Plasma phospho-tau181 as a biomarker for Alzheimer's disease: development and validation of a prediction model using data from four prospective cohorts

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SUMMARY

Background

Cerebrospinal fluid (CSF) and positron emission tomography (PET) biomarkers of amyloid-β (Aβ) and tau are accurate for detecting Alzheimer's disease pathology but invasiveness, high-cost, and limited availability hamper widespread clinical diagnostic use. CSF phosphorylated-tau181 (p-tau181) is highly specific for Alzheimer's disease pathology. We aimed to assess whether blood p-tau181 can differentiate Alzheimer's disease dementia from unimpaired cognitive function, mild cognitive impairment (MCI) due to Alzheimer's disease, and other neurodegenerative diseases; detect whether a tau or amyloid PET scan is abnormal; and predict future cognitive decline and hippocampal atrophy.

Methods

We developed and validated an ultrasensitive blood immunoassay for p-tau181. Assay performance was evaluated in four clinic-based prospective cohorts. The discovery cohort comprised 19 patients with Alzheimer's disease and 18 age-matched controls. Two validation cohorts (TRIAD, *n*=226 and BioFINDER-2, *n*=763) included cognitively unimpaired elderly people aged 63-69 years, patients with MCI, Alzheimer's disease, and frontotemporal dementia, as well as healthy young adults (mean age 23 years) in TRIAD and patients with other neurodegenerative disorders in BiOFINDER-2. The final primary-care cohort comprised 105 controls from the community without a diagnosis of a neurological condition and patients referred from primary care physicians for specialist care. Concentrations of plasma p-tau181 were compared with established CSF and PET biomarkers and longitudinal measurements, using Spearman correlation, area under the curve (AUC), and linear regression analyses.

Findings

Plasma p-tau181 showed gradual increases along the Alzheimer's disease continuum, from Aβ-negative young adults and cognitively unimpaired (CU)-elderly over Aβ-positive CU-elderly and mild-cognitive impaired (MCI) cases to Aβ-positive MCI and Alzheimer's disease dementia (P<0·0001, Alzheimer's disease versus all others). Plasma p-tau181 distinguished Alzheimer's disease dementia from Aβ-negative young adults (AUC=99·40%) and CU-elderly (AUC=90·21%-98·24%), as well as other neurodegenerative disorders, including frontotemporal dementia (AUC=82·76-100%), vascular dementia (AUC=92·13%), progressive supranuclear palsy/corticobasal syndrome (AUC=88·47%), and Parkinson's disease/multiple systems atrophy (AUC=84·81%). Plasma p-tau181 was associated with PET-measured cerebral tau (AUC=82·37-93·11%) and Aβ (AUC=76·14-88·09%) pathologies, and one-year cognitive decline and hippocampal atrophy (P<0·05). In primary-care, plasma p-tau181 discriminated Alzheimer's disease from young adults (AUC=100%) and CU-elderly (AUC=84·44%). Plasma p-tau181 outperformed each of age, *APOE* ε4 genotype carriage, age and *APOE* ε4 combined, and other plasma biomarkers (total-tau, $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ and total-tau/$A\beta_{1\text{-}42}$) in predicting each of Alzheimer's disease diagnosis, tau PET and Aβ PET positivity.

Interpretation

Blood p-tau181 predicts tau and Aβ pathologies, differentiates Alzheimer's from other neurodegenerative disorders, and identifies Alzheimer's disease across the clinical continuum in both primary-care and specialist settings. Blood p-tau181 may be a simple, accessible and scalable test for screening and diagnosis of Alzheimer's disease.

Funding

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KEYWORDS

Alzheimer's disease; tauopathies; phosphorylated tau-181; blood; plasma; tau PET; amyloid PET; diagnostic accuracy; sensitivity and accuracy

RESEARCH IN CONTEXT

Evidence before this study

Diagnosing Alzheimer's disease is challenging, partly due to the closely related pathological features shared with other neurodegenerative diseases. Presently, a definite diagnosis of Alzheimer's disease can only be established by *post mortem* pathological examination focusing on two main pathological hallmarks: (i) amyloid plaques consisting of aggregated amyloid beta (Aβ) peptides, and (ii) neurofibrillary tangles made of abnormally phosphorylated tau protein. In living individuals, Alzheimer's disease diagnosis relies on two main approaches: (i) imaging of the accumulation of tau tangles and Aβ plaques in the brain using positron emission tomography (PET), and (ii) measuring brain-specific biochemical changes in CSF reflecting tau and Aβ pathophysiology. However, tau PET is expensive and only available in specialised medical centres. In 1995, our group developed two immunoassays for quantifying tau in CSF, one for measuring pathological tau phosphorylated at threonine-181 (p-tau181) and the other for the neuronal injury marker "total tau." These assays, targeting mid-region tau species, were subsequently developed into commercial kit assays, and have recently been approved by the United States Food and Drugs Administration to support diagnosis and candidate drug testing. The assays have been used in hundreds of published independent clinical studies. In reviewing

