complex and dynamic process, beginning with the early accumulation of amyloid- β , followed by tau deposition and neurodegeneration.¹ These pathological changes can be detected *in vivo* using CSF analyses, PET and MRI. However, despite being clinically useful, these techniques are invasive, expensive or time-consuming, which limits their use in clinical practice or their availability across the same individuals.² Thus, there is an urgent need for blood-based biomarkers that overcome these limitations, which can be widely used in primary care settings as well as clinical trials to detect Alzheimer's disease pathology.

In line with this, plasma biomarkers have recently emerged as non-invasive, cost-effective and accessible tools to assess the pathological changes that occur over the disease course.³ For instance, plasma amyloid- β 42/40 (A β 42/40) levels are thought to reflect amyloid- β deposition and have been shown to correlate with brain amyloidosis both in cognitively normal as well as cognitively impaired individuals.^{4–6} Plasma phosphorylated-tau 181 (P-tau181) and 217 (P-tau217) might reflect both amyloid and tau deposition in the brain, correlating with increased amyloid-PET and tau-PET signals.^{7–10} Finally, plasma neurofilament light chain (NfL) levels are thought to reflect axonal injury and neurodegeneration, being increased not only in Alzheimer's disease¹¹ but also other neurodegenerative diseases^{12,13} and correlating with measures of brain atrophy on structural MRI.^{14,15} While these studies have shown an association between plasma biomarkers with some of the pathological processes associated with Alzheimer's disease using a cross-sectional study design, it is currently unclear whether baseline plasma biomarker levels can be used to predict longitudinal amyloid accumulation, tau deposition, brain atrophy as well as cognitive decline in the same individuals. Recent studies have shown that plasma P-tau181 is associated with global cognitive decline and hippocampal atrophy⁸ as well as temporal cortical thinning and cortical hypometabolism in individuals with a positive amyloid PET scan.¹⁶ In addition, high levels of plasma P-tau217 were recently found to correlate with increases in tau PET in the entorhinal cortex in subjects with a normal tau PET scan at baseline¹⁷ and longitudinal increases in this marker were associated with worse cognition and brain atrophy.¹⁸ However, the prognostic value of different plasma biomarkers, including amyloid burden, and tau accumulation, as well as temporal brain atrophy and cognitive decline, and whether they have an independent ability to anticipate future pathological and clinical changes associated with Alzheimer's disease remains unclear. This would be important, particularly in non-demented individuals, who are most likely to

benefit from future disease-modifying therapies if their increased risk of developing the disease can be identified in advance.¹⁹

To answer this question, in this study we examined individuals with baseline plasma A β 42/40, P-tau181, P-tau217 and NfL, in addition to longitudinal amyloid-PET, tau, structural MRI and cognitive measures. Our main goal was to determine which of these plasma biomarkers was the best independent predictor of future imaging and cognitive changes in non-demented individuals. Moreover, we compared the predictive ability of continuous plasma biomarker values with binary plasma biomarker values, which can be more easily implemented in the clinic and for decision-making in clinical trials. To examine the specificity of our findings we also studied these biomarkers in patients with a non-Alzheimer's neurodegenerative disease. Finally, we compared the predictive value of the plasma biomarkers to those obtained from the same biomarkers measured in the CSF.

Materials and methods

Participants

This study included 317 individuals from the Swedish BioFINDER-2 cohort (NCT03174938), an ongoing longitudinal study designed to develop new markers for the early diagnosis of Alzheimer's disease and other neurodegenerative disorders. Subjects with baseline plasma and CSF levels of A β 42/40, P-tau181, P-tau217 and NfL in addition to longitudinal Mini-Mental State Examination (MMSE) scores, ¹⁸F-RO948 PET, ¹⁸F-flutemetamol PET and structural MRI were included. All subjects had two to four longitudinal PET scans, MRI scans and cognitive evaluations over a period of 2 years. Amyloid status was established using CSF A β 42/40 levels with a previously established cut-off of <0.752, which was defined with mixture modelling.²⁰

The BioFINDER-2 study enrolls participants in five subcohorts (NCT03174938). Subcohorts 1 and 2 include neurologically and cognitively healthy elderly subjects, who were required to: (i) be 45–65 years old (subcohort 1) or 66–100 years old (subcohort 2); (ii) not have cognitive symptoms as assessed by a physician specialized in cognitive disorders; (iii) have an MMSE score between 27 and 30; (iv) not fulfil the criteria for mild or major neurocognitive disorder (mild cognitive impairment or dementia) according to DSM-51; and (v) be fluent in Swedish. The recruitment process of this cohort was designed to have 50% APOE ϵ 4 carriers.

Cohort 2 comprises participants with subjective cognitive decline or mild cognitive impairment, who were required to: (i) be 40–100 years old; (ii) have been referred to the memory clinics because of cognitive symptoms; (iii) have an MMSE score of 24–30 points; (iv) not

fulfil the criteria for any dementia (major neurocognitive disorder) according to DSM-51; and (v) be fluent in Swedish. In accordance with the research framework by the National Institute on Aging-Alzheimer's Association (NIA-AA),²¹ study participants with subjective cognitive decline were considered to be cognitively unimpaired. Participants were classified as having mild cognitive impairment if they performed worse than -1.5 standard deviations (SD) in any cognitive domain according to age and education stratified test norms.

Cohort 3 consists of participants with dementia due to Alzheimer's disease, who were required to: (i) be 40–100 years old; (ii) have been referred to the memory clinics because of cognitive symptoms; (iii) have an MMSE score of ≥ 12 points; (iv) fulfil the DSM-5 criteria for dementia (major neurocognitive disorder) due to Alzheimer disease; and (v) be fluent in Swedish. Clinical Alzheimer's disease dementia was diagnosed according to the DSM-5 criteria for major neurocognitive disorder. All patients with Alzheimer's disease were amyloid- β -positive in agreement with the updated NIA-AA criteria for Alzheimer's disease.²¹

Cohort 4 covers other non-Alzheimer's disease dementias and neurodegenerative disorders. Inclusion criteria were: (i) aged 40–100 years; (ii) fulfilment of criteria for dementia (major neurocognitive disorder) due to frontotemporal dementia, Parkinson's disease with dementia,²² subcortical vascular dementia,²² Parkinson's disease,²³ progressive supranuclear palsy,²⁴ multiple system atrophy,²⁵ corticobasal syndrome²⁶ or semantic variant primary progressive aphasia²⁷; and (iii) fluent in Swedish. Patients with amyloid pathology were excluded from this group to ensure there was no underlying concomitant Alzheimer's disease pathology.