such previous work, we searched PubMed for all articles published from database inception to January 20, 2020, without language restrictions, using the keywords “tau”, “phosphorylated tau”, “CSF tau”, “CSF biomarker”, “Alzheimer’s disease”, “plasma tau”, “amyloid”, “MRI”, “PET”, “cognitive decline” and “hippocampal atrophy”. We found that CSF p-tau181, but not “total tau,” is highly specific for Alzheimer’s disease; this biomarker is not altered in neurodegenerative diseases without Alzheimer co-pathology. Moreover, CSF p-tau181 correlates strongly with cognitive impairment, hippocampal atrophy, Aβ and tau PET. However, the usability of CSF p-tau181 is restricted by the need of a lumbar puncture. Due to this shortcoming, there is a need for an easily accessible p-tau181 blood test that can reliably detect key Alzheimer’s disease pathophysiological processes to enable research, diagnosis and drug development. Nonetheless, attempts to develop a reliable a blood p-tau181 assay have been challenging due to the very low concentrations in blood samples. Furthermore, initial unsuccessful efforts were concentrated on applying the established mid-region CSF p-tau181 immunoassays directly on blood. Recent evidence has shown that tau in blood and CSF may be processed differently, with mainly N-terminal forms of tau present in measurable quantities in blood. A few studies, each targeting different tau species, have described blood p-tau181 immunoassays showing encouraging results in limited patient cohorts. However, some of these assays lack the analytical sensitivity for examining cognitively unimpaired individuals some of whom may be in the preclinical phase of Alzheimer’s disease. Moreover, it is unclear if the published blood p-tau181 assays detect either Alzheimer-specific tau pathology similar to CSF p-tau181 or tau pathology that is common to all neurodegenerative diseases characterized by the presence of pathological tau.

Added value of this study

In this study, we present a blood-based immunoassay measuring p-tau181 on a novel N-terminal form of tau that is distinct from the mid-region forms targeted by the established CSF assays. This assay was validated to be specific for the p-tau181 site, does not capture non-

phosphorylated tau species, and shows excellent diagnostic performance for Alzheimer's disease in both plasma and serum. Due to its high-sensitivity, the assay was able to measure blood p-tau181 in 1,131 study participants, including healthy young adults aged ~23 years. Blood p-tau181 was measurable in CSF, and correlated strongly with both mid-region CSF p-tau181 and tau PET, indicating that all three methods recognise brain-derived tau. The blood p-tau181 assay identified incipient Alzheimer's disease at the very early stages by differentiating between cognitively unimpaired (CU) elderly individuals without brain Aβ aggregates from CU elderly with Aβ-pathology. Furthermore, plasma p-tau181 demonstrated high diagnostic accuracy for Alzheimer's disease, showing stepwise increases along the whole Alzheimer's disease continuum; the assay discriminated Aβ-positive CU elderly and Aβ-positive mild cognitive impaired (MCI) cases from Aβ-negative CU elderly and young adults. Importantly, replication of the excellent diagnostic performance of blood p-tau181 to identify Alzheimer's disease in independent cohorts classified differently (either using CSF core biomarkers only, clinical diagnosis only, or clinical diagnosis in addition to CSF core biomarkers as well as tau and Aβ PET) suggests that plasma p-tau181 has robust performance irrespective of the classification method used. Similar to mid-region CSF p-tau181, our blood p-tau181 appeared specific to Alzheimer's disease, differentiating it from other neurodegenerative diseases with high accuracy. In addition, blood p-tau181 predicted cognitive decline and hippocampal atrophy over a period of one-year, making it suitable as an Alzheimer's disease progression marker that can also be used as an outcome measure in clinical trials. Furthermore, plasma p-tau181 performed better than the most well-known Alzheimer's disease risk factors, that is, age and *APOE* ε4 – both singly and combined – as well as other plasma biomarkers (total tau, $A\beta_{1-42}$, $A\beta_{1-42}/A\beta_{1-40}$ and total-tau/$A\beta_{1-42}$) in predicting each of Alzheimer's disease diagnosis, as well as increased tau PET and Aβ PET.