Exclusion criteria for all subcohorts were: (i) having significant unstable systemic illness that makes it difficult to participate in the study; (ii) current significant alcohol or substance misuse; and (iii) refusing lumbar puncture, MRI or PET.

The Regional Ethical Review Board of Lund University, the Swedish Medicines and Products Agency, and the Radiation Safety Committee of Skåne University Hospital in Sweden approved the study and written, informed consent was obtained from all participants according to the Declaration of Helsinki.

Measurement of plasma and CSF biomarkers

Blood was collected from all participants using EDTA-plasma tubes (Vacutainer[®] K2EDTA tube, BD Diagnostics), which were centrifuged (2000g, $+4^{\circ}\text{C}$) for 10 min, transferred into 50 ml polypropylene tubes and mixed. Then, 1 ml was aliquoted into 1.5 ml polypropylene tubes and stored at -80°C within 30–60 min of collection. All plasma samples underwent one freeze-thaw cycle when 200 μl were further aliquoted into 0.5 ml Eppendorf tubes (Eppendorf Nordic A/S) and stored at -80°C .⁹ The CSF from the same individuals was collected through lumbar puncture using standardized procedures, as described elsewhere.²⁸ To establish the concentrations of the plasma and CSF markers of interest to this study, we used: (i) EUROIMMUN immunoassays (EUROIMMUN AG)²⁹ for plasma A β 42 and A β 40; (ii) Meso Scale Discovery (MSD) immunoassays for CSF A β 42⁶ and A β 40⁹ (MSD); (iii) CSF and plasma P-tau181³⁰ and CSF and plasma P-tau 271⁹ immunoassays developed at Lilly Research Laboratories; and (iv) a single molecule array (Simoa) assay for CSF and plasma NfL (Quanterix).¹²

APOE genotyping

APOE genotypes were determined in DNA extracted from a 5 ml aliquot of EDTA blood using PCR amplification complemented by hybridization using TaqMan[™] probes.

Imaging acquisition

All subjects underwent longitudinal ^{18}F -RO948 PET on a GE Discovery scanner and structural MRI on a Siemens Prisma 3 T scanner. In addition, 85 non-demented individuals also had longitudinal ^{18}F -flutemetamol PET scans on a Philips Gemini TF 16 scanner.

^{18}F -RO948 PET images were acquired 70–90 min after injection of 370 MBq ^{18}F -RO948, reconstructed using VPFX-S (ordered subset expectation maximization combined with corrections for time-of-flight and point spread function) with six iterations and 17 subsets with 3 mm smoothing, a standard Z filter and 25.6-cm field of view (256×256 matrix).

Structural T_1 -weighted images were acquired using a magnetization-prepared rapid gradient echo (MPRAGE) sequence using the following parameters: 178 slices, repetition time: 1950 ms, echo time: 3.4 ms, inversion time: 900 ms, flip angle: 9° , 1 mm isotropic voxels. In addition, fluid-attenuated inversion recovery (FLAIR) images were also acquired at baseline with the following parameters: 176 slices, repetition time: 5000 ms, echo time: 393 ms, inversion time: 1800 ms, 1 mm isotropic voxels.

Finally, ^{18}F -flutemetamol PET images were acquired 90 to 110 min after injection of 185 MBq ^{18}F -flutemetamol and reconstructed into 4×5 frames using the line-of-response row-action maximum-likelihood algorithm.

Longitudinal imaging preprocessing

All ^{18}F -RO948 and ^{18}F -flutemetamol PET images were motion-corrected, time-averaged and coregistered to their corresponding skull stripped, longitudinally preprocessed T_1 -weighted images. ^{18}F -RO948 images were further normalized by a reference region consisting of the inferior cerebellar grey matter,³¹ whereas ^{18}F -flutemetamol scans were normalized using a reference region that included the whole cerebellum, brainstem and eroded subcortical white matter.³² Structural MRI images were preprocessed using the longitudinal analysis pipeline of FreeSurfer (version 6.0, <https://surfer.nmr.mgh.harvard.edu/>). Briefly, after running the cross-sectional pipeline on each time point, an unbiased within-subject template was created. Several preprocessing steps such as skull stripping, Talairach transforms, atlas registration as well as spherical surface maps and parcellations were then performed with common information from the within-subject template.^{33–35}

FLAIR images were preprocessed using the Lesion Segmentation Toolbox³⁶ implemented in SPM8 (<https://www.fil.ion.ucl.ac.uk/spm/>) to generate total white matter lesion volumes for each individual, which were included in secondary analyses.

Longitudinal imaging analyses

To determine longitudinal changes in amyloid deposition on ^{18}F -flutemetamol PET, tau accumulation on ^{18}F -RO948 PET and brain atrophy on structural MRI, we used two different approaches: one based on regions of interest and the other based on whole brain voxel-wise analyses.

For the first approach, we calculated: (i) the amyloid- β PET standardized uptake value ratio (SUVR) for a global composite region that included the caudal anterior cingulate, frontal, lateral parietal and lateral temporal gyri³²; (ii) the tau SUVR of a composite region consisting of the entorhinal, fusiform, parahippocampus and inferior temporal gyri, corresponding to Cho stages I–IV³⁷; (iii) the mean cortical thickness of a meta-temporal region of interest that included the entorhinal, fusiform, inferior and middle temporal gyri on

structural MRI³⁸; and (iv) the average hippocampal volumes and corresponding intracranial volumes on structural MRI.

For the second approach, we created slope images for ¹⁸F-flutemetamol PET and ¹⁸F-RO948 PET images by subtracting the last longitudinal image to the first image of each individual. These difference maps were subsequently smoothed using a Gaussian kernel of 8 mm and voxel-wise analyses were carried out using the smoothed maps. To conduct voxel-wise analyses with the structural MRI scans, we used the longitudinal registration pipeline of the statistical parametric mapping software SPM12 (<https://www.fil.ion.ucl.ac.uk/spm/>). For each participant, the T₁-weighted scans were registered and bias-corrected using the longitudinal registration tool. An average T₁-weighted image was created for each individual and subsequently segmented into grey matter. The segmented grey matter maps were then multiplied by the Jacobian difference maps. Finally, using the forward deformation fields calculated in the previous steps, the resulting images were normalized to MNI space and smoothed using a Gaussian kernel of 12 mm.

Statistical analyses

Statistical analyses were carried out using SPSS 25.0 (IBM Corp.) and R (version 3.5.1). To test whether baseline biomarker levels were associated with longitudinal changes in brain imaging and cognition we used univariate and multivariate linear mixed effect models. These models used global amyloid SUVR, temporal tau SUVR, temporal thickness, hippocampal volumes or global cognition (MMSE) as dependent variables and the plasma biomarkers, time, age, sex, amyloid status, APOE ε4 carriership, presence of cognitive impairment, years of education (for cognitive variables) and intracranial volume (for volumetric variables) as fixed effects. We also included the interaction between biomarker levels and time (together with the main effects), and random effects for intercepts. Separate models were built for each plasma variable. The univariate models included only one plasma biomarker each. Model fits were compared (for the same outcomes) using an ANOVA and the Akaike information criterion (AIC) was reported for each model. In addition, the effect sizes of each predictor were also calculated using Cohen's *d*.

The multivariate models included all plasma biomarkers simultaneously as predictors. The aim of these models was to determine which plasma biomarker had a superior ability in predicting longitudinal changes, independently of the other biomarkers, which was the main aim of our study. In all multivariate models, the variance inflation factor (>5) was calculated to ensure there was no multicollinearity amongst the included variables. To explore the results obtained in the multivariate linear mixed models with imaging variables, we also performed voxel-wise multiple regression analyses using the longitudinal PET and MRI images in SPM12, including the significant plasma biomarkers as the variables of interest and age, sex, presence of cognitive impairment, amyloid status, APOE ε4 carriership and intracranial volume (MRI analyses) as covariates.

Finally, since binary biomarker values are easier to implement in clinical practice compared to continuous values, we dichotomized the plasma biomarkers into normal and abnormal. To do this, we used the Youden index to define optimal cut-offs in Aβ₄₂/40, P-tau₁₈₁, P-tau₂₁₇ and NfL plasma levels that could discriminate a sample of 121 amyloid-β-negative cognitively normal individuals of BioFINDER-2 from the 123 amyloid-β-positive demented patients with Alzheimer's disease described in Table 1, which were the following: 0.16 pg/ml for plasma Aβ₄₂/40, 7.48 pg/ml for plasma P-tau₁₈₁, 3.04 pg/ml for plasma P-tau₂₁₇ and 17.58 pg/ml for plasma NfL. These cut-offs were then applied to the non-

demented subjects of our cohort to divide them into normal and abnormal biomarker groups. We then repeated the linear mixed models with the binary biomarkers as predictors to assess whether they could predict changes in brain imaging and cognition, similarly to the continuous biomarkers.

All the analyses conducted in R and SPSS were adjusted for multiple comparisons using false discovery rate (FDR) corrections ($q < 0.05$, two-tailed).³⁹ Similarly, the voxel-wise analyses using PET and MRI images were adjusted for multiple comparisons with topological FDR corrections in SPM12 ($P < 0.05$, two-tailed).⁴⁰

Data availability

Anonymized data will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and providing that the data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the Ethical Review Board of Sweden and Region Skåne, which should be regulated in a material transfer agreement.

Results

Study participants

In total, 317 participants were included in this longitudinal study, of which 159 were non-demented (52 cognitively normal, 44 with subjective cognitive decline and 63 with mild cognitive impairment), 123 had dementia due to Alzheimer's disease and 35 had non-Alzheimer's neurodegenerative diseases (Table 1). Among individuals with mild cognitive impairment, 30 had a single domain amnesic cognitive profile, 19 had a single domain non-amnesic profile, 13 had a multidomain amnesic profile and 1 had a multidomain non-amnesic cognitive profile.

In this study, the main analyses were conducted in all non-demented individuals, whereas the Alzheimer's disease dementia and non-Alzheimer's groups were only included in sensitivity analyses. In the non-demented group, the majority of subjects had longitudinal ¹⁸F-RO948 tau PET and structural MRI (120 participants: 45 cognitively normal, 32 with subjective cognitive decline, 43 with mild cognitive impairment) and a subsample also had longitudinal ¹⁸F-flutemetamol PET (84 participants: 37 cognitively normal, 23 with subjective cognitive decline, 24 with mild cognitive impairment). In the non-Alzheimer's group, a subsample of 14 patients also had longitudinal ¹⁸F-RO948 tau PET and structural MRI.

As expected, non-demented individuals had higher cognitive scores, plasma Aβ₄₂/40 levels, hippocampal volumes and temporal thickness in addition to lower NfL levels compared to the other two groups. Further, they also had higher P-tau₁₈₁, P-tau₂₁₇ and tau-PET signals compared to the non-Alzheimer's group. The number of visits and longitudinal follow-up time for each assessment are described below as median followed by interquartile range (IQR). Additional information about the time to each follow-up can be found in Table 1. For the imaging and clinical variables that were significantly associated with baseline plasma biomarkers in the multivariate linear mixed effects models, we included the plots with the mean longitudinal trajectories (Figs 1–4) in addition to the spaghetti plots with the individual longitudinal trajectories (Supplementary Fig. 1). Finally, we also included the results of analyses performed in amyloid-positive non-demented individuals in Supplementary material and compare the results obtained in this group with the ones observed in the whole non-demented sample in the 'Discussion' section.