Implications of all the available evidence

The blood p-tau181 assay described in this study may represent the first simple, practical and scalable test for the diagnosis of Alzheimer's disease. This technology has immediate applications for diagnosis and recruitment for disease-modifying trials. This assay has the potential to be incorporated in clinical practice as a rapid screening test to identify or rule out Alzheimer's disease pathophysiology and guide therapy and clinical management of patients with suspected neurodegenerative disorders.

INTRODUCTION

With over 50 million sufferers worldwide, the cost of dementia care reached a trillion US dollars in 2018[1]. Amyloid-β (Aβ) and tau pathology are the defining pathological features of Alzheimer's disease[2]. *In vivo* detection of these processes is central to disease diagnosis[3], its biological definition[4], and for selecting individuals for clinical trials[5]. Although cerebrospinal fluid (CSF) and positron emission tomography (PET) biomarkers of Aβ and tau are highly accurate for detecting Alzheimer's disease pathology, their costs and limited availability hamper their feasibility for use in clinical diagnostic practice and for screening in clinical trials[6].

The accessibility and cost-effectiveness of blood-based biomarkers make them highly attractive for first-line clinical use and to facilitate clinical trial recruitment and monitoring[7] Blood neurofilament light chain, a marker of neuronal injury, is increased in Alzheimer's disease[8], but this biomarker has low specificity, since abnormal increases are reported also in several other neurodegenerative disorders such as multiple system atrophy, corticobasal syndrome, and progressive supranuclear palsy[9].

Other advances include mass spectrometry-based assays for plasma Aβ ($A\beta_{1-42}/A\beta_{1-40}$), that reflect brain amyloidosis[10,11]. However, these assays have limitations, including substantial peripheral Aβ expression[12] giving less pronounced decreases and larger overlap of $A\beta_{1-42}/A\beta_{1-40}$ in plasma than in CSF between Aβ-PET positive and negative individuals[10]. Furthermore, brain amyloidosis is present in 10-30% of cognitively unimpaired (CU) individuals[13]. On the contrary, CSF phosphorylated-tau181 (p-tau181) is a highly specific pathological marker of Alzheimer's disease that remains normal in other dementias[14]. Thus, a blood test for p-tau181 would be a major advance for diagnostics. Some previous studies using immunoassays targeting distinct tau species reported promising results for blood p-tau181 as a biomarker for Alzheimer's disease[15–18]. However, some of these assays lack the analytical sensitivity for examining preclinical and CU individuals, and it is unclear if Alzheimer-specific tau pathology is detected. In this study, we report, in four independent populations, the performance of an ultra-sensitive immunoassay for blood p-tau181 that can be implemented for a practical assessment of *in vivo* Alzheimer's disease pathophysiology. We studied whether blood p-tau181 can: (i) differentiate Alzheimer's disease dementia from CU, mild cognitive impairment (MCI) due to Alzheimer's disease, and other neurodegenerative diseases; (ii) detect whether a tau or amyloid PET scan is abnormal; and (iii) predict future cognitive decline and hippocampal atrophy.

METHODS

Study participants

We used four independent clinic-based prospective cohorts recruiting consecutive cases. The discovery cohort included Alzheimer's disease patients (*n*=19) with typical Alzheimer's disease core CSF biomarkers profile (specifically CSF $A\beta_{1-42}$ <530 ng/L, p-tau181 >60 ng/L, and total-tau >350 ng/L[19]), and age-matched controls (*n*=18) who were patients examined at the memory

or neurology clinics for minor neurological or psychiatric symptoms, and with both basic and core CSF biomarkers levels within normal ranges.

Two independent validation cohorts were evaluated, from the TRIAD (*n*=226, McGill University, Canada) and the Swedish BioFINDER-2 (*n*=763, Lund University, Sweden) studies. Participants in both cohorts underwent detailed assessments including CSF ($A\beta_{1-42}$, p-tau181, and total-tau), and PET (tau and Aβ) biomarkers, as well as detailed clinical and cognitive evaluations. Both cohorts included CU elderly, MCI, Alzheimer's disease dementia, and frontotemporal dementia patients. In addition, TRIAD included young adults while BiOFINDER-2 had other neurodegenerative disorders.

Finally, we tested the feasibility of using the assay as a rapid screening tool in a primary-care cohort (*n*=105) that included controls from the community without diagnosis of a neurological condition and patients referred from primary care physicians for specialist care. These patients had received clinical diagnosis in the primary-care setting, but were yet to undergo biomarker and clinical assessments in specialist centers.