Table 1 Baseline cohort characteristics

	Non-demented (n = 159) (52 CN, 40 SCD, 67 MCI)	Non-AD dementia (n = 35)	AD dementia (n = 123)	P-value
Age	69.2 (42.4–87.5)	73.0 (57.6–87.3)	73.9 (52.8–87.6)	<0.001
Sex, male/female	87/72	24/11	56/67	0.927
Education	13.1 (7–33)	11.3 (7–22)	12.3 (4–25)	0.162
MMSE	28.0 (23–30)	23.5 (17–30)	20.1 (8–28)	<0.001
Amyloid- β positivity, %	8.2 CN, 22.6 SCD, 33.3 MCI	0	100	<0.001
APOE ϵ 4 carriership, %	54.7	25.7	72.4	<0.001
Plasma A β 42/40, pg/ml	0.18 (0.09–1.9)	0.17 (0.12–0.21)	0.16 (0.09–0.63)	0.395
Plasma P-tau181, pg/ml	7.9 (1.1–24.8)	8.7 (2.9–26.8)	13.1 (3.7–57.4)	<0.001
Plasma P-tau217, pg/ml	2.81 (0.5–36.0)	3.49 (0.5–40.6)	7.36 (0.7–21.4)	<0.001
Plasma NfL, pg/ml	17.5 (4.0–70.1)	24.2 (9.2–62.7)	26.9 (9.7–254.4)	<0.001
CSF A β 42/40, pg/ml	0.73 (0.30–1.46)	1.08 (0.8–1.3)	0.48 (0.21–0.79)	<0.001
CSF P-tau181, pg/ml	81.3 (12.4–289.3)	41.29 (17.0–81.0)	87.8 (18–193)	<0.001
CSF P-tau217, pg/ml	167.3 (8.6–785.8)	84.5 (19.7–499.5)	608.1 (65.3–2015.2)	<0.001
CSF NfL, pg/ml	1208.2 (250–10600)	1743.1 (390.0–4600.0)	2071.1 (330–9330)	<0.001
Amyloid-PET global composite SUVR ^a	0.79 (0.53–1.31)	–	–	–
Tau temporal composite SUVR ^b	1.27 (0.96–2.52)	1.09 (0.94–1.25)	2.26 (1.19–4.51)	<0.001
Temporal cortical thickness ^p	2.66 (2.17–3.11)	2.56 (1.84–2.95)	2.38 (1.58–2.74)	<0.001
Hippocampal volumes ^b	3583.4 (2199.5–4818.7)	3328.9 (1779.8–4289.3)	2791.6 (2023.7–3907.8)	<0.001
Time to longitudinal amyloid-PET	1.56 (0.92–1.96)	–	–	–
Time to longitudinal tau				
Second scan	1.26 (0.03–2.06)	1.17 (0.01–2.02)	–	0.003
Third scan	1.59 (0.85–1.87)	1.20 (1.10–1.39)	–	0.012
Fourth scan	1.60 (1.52–1.68)	–	–	–
Time to longitudinal MRI				
Second scan	1.26 (0.03–2.06)	1.17 (0.01–2.02)	–	0.002
Third scan	1.59 (0.85–1.87)	1.20 (1.10–1.39)	–	0.012
Fourth scan	1.60 (1.52–1.68)	–	–	–
Time to longitudinal cognitive assessment				
Second evaluation	1.21 (0.45–1.75)	1.05 (0.76–1.41)	–	0.217
Third evaluation	1.99 (1.65–2.68)	1.93 (1.21–2.48)	–	0.409

Data are presented as median (range) unless otherwise described. P-values were derived from Kruskal-Wallis tests for continuous non-normally distributed measures and chi-squared tests for categorical measures. Amyloid- β positivity was determined using a cut-off <0.8 using CSF A β 42/40. AD = Alzheimer's disease; CN = cognitively normal; MCI = mild cognitive impairment; SCD = subjective cognitive decline.

^aAmyloid-PET was only available for a subsample of subjects in the non-demented group (n = 86).

^bTau-PET and MRI data were only available for a subsample of subjects of the non-demented group (n = 120) and of the non-Alzheimer's disease group (n = 14).

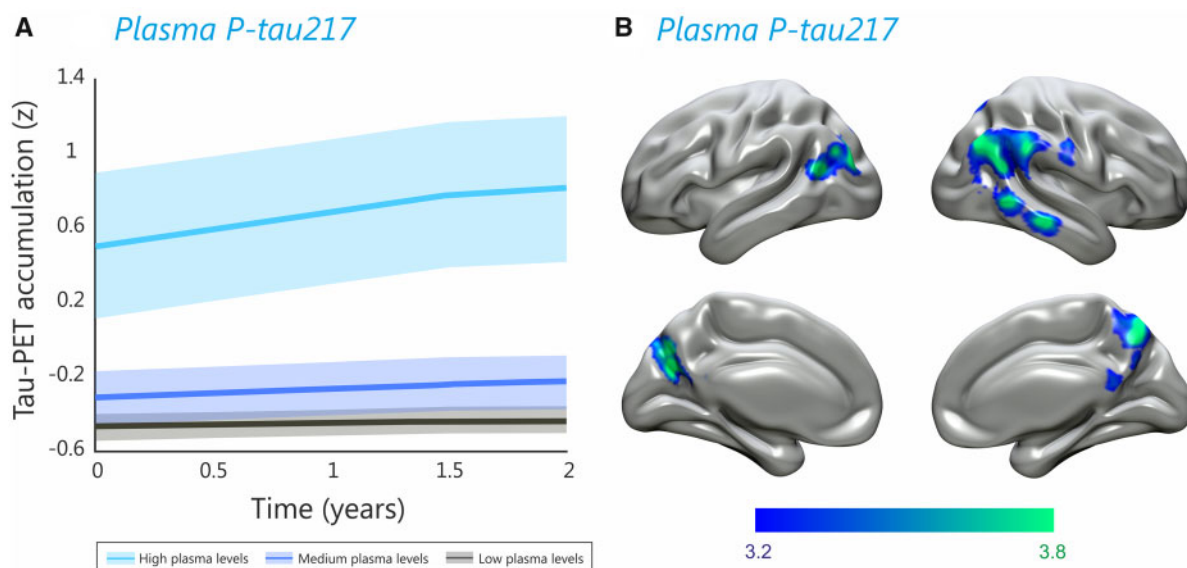


Figure 1 Plasma P-tau217 levels independently predict longitudinal tau accumulation in non-demented individuals. Predicted trajectories for temporal tau accumulation (z-scores) in relation to baseline plasma P-tau217. (A) The models were fit using continuous P-tau217 values but for illustration purposes the plots show the trajectories for individuals with high, medium and low plasma P-tau217 tertiles. (B) The voxel-wise analyses using longitudinal tau images showed a positive correlation between plasma P-tau217 and increased tau accumulation in temporal and parietal areas, after FDR corrections.

Relationship between plasma biomarkers and longitudinal tau in non-demented participants

To test whether baseline plasma biomarkers correlate with changes in tau burden over time (number of visits: median = 2, IQR = 1; follow-up time: median = 1.6, IQR = 0.7), for each biomarker we tested linear mixed-models with temporal tau SUVR as the outcome and the interaction between the plasma biomarker and time as a predictor, adjusting for age, sex and presence of cognitive impairment. These analyses showed that all baseline plasma biomarkers predicted greater longitudinal temporal tau accumulation (Supplementary Table 1). The model with plasma P-tau217 had a significantly better fit to the data compared to the other models, as reflected by the lowest AIC value (P-tau217: 270.6, P-tau181: 291.8, A β 42/40: 305.3, NfL: 306.0) (Supplementary Table 1). In addition, it also displayed the largest effect size (P-tau217 Cohen's *d*: 0.87) compared to the other biomarkers (P-tau181: 0.60, A β 42/40: -0.45, NfL: 0.44) (Supplementary Table 1).

When the interactions between time and each plasma biomarker were included in the same multivariate model, only P-tau217 remained as a significant independent predictor of longitudinal temporal tau accumulation ($t = 3.947$, $P < 0.001$) (Fig. 1A and Supplementary Fig. 1A), after verifying there were no multicollinearity issues in this model (Supplementary Table 2). These results were also confirmed by the voxel-wise analyses, which showed that P-tau217 correlated with greater tau accumulation in temporal regions in addition to lateral parietal and medial parietal areas (Fig. 1B), which overlapped with several brain areas showing increased longitudinal tau-PET deposition in our cohort (Supplementary Fig. 2). This could potentially be due to the limited variability in plasma P-tau217 values in our sample with tau-PET data, which consisted of 120 individuals. With larger sample sizes, most likely the overlap between the areas showing a correlation of P-tau217 and tau-PET with the areas showing longitudinal tau-PET changes would be greater due to higher variability in plasma P-tau217 values.

The results of significant interactions between all the predictors in the multivariate models can be found in the Supplementary material.

Relationship between plasma biomarkers and longitudinal amyloid-PET in non-demented participants

For individuals who underwent longitudinal amyloid-PET, we used linear mixed models to evaluate whether the plasma markers were also associated with amyloid changes over time in a neocortical composite region (number of visits: median = 1.5, IQR = 1; follow-up time: median = 0.5, IQR = 1.7). These analyses showed that plasma A β 42/40, P-tau217 and NfL, but not P-tau181, predicted longitudinal amyloid deposition, with the model including P-tau217 as a predictor having the lowest AIC values (P-tau217: 74.5, P-tau181: 97.6, A β 42/40: 90.1, NfL: 90.7) (Supplementary Table 1). The analysis of the effect sizes showed that A β 42/40 was the strongest predictor (Cohen's *d*: -0.81), followed by P-tau217 (Cohen's *d*: 0.73), NfL (Cohen's *d*: 0.65) and P-tau181 (Cohen's *d*: 0.42) (Supplementary Table 1).

However, when all biomarkers were included in the same model, only plasma A β 42/40 was a significant predictor of amyloid accumulation ($t = -2.578$, $P = 0.012$), suggesting that this marker is the only one that is associated with amyloid pathology independently of the other biomarkers (Fig. 2 and Supplementary Fig. 1B). There was no multicollinearity between all the predictors (Supplementary Table 2). The voxel-wise analyses did not show

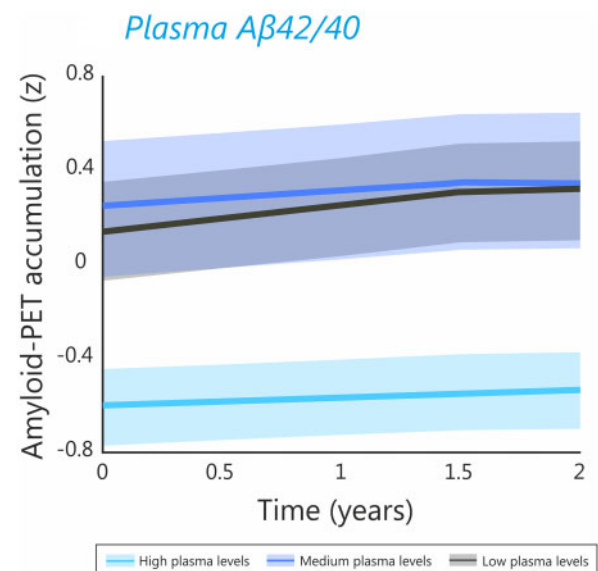


Figure 2 Plasma A β 42/40 levels independently predict longitudinal amyloid-PET deposition in non-demented individuals. Predicted trajectories for global amyloid-PET accumulation (z-scores) in relation to baseline plasma A β 42/40 in a subsample ($n = 86$) with available amyloid-PET data. The models were fit using continuous A β 42/40 values but for illustration purposes the plots show the trajectories for individuals with high, medium and low individuals with high, medium and low plasma A β 42/40 tertiles. Plasma A β 42/40 did not show significant results in the voxel-wise analyses, after adjusting for multiple comparisons.

significant results between plasma A β 42/40 levels and amyloid accumulation, after adjusting for multiple comparisons.