All studies were approved by the relevant ethical committees, and written informed consent obtained for all participants where necessary. For further details about the study participants, see the appendix (pp 5-6).

Outcomes

In the discovery cohort, CSF p-tau181, total tau and $A\beta_{1-42}$ were measured during February to March 2019 using the established Innotest® ELISA assays from Fujirebio, as described previously[20]. Biomarker-positive Alzheimer's disease diagnosis was achieved using previously-defined cut-offs[19]. The fully-automated LUMIPULSE® G1200 (Fujirebio) was used

to measure CSF p-tau181, total-tau and $A\beta_{1-42}$ for the TRIAD and the primary-care cohorts during August to December 2019. For BioFINDER-2, the Mesoscale Discovery assays were used to measure CSF $A\beta_{1-42}$ and $A\beta_{1-40}$.

In the TRIAD cohort, individuals were assessed using 3T magnetic resonance imaging (MRI) as well as Aβ [^{18}F]AZD4694 PET and tau [^{18}F]MK-6240 PET during April 2017 to June 2019. In the BioFINDER-2 cohort, individuals had MRI, Aβ [^{18}F]flutemetamol PET, and tau [^{18}F]RO948 PET during May 2017 to October 2019. Postmortem Braak staging suggests that the accumulation of tau neurofibrillary tangles in Alzheimer's disease follows a typical pattern that begins in the transentorhinal cortex (stage I-II), spreading to limbic (III-IV), and isocortical (V-VI) regions[21]. We segregated individuals into Braak-staged groups based on *in vivo* tau PET deposition in regions corresponding to Braak I-II, Braak III-IV, and Braak V-VI. Tau PET SUVR was measured regionally in the transentorhinal (stage I-II), limbic (III-IV), and isocortical (V-VI) Braak regions, as previously described[22], as well as globally in a composite area including the whole cortex (Braak stage I-VI regions), and tau positivity defined as 2.5 standard deviations (SD) higher than the mean SUVR of Aβ-negative cognitively unimpaired (CU) elderly. Further details are available in the appendix (pp 6-7).

In individuals in TRIAD (*n*=88) who had baseline plasma p-tau181 measures as well as both baseline and one-year follow-up Mini Mental State Examination (MMSE) scores and structural MRI assessments, we evaluated the associations between baseline plasma p-tau181 concentrations and one-year longitudinal change in cognitive function and neurodegeneration, using linear regression analyses. MMSE is a neuropsychiatric test of cognitive function whilst structural MRI provides insights into brain atrophy. Brain atrophy was measured with the analysis of gray matter density on T1-weighted MRI images using voxel-based morphometry.

The linear regression analyses accounted for the following potential confounding variables: age, gender, *APOE* ε4 genotype carriage, and years of education.

Predictors

Plasma p-tau181 for the four cohorts was measured during May to December 2019 (one run for each cohort) in a blinded manner, on the Simoa HD-1 (Quanterix). The AT270 mouse monoclonal antibody (#MN1050, Invitrogen) specific for the threonine-181 phosphorylation site[23] was coupled to paramagnetic beads (#103207, Quanterix) and used for capture. This antibody recognizes the tau sequence 176-PPAPKT(p)P-182 phosphorylated specifically at threonine-181[24]. As detector, we used the anti-tau mouse monoclonal antibody Tau12 (#806502, BioLegend), which binds the N-terminal epitope 6-QEFEVMEDHAGT-18 on human tau protein[25]. Both amino acid numbering follow that of full-length tau 1-441 (Uniprot ID #P10636-8). The detection antibody was conjugated to biotin (#A3959, Thermo Scientific) following the manufacturer's recommendations. Full-length recombinant tau-441 phosphorylated *in vitro* by glycogen synthase kinase 3β (#TO8-50FN, SignalChem) was used as the calibrator. For detailed analytical procedures and assay validation, see the appendix (pp 7-9).