Relationship between plasma biomarkers with brain atrophy in non-demented participants

To assess whether plasma biomarkers could also predict longitudinal structural changes in brain areas that are known to be vulnerable to Alzheimer's disease such as temporal cortical areas and the hippocampus,⁴¹ we conducted the linear mixed model analyses using the cortical thickness of a composite temporal region³⁸ or the average hippocampal volumes as the outcome (number of visits: median = 2, IQR = 1; follow-up time: median = 1.6, IQR = 0.7). These analyses showed that baseline plasma P-tau217 and NfL levels predicted more severe temporal cortical thinning over time, whereas all plasma biomarkers predicted greater hippocampal volume loss. The comparisons between the models showed that plasma P-tau217 was the best predictor of both temporal thinning (AIC P-tau217: 352.7, P-tau181: 372.6, A β 42/40: 372.5, NfL: 365.1) and hippocampal atrophy (AIC P-tau217: 96.7, P-tau181: 116.3, A β 42/40: 121.4, NfL: 102.0) (Supplementary Table 1). In line with this, P-tau217 had the largest effect size (Cohen's *d*: -0.63) in the predictions of temporal thinning compared to NfL (Cohen's *d*: -0.48), P-tau181 (Cohen's *d*: -0.21) and A β 42/40 (Cohen's *d*: 0.11), whereas P-tau217 and NfL had the largest effects sizes (both with Cohen's *d*: -0.79) in the predictions of hippocampal atrophy compared to P-tau181 (Cohen's *d*: -0.53) and A β 42/40 (Cohen's *d*: 0.39) (Supplementary Table 1).

When all plasma biomarkers were included in the same model, P-tau217 was the only significant independent predictor of temporal cortical thinning ($t = -3.048$, $P = 0.003$) (Fig. 3A and Supplementary Fig. 1C), whereas both P-tau217 and NfL were significant predictors of hippocampal volume loss (P-tau217: $t = -2.958$, $P = 0.004$; NfL: $t = -2.794$, $P = 0.006$) (Fig. 3B, C and Supplementary Fig.

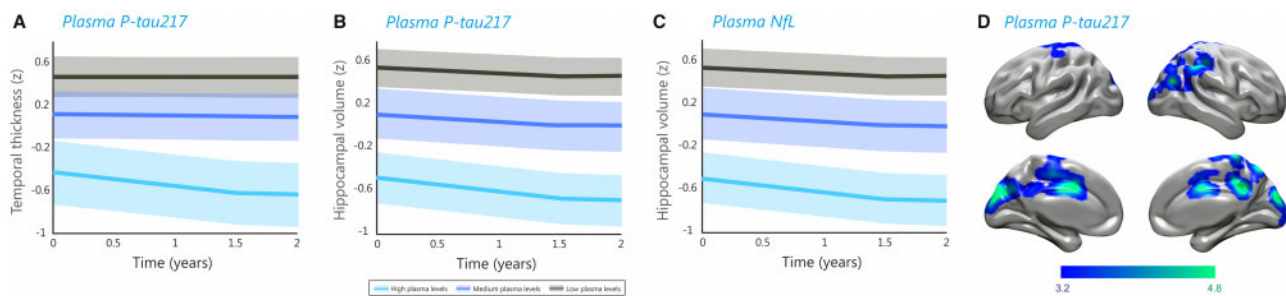


Figure 3 Plasma P-tau217 and NfL levels independently predict longitudinal brain atrophy in non-demented individuals. Predicted trajectories for temporal cortical thickness and hippocampal volumes (z-scores) in relation to baseline plasma P-tau217 and plasma NfL. (A–C) The models were fit using continuous P-tau217 and NfL values but for illustration purposes the plots show the trajectories for individuals with high, medium and low plasma P-tau217 tertiles for (A) temporal cortical thinning and (B) hippocampal volume loss as well as (C) individuals with high, medium and low plasma NfL tertiles. (D) The voxel-wise analyses showed a positive correlation between plasma P-tau217 and longitudinal brain atrophy in parietal and occipital areas, after FDR corrections. Plasma NfL did not show significant results in the voxel-wise analyses, after adjusting for multiple comparisons.

1D and E). There was no multicollinearity between all the predictors (Supplementary Table 2). These results were further confirmed by the voxel-wise analyses, which show that P-tau217 was associated with greater parietal, cingulum and occipital atrophy (Fig. 3D), in line with this biomarker being highly sensitive to both cortical and subcortical atrophy. The results of significant interactions between all the predictors in the multivariate models can be found in the Supplementary material.

Relationship between plasma biomarkers and cognitive decline in non-demented participants

To determine the clinical value of plasma biomarkers to predict decline in global cognition, we conducted linear mixed model analyses using the MMSE scores as the outcome (number of visits: median = 2, IQR = 2; follow-up time: median = 1.0, IQR = 1.8). The results of these analyses showed that plasma P-tau181 and P-tau217 levels were associated with longitudinal decline in cognition, in contrast to A β 42/40 and NfL. The comparisons between the previous significant models showed that the one with P-tau217 had again the best fit to the data (AIC P-tau217: 166.4, P-tau181: 170.6, A β 42/40: 178.0, NfL: 176.3) (Supplementary Table 1), even after adjusting for the different cognitive profiles (amnestic, non-amnestic, single domain, multidomain) in individuals with mild cognitive impairment (AIC P-tau217: 154.6, P-tau181: 158.4, A β 42/40: 165.7, NfL: 157.1). In line with this, P-tau217 showed the largest effect sizes (Cohen's *d*: -0.49) in the predictions of MMSE scores compared to P-tau181 (Cohen's *d*: -0.46), NfL (Cohen's *d*: -0.32) and A β 42/40 (Cohen's *d*: 0.21) (Supplementary Table 1).

When all biomarkers were included in the same multivariate model, only P-tau217 was a significant independent predictor of cognitive decline ($t = -2.275$, $P = 0.024$) (Fig. 4 and Supplementary Fig. 1F). There was no multicollinearity between all the predictors (Supplementary Table 2). The results of significant interactions between all the predictors in the multivariate models can be found in the Supplementary material.

We also conducted additional analyses to assess whether baseline white matter lesion volumes influenced cognitive decline. The results showed that they were not significant predictors of cognitive decline in the individuals of our cohort ($t = -0.071$, $P = 0.944$), even when added as interaction terms in the models with the plasma markers (P-tau217 model: $t = 1.432$, $P = 0.156$; P-tau181 model: $t = 1.354$, $P = 0.179$; A β 42/40 model: $t = 1.476$, $P = 0.143$; NfL model: $t = 1.382$, $P = 0.170$).

Binary plasma biomarkers are associated with longitudinal imaging changes and cognitive decline

We dichotomized the plasma biomarkers into normal and abnormal using the Youden index (see 'Materials and methods' section). Similar to the analyses using the continuous biomarkers as predictors, we found that binarized A β 42/40 (normal versus abnormal levels) was the only independent predictor of amyloid PET accumulation ($t = 2.560$, $P = 0.012$). Moreover, binarized P-tau217 was the only independent predictor of temporal cortical thinning ($t = -3.072$, $P = 0.003$), and together with binarized P-tau181 levels, also independently predicted tau accumulation (P-tau217: $t = 4.289$, $P < 0.001$; P-tau181: $t = 2.795$, $P = 0.006$), hippocampal atrophy (P-tau217: $t = -2.978$, $P = 0.003$; P-tau181: $t = -2.325$, $P = 0.021$) and cognitive decline (P-tau217: $t = -3.208$, $P = 0.002$; P-tau181: $t = -3.088$, $P = 0.002$).

Elevated plasma A β 42/40 and P-tau217 are not associated with changes in non-Alzheimer's disorders

To test whether our results are specific for Alzheimer's disease, we also conducted the linear mixed model analyses in a group of amyloid-negative patients with a non-Alzheimer's neurodegenerative disorder (frontotemporal dementia, Parkinson's disease with dementia, subcortical vascular dementia, progressive supranuclear palsy, multiple system atrophy, corticobasal syndrome or semantic variant primary progressive aphasia). These analyses showed that NfL levels were associated with more severe cognitive decline ($t = -2.784$, $P = 0.008$). The other plasma markers did not predict longitudinal changes in brain imaging or cognitive measures in this non-Alzheimer's group (Supplementary Table 3).

Comparison with CSF biomarkers

To assess whether CSF A β 42/40, CSF P-tau181, CSF P-tau217 and CSF NfL have a similar predictive ability compared to their plasma counterparts, we compared the models with CSF and plasma predictors for each longitudinal outcome using ANOVA. These analyses revealed there were no significant differences between the models with CSF A β 42/40 and plasma A β 42/40 (Supplementary Table 4). On the other hand, CSF P-tau181 was a worse predictor (higher AIC values) of cognitive decline than plasma P-tau181 but performed equally well in the prediction of all other outcomes (Supplementary Table 5). Moreover, CSF P-tau217 was a worse predictor (higher AIC values) of tau accumulation, temporal cortical thinning and hippocampal atrophy compared to plasma P-tau217

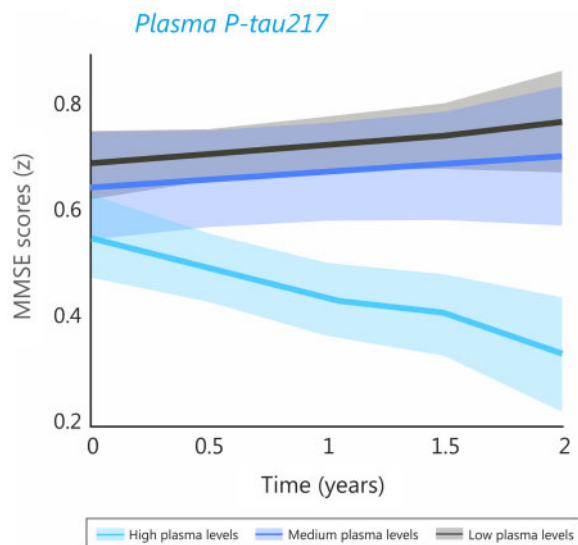


Figure 4 Plasma P-tau217 levels independently predict cognitive decline in non-demented individuals. Predicted trajectories for global cognitive decline measured with the MMSE (z-scores) in relation to baseline plasma P-tau217. The models were fit using continuous P-tau217 values but for illustration purposes the plots show the trajectories for individuals with high, medium and low plasma P-tau217 tertiles.

but performed equally well in predicting amyloid accumulation or cognitive decline (Supplementary Table 6). Finally, CSF NfL was a worse predictor of temporal cortical thinning, amyloid accumulation and hippocampal volume (higher AIC values) but performed equally well in predicting tau or cognitive decline compared to plasma NfL (Supplementary Table 7). Altogether, these results suggest that CSF biomarkers performed equally well or worse in the predictions of longitudinal imaging and cognitive changes compared to the plasma biomarkers.

Discussion

With the potential development of new disease-modifying therapies for Alzheimer's disease, a uniform, simple and widely accessible test is urgently needed to identify high-risk individuals who should be further evaluated for treatment.¹⁰ Blood-based biomarkers are non-invasive and cost-effective tools that could be used to identify such individuals. However, it is currently unclear whether these biomarkers are able to predict longitudinal pathological changes associated with Alzheimer's disease. In this study, we show that plasma A β 42/40 is an independent predictor of future amyloid accumulation, whereas plasma P-tau217 is an independent predictor of tau accumulation, temporal cortical thinning, hippocampal atrophy and global cognitive impairment in non-demented individuals. Moreover, the plasma biomarkers performed equally well or better than the corresponding biomarkers in CSF. These findings suggest that plasma A β 42/40 and P-tau217 could be candidate prognostic markers to predict future brain abnormalities and clinical deficits associated with Alzheimer's disease.

Several studies have begun to assess the utility of blood-based markers to detect the underlying amyloid and tau pathological processes that characterize Alzheimer's disease. For instance, plasma A β 42/40 concentrations have been shown to identify baseline amyloid status defined on amyloid-PET scans.^{4–6} In addition, recent cross-sectional studies have shown that the novel plasma P-tau181 and P-tau217 markers are increased since early stages of Alzheimer's disease, correlating with tau deposition on tau scans

and discriminating Alzheimer's from non-Alzheimer's disorders.^{7,9,17} Our study extends these cross-sectional findings by showing that baseline plasma biomarkers can furthermore predict longitudinal amyloid and tau accumulation in non-demented individuals. Specifically, we observed that although several plasma biomarkers were associated with amyloid accumulation in the univariate models, plasma A β 42/40 was the only biomarker that independently predicted increased global amyloid deposition over time in the multivariate models. These results are in line with amyloid-PET deposition being closely related with amyloid levels in the blood.⁴ In addition, we also found that blood P-tau217 levels at baseline was an independent predictor of increased tau accumulation over time and furthermore correlated with voxel-wise tau accumulation in temporal and parietal regions. We have recently shown that plasma P-tau217 has a significantly higher diagnostic accuracy for Alzheimer's disease than other plasma biomarkers, being associated with the density of tau tangles and becoming elevated as early as 20 years before disease onset in familial mutation carriers.⁹ Thus, it is plausible that plasma P-tau217 is also a better indicator of longitudinal tau accumulation, even when compared to plasma P-tau181. This would be in line with recent studies showing that CSF P-tau217 is more strongly associated with tau deposition than CSF P-tau181^{30,42} and might reflect better the pathological state of tau associated with the formation of paired helical tau filaments.⁴³