We used area under the curve (AUC) analyses to compare the ability of plasma p-tau181 and two of the most well-known risk factors for Alzheimer's disease (age and *APOE* ε4 genotype carriage) to correctly identify: (i) Alzheimer's disease diagnosis, (ii) increased Aβ PET, and (iii) elevated tau PET uptake. *APOE* ε4 genotyping was performed using the TaqMan real-time polymerase chain reaction assay externally at Applied Biosystems (California, United States of America). Furthermore, the performance of plasma p-tau181 to accurately identify Alzheimer's disease diagnosis and increased Aβ and tau PET was compared with other plasma biomarkers (total-tau, $A\beta_{1-42}$, $A\beta_{1-42}/A\beta_{1-40}$ and total-tau/$A\beta_{1-42}$) using AUC analyses. Plasma total tau, $A\beta_{1-}$

$_{42}$, and $A\beta_{1-40}$ were measured using the Neuro 3-Plex A kit available commercially from Quanterix (#101995), following the manufacturer's instructions.

Statistics

The prospective clinical cohorts are continuously recruiting patients, and for this study we included all individuals and patients with samples available for analysis. Statistical analyses were performed using R v3.1.2, MATLAB v9.2 with VoxelStats package[28], and SPSS v26. Only individuals with complete data were included in each specific analysis. Unpaired t-test and analysis of variance with Tukey's multiple comparisons test were used to compare continuous variables between groups. Chi-square test was used to compare dichotomous variables between groups. Receiver operating curves (ROC) contrasting groups provided the area under the curve (AUC) for a diagnosis of Alzheimer's disease or biomarker positivity. AUC, sensitivity, specificity and the representative best value for accuracy at an optimal cut-off value were used to determine biomarker performance. Spearman's rank correlation tested associations between biomarkers. No covariates were used in the aforementioned models. Linear regression models tested the associations between plasma p-tau181 and baseline and one-year change in cognition (MMSE score) and structural imaging (hippocampus gray matter density) data. The linear regressions were corrected for age, gender, APOE ε4 status and years of formal education. Significance was reported if $P < 0.05$. Further details may be found in the appendix (p 9).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The joint first and the joint last authors had access to all the data and had final responsibility for the decision to submit for publication.

RESULTS

We studied 37 individuals in the discovery cohort, 226 and 763 in the first and second validation cohorts, respectively, and a further 105 in the primary-care cohort (n=1,131 individuals). Blood p-tau181 concentrations were not affected by gender (P>0·05). Demographics of the discovery and primary-care cohorts are available in Table 1. Demographic characteristics of the TRIAD and BioFINDER-2 populations are presented in Table 2 as well as Tables S1 and S2 (appendix pp 19-20).

The blood p-tau181 assay (Figure S1; appendix p 10) demonstrated high analytical performance (Table S3; appendix p 21), with high precision within and between runs, and between different batches of reagents (Tables S4 and S5; appendix pp 22-24). Mass spectrometric studies showed that the assay specifically measures N-terminal to mid-domain forms of tau phosphorylated at threonine-181, and does not recognise non-phosphorylated forms of tau (Figure S2; appendix p 11).

In the discovery cohort, the mean p-tau181 in paired serum and plasma were approximately two- and three-fold increased in CSF biomarker-positive Alzheimer's disease patients compared with controls, respectively (P<0·0001; Figure 1A). P-tau181 concentrations in paired serum and plasma correlated well with one another (r=0·8150,P<0·0001; Figure S3; appendix p 12). P-tau181 in blood showed high performance for the diagnosis of Alzheimer's disease (serum, AUC=95·91%; plasma, AUC=90·06%; Figure 1B and Figure S4; appendix p 13), suggesting that plasma and serum are equally suitable for p-tau181 analysis.

In TRIAD, plasma p-tau181 was increased in the CSF Aβ-positive Alzheimer's disease dementia group compared to all other diagnostic groups (P<0·0001; Figure 1C). Plasma p-

tau181 concentrations in Aβ-positive CU elderly as well as Aβ-negative and Aβ-positive MCIs were higher than in the young, frontotemporal dementia and Aβ-negative CU elderly cases (P<0·05; Figure 1C and Table S1; appendix p 19). Plasma p-tau181 discriminated Alzheimer's disease from frontotemporal dementia (AUC=100%), young and CU elderly (AUC and accuracy>95%), and MCI (AUC >85% and accuracy >80%; Figure 1D and Figure S4; appendix p 13). Plasma p-tau181 distinguished Aβ-positive CU elderly from Aβ-negative CU elderly (AUC=81·02%), and young adults (AUC=89·90%) (Figure S5; appendix p 14).