In addition to being a reliable marker for longitudinal tau accumulation, our multivariate models show that plasma P-tau217 also independently predicted temporal cortical thinning and hippocampal atrophy in non-demented individuals, in contrast to NfL, which only independently predicted hippocampal atrophy. These results were further confirmed by the voxel-wise analyses, which showed that plasma P-tau217 levels correlated with longitudinal voxel-wise atrophy in parietal, cingulum and occipital regions, being therefore sensitive to both cortical and subcortical brain atrophy. It is well established that brain atrophy in Alzheimer's disease begins in the hippocampus and temporal areas, which then spreads to medial parietal regions and surrounding areas due to the connections between the hippocampus and the posterior cingulate.⁴³ The associations between P-tau217 levels with longitudinal atrophy in the hippocampus, other temporal areas and parietal regions seem to reflect this atrophy pattern. This higher sensitivity of P-tau217 to brain atrophy in areas that are typical of Alzheimer's disease could be related to the vulnerability of these areas to tau accumulation. Previous studies have shown that tau burden in temporal brain areas correlates with volume loss within the same areas in early stages of the disease,^{44–46} which could be due to the toxic effects of tau on neuronal volume and dendritic complexity.^{47,48} These anatomical changes in temporal areas eventually lead to global cognitive decline,^{1,49,50} which in our study was also independently predicted by baseline plasma P-tau217 levels. Thus, altogether, these findings suggest that plasma P-tau217 is a sensitive marker of multiple changes associated with Alzheimer's disease, including tau burden, brain atrophy and clinical decline. This was further strengthened by the comparison between the univariate models with single biomarkers, which showed that the models with P-tau217 had the best fit to the data. Finally, the lack of ability of plasma P-tau217 or plasma A β 42/40 to predict cognitive decline in non-Alzheimer's patients, in contrast to NfL, suggest that P-tau217 and A β 42/40 are not only sensitive but also quite specific to Alzheimer's disease. Moreover, we also found that P-tau217 and A β 42/40 did not predict changes in tau accumulation or brain atrophy in the non-Alzheimer's group. However, this finding must be interpreted with caution as only 14 of 35 patients with a non-Alzheimer's disorder underwent

longitudinal tau-PET and MRI, which most likely limited the statistical power of these analyses.

Of note, in the current study, we performed the main analyses in the whole non-demented group independently of amyloid-status since plasma biomarkers are most useful in clinical practice and trials where PET imaging or CSF lumbar puncture have not already been done due to the low accessibility, high cost or invasiveness of these procedures.⁵² In other words, plasma biomarkers are probably most useful in contexts where the amyloid status of a patient is unknown and not in deeply phenotyped populations where amyloid (and maybe tau) CSF or PET examinations have already been performed.

In fact, it has been recently proposed that plasma biomarkers should be utilized in clinical trials of Alzheimer's disease as a pre-screening tool to identify individuals who have not yet undergone CSF or PET in order to identify cases with a higher risk of developing this disorder.⁵² For instance, in an anti-tau trial with tau PET and cognition as outcomes, based on our results, one could use plasma P-tau217 to identify those with high risk of accumulating tau and exhibiting cognitive decline over time. However, before entering the trial, a tau PET scan would need to be done and probably also an amyloid PET scan, but both PET acquisitions would only be performed in individuals with high P-tau217 levels. Hence, by excluding subjects with low plasma P-tau217 levels from PET scanning, the costs associated with these clinical trials would be significantly reduced.

From a clinical point of view, we think that both plasma A β 42/40 and P-tau217 might be used in the future as initial tests in primary healthcare settings where CSF and PET are not available to identify individuals with a higher risk of subsequent amyloid accumulation, tau accumulation, brain atrophy and cognitive decline. These high-risk individuals could then be referred to specialized memory clinics to undergo a more thorough diagnostic work-up before starting relevant treatments.

Our study has several strengths, including the large number of participants with several plasma biomarkers and longitudinal amyloid-PET, tau, structural MRI as well as cognitive measures. In addition, all subjects had CSF A β 42/40, P-tau181, P-tau217 and NfL levels, which showed either similar or worse predictive ability compared to the plasma biomarkers. Finally, we also performed our analyses using binary plasma biomarker levels, which showed a similar ability to predict longitudinal imaging and clinical changes compared to the continuous plasma levels, indicating they could be easily implemented in clinical practice. However, a few limitations should also be recognized such as the fact that many subjects had not yet undergone longitudinal amyloid-PET scanning at the time of the study, which could explain why no significant results were found in the associations between plasma A β 42/40 levels and voxel-wise amyloid accumulation after adjusting for multiple comparisons. In addition, we did not have an independent cohort that we could use to confirm our findings, but we are planning to do this in the future when the plasma markers and longitudinal imaging techniques we used become available in other relevant longitudinal cohorts. Finally, the small number of subjects included in the non-Alzheimer's group, which included diverse diagnoses with more homogenous amyloid levels is also a limitation. Future studies assessing our panel of plasma biomarkers in separate and larger groups with different non-Alzheimer's disorders are needed in order to establish the specificity of plasma A β 42/40 and P-tau217 for Alzheimer's disease.

In summary, here we show that baseline plasma A β 42/40 and P-tau217 levels are independent predictors of pathological changes that occur over the course of Alzheimer's disease. These biomarkers performed equally well or better than the corresponding biomarkers in CSF. These findings indicate that plasma A β 42/40

and P-tau217 could be used as important prognostic tools to estimate the progression of the disease in clinical practice, allowing for a more accurate patient management and better disease monitoring in future clinical trials.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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