In BioFINDER-2, plasma p-tau181 levels gradually increased across the entire Alzheimer's disease clinical continuum, being lowest in the CSF Aβ-negative CU elderly and Aβ-negative MCI groups followed by the Aβ-positive CU elderly and Aβ-positive MCI groups, and then the (CSF Aβ-positive) Alzheimer's disease dementia group (Figure 1E). Plasma p-tau181 was increased in Alzheimer's disease dementia compared with each of the MCI and CU elderly groups (P<0·0001), and discriminated Alzheimer's disease from Aβ-negative CU elderly and Aβ-negative MCI (AUC=90·21% and 86·51%, respectively (Figure IF). Moreover, plasma p-tau181 was increased in Alzheimer's disease compared with other (Aβ-negative) neurodegenerative disorders (P<0·0001). Plasma p-tau181 concentrations separated Alzheimer's disease from vascular dementia (AUC=92·13%), progressive supranuclear palsy/corticobasal syndrome (AUC=88·47%), behavioral variant frontotemporal dementia/primary progressive aphasia (AUC=82·76%), and Parkinson's disease/multiple systems atrophy (AUC=81·90%; Figure 1F). Data when including clinically diagnosed non-Alzheimer's disease cases who proved to be Aβ-positive (i.e., having concomitant Alzheimer's disease-type pathology) are shown in Figure S6 (appendix p 15).

In the primary-care cohort, plasma p-tau181 concentration increased progressively from young to CU elderly, MCI, and clinically diagnosed Alzheimer's disease patients with unknown CSF

and PET biomarker status (Figure 1G). Plasma p-tau181 discriminated Alzheimer's disease dementia from young individuals (accuracy=100%), CU elderly (AUC=84·44% and accuracy >90%), but not from MCI (AUC=55·00%) (Figure 1H and Figure S4; appendix p 13).

In TRIAD, plasma p-tau181 strongly correlated with tau [^{18}F]MK-6240 PET across the cortex with the highest association in the temporal lobe (Figure 2A), and also with Aβ [^{18}F]AZD4694 PET across the cortex with the highest association in the precuneus, frontal cortex, and striatum (Figure 2B). Plasma p-tau181 strongly predicted [^{18}F]MK-6240 PET positivity (AUC and accuracy >90%, Figure 3A) and [^{18}F]AZD4694 PET positivity (AUC=88·09% and accuracy >80%, Figure 3B). Additionally, plasma p-tau181 discriminated individuals positive for both [^{18}F]MK-6240 and [^{18}F]AZD4694 from individuals negative for at least one of the PET biomarkers (AUC and accuracy >90%,Figure 3C). Plasma p-tau181 correlated with tau PET uptake across all Braak stages (Figure S7; appendix p 16). Plasma p-tau181 correlated better with both tau PET and Aβ PET in Aβ-positive cases than in Aβ-negative individuals (see legend to Figure 2). Plasma p-tau181 correlation with tau and Aβ PET stratified by clinical diagnosis are shown in Table S6 (appendix p 25). Plasma p-tau181 increased with disease severity measured by tau PET uptake (Figure 3D), and also correlated with duration of symptoms within the Alzheimer's disease dementia group, calculated as age at blood collection minus age of onset (r=0·3627, *P*=0·0252). Importantly, among tau PET-negative individuals (Braak 0), plasma p-tau181 distinguished Aβ-positive from Aβ-negative cases (AUC=84·82%;Figure 3E).

In BioFINDER-2, plasma p-tau181 correlated with [^{18}F]RO948 in Aβ-positive cases (i) Braak I-II ROI (r=0·445,*P*<0·001), ii) Braak III-IV (r=0·488,*P*<0·001), iii) Braak V-IV (r=0·446,*P*<0·001). Plasma p-tau181 differentiated tau PET-positive individuals from tau PET-negative participants with high accuracy (AUC=83·08%, 85·08% and 84·70% for tau PET

Braak I-II, III-IV and V-VI ROI respectively; Figure S8, appendix p 17). Additionally, plasma p-tau181 was higher for Aβ PET-positive cases than Aβ PET-negative participants (P<0·0001).

In the discovery cohort, plasma and serum p-tau181 (Simoa) were highly correlated with Innotest CSF p-tau181 (r=0·7055-0.7937,P<0·0001;Fig. S9A) and CSF $A\beta_{1-42}$ (r= -0·5936 – -0·6830, P<0·0001; Figure S9A, appendix p 18). In TRIAD, plasma p-tau181 correlated well with CSF p-tau181, measured with either Lumipulse or Simoa; for details see Figure S9B (appendix p 18). Simoa and Lumipulse CSF p-tau181 correlated strongly (r=0·8666,P <0·0001;Figure S9B; appendix p 18). Simoa p-tau181 measured in paired plasma and CSF from the same individuals (Figure S9B; appendix p 18) gave mean plasma p-tau181 to CSF p-tau181 ratio of ~5%.

Plasma p-tau181 was a better predictor of Alzheimer's disease diagnosis and increased Aβ PET than each of age, *APOE* ε4 allele, and age and *APOE* ε4 carriage combined. Adding age and *APOE* ε4 status provided only marginal or no improvements to the predictive accuracies of plasma p-tau181 (Tables S7-S8; appendix p 25). Similarly, plasma p-tau181 predicted elevated tau PET better than each of age, *APOE* ε4, and age and *APOE* ε4 status combined in all Braak ROI when considering the entire cohort, as well as within the Alzheimer's disease cases, and the non-demented groups (Braak III-IV and V-VI; Tables S9-S11, appendix p 26). In *APOE* ε4-stratified analysis, plasma p-tau181 remained a much better predictor of Alzheimer's disease as well as increased Aβ PET and tau PET than age, in both carriers and non-carriers (Tables S7-S11; appendix pp 25-26).

Plasma p-tau181 was a more accurate predictor of: (i) Alzheimer's disease, (ii) increased Aβ PET, and (iii) elevated tau PET (Braak I-VI), than each of plasma $A\beta_{1-42}$, $A\beta_{1-42}$ /$A\beta_{1-40}$, total-tau, and total-tau/$A\beta_{1-42}$ (Table S12; appendix p 27).

A subset of individuals in TRIAD (n=88) had one-year follow-up structural MRI and cognitive assessment. After correcting for age, gender, *APOE* ε4 and years of education, plasma p-tau181 correlated with one-year worsening in MMSE (P≤0·0015,Figure 4A-B), and with both baseline and one-year change hippocampal atrophy (P<0·0001 and 0·05 respectively, Figure 4C-D; for analysis by diagnostic group, see Table S13; appendix p 27).

DISCUSSION

We report a high-performance blood p-tau181 assay that identified brain tau pathology and showed increased levels in individuals having Aβ pathology but were still tau PET-negative. Moreover, plasma p-tau181 demonstrated high diagnostic accuracy for Alzheimer's disease in four independent cohorts, discriminated Aβ-positive CU elderly and Aβ-positive MCI cases from Aβ-negative CU elderly and young adults, and also showed high performance to identify clinically diagnosed Alzheimer's disease patients with unknown brain amyloid status in the primary-care setting. Furthermore, blood p-tau181 differentiated Alzheimer's disease from several other neurodegenerative diseases with high performance. In addition, blood p-tau181 predicted cognitive decline and hippocampal atrophy over a period of one-year.

The specificity of p-tau181 to Alzheimer's disease, as shown in CSF[14], corroborated also in blood in the present study, makes it highly desirable biomarker for clinical use. Previous studies, using plasma p-tau181 assays developed on different technology platforms, have reported moderate accuracy of plasma p-tau181 in discriminating Alzheimer's disease from non-demented controls[15–18]. However, these assays have not been applied to large, independent cohorts including non-Alzheimer neurodegenerative disorders. Therefore, it is unclear if any of these assays, each targeting a distinct form of tau, is specific to tau pathology in Alzheimer's disease; one assay has shown similar increases in frontotemporal dementia, Parkinson's disease, progressive supranuclear palsy and multiple system atrophy[26]. Two assays were not sensitive

enough for measuring p-tau181 levels in many participants, including control patients[16,18]. In addition, some assays were validated specifically for plasma[16,17], limiting the choice of matrix. The ultra-sensitive assay presented in this study measures specific N-terminal p-tau181 species, as verified by mass spectrometry experiments, and the detection in CSF and strong correlations between blood and CSF levels of CSF p-tau181 support that it specifically measures brain-derived p-tau181. Importantly, blood p-tau181 discriminated Aβ-negative CU elderly cases from Aβ-positive CU elderly and Aβ-positive MCI cases, suggesting that plasma p-tau181 can model the whole Alzheimer's disease continuum. Furthermore, similar to CSF p-tau181[27], blood p-tau181 separated Alzheimer's disease from other neurodegenerative disorders with high accuracy, indicating that this assay may be a specific marker of tau pathology in Alzheimer's disease. Importantly, the assay distinguished Alzheimer's disease from phenotypes of primary tauopathies including progressive supranuclear palsy and corticobasal syndrome, both with concomitant tau pathology also seen in Alzheimer's disease. Together, the results indicate that blood p-tau181 has the specificity and scalability required for effective population screening in Alzheimer's disease.

The blood p-tau181 test displayed high accuracy for predicting *in vivo* tau tangles and a predictive power to detect Aβ plaque-positive cases comparable to high-performance mass spectrometry-based Aβ plasma assays[10,11]. Notably, blood p-tau181 identifies individuals with brain tau and Aβ pathology with up to >90% AUC. The strong correlation between plasma p-tau181 and Aβ PET (Figure 2) together with the increased plasma p-tau181 in Aβ PET-positive but tau PET-negative (Braak 0) individuals suggests that this new test detects Alzheimer's disease-type pathology in the very early disease stages. This finding also suggests potential biological links between tau production and Aβ plaques, in that plasma p-tau181 may detect a neuronal reaction to initial Aβ aggregation[28], supporting the amyloid cascade hypothesis. Significantly, the high accuracy of our blood assay to identify brain tangle and plaque

pathologies, both singly and jointly, makes it an ideal biomarker satisfying biological and clinical definitions of Alzheimer's disease[4]. The blood p-tau181 assay thus constitutes an unprecedented advance for rapidly identifying *in vivo* Alzheimer's disease pathophysiology, and could become a cost- and time-saving first-line test for the evaluation of patients with suspected Alzheimer's disease, irrespective of disease stage. The overlap between MCI and Alzheimer disease participants in the primary-care cohort may likely be driven by MCI patients already having Alzheimer disease dementia phenotypes, which cannot be excluded in this cohort without detailed PET or CSF biomarker data. The multi-centric design, the larger and more diverse population (compared to the other cohorts) and the different PET ligands used in BioFINDER-2 may account for the slightly lower AUCs for this cohort. Nonetheless, this cohort likely reflects the heterogeneous patient populations seen in the primary-care clinic. The overall excellent performance of blood p-tau181 in all cohorts studied indicates that this test is useful for supporting Alzheimer's disease diagnosis.

The association between baseline blood p-tau181 and one-year cognitive deterioration as well as rate of hippocampal atrophy suggest that the new p-tau181 blood test also can serve as a predictor of disease progression, and thus may be used to select individuals most likely to progress during the typically short clinical trial periods. The correlation between plasma p-tau181 and [^{18}F]MK-6240 tau PET in the TRIAD cohort showed almost a bi-modal distribution, with p-tau181 increasing rather steeply within CU and MCI cases, and then plateauing in Alzheimer's disease dementia cases, despite increasing tau PET ligand retention. These findings suggest that plasma p-tau181 increases during the very early stages of tau pathology accumulation, supported by the high plasma p-tau181 in Aβ PET-positive individuals who were still tau PET-negative (Braak stage 0; Figure 3E). However, plasma p-tau181 does not appear to increase further in cases with moderate to severe tau pathology. Similar observations were made in a previous study, reporting a poor correlation between p-tau181 and [^{18}F]AV1451 tau

PET in Alzheimer's disease dementia, but more robust correlations in Aβ-positive CU and MCI cases[15]. In contrast to tau PET, we showed high correlations between plasma and CSF p-tau181 irrespective of disease stage and the immunoassay method used, indicating that p-tau181 in both biofluids directly reflect brain tau phosphorylation state that may not directly translate to tau aggregation status measured by PET.

Our results show significantly novel data. Firstly, a previous study[16] showed a modest correlation (r=0·45) between plasma p-tau181 and CSF p-tau181(Innotest) in a small cohort (n=11 participants). However, no prior study has demonstrated that the plasma analyte measured by their p-tau181 assay can also be measured in serum and CSF. Moreover, one study showed that plasma p-tau181 predicts increased Aβ PET with 80% AUC in CU, MCI and Alzheimer's disease participants combined, but did not present whether plasma p-tau181 predicts tau PET positivity[15]. Another study, using a discontinued commercial assay, reported poor performance for plasma p-tau181[18]. On the contrary, we showed that our plasma p-tau181 is an excellent predictor of both amyloid PET and tau PET, validating these findings in two large cohorts, each using a distinct set of PET ligands. Furthermore, contrary to the immunomagnetic reduction p-tau181 assay[17,26], our blood p-tau181 assay appears specific to Alzheimer's disease-type tau pathology, showing no significant increases in several other tauopathies. This emphasizes that not only is tau phosphorylation at threonine-181 important but the species on which this phosphorylation site occurs is critical. Importantly, blood p-tau181 has potential uses in three clinical settings – primary-care, clinical diagnosis and biomarker-based diagnosis. The extensive validation has established for the first time that plasma and serum are similarly suitable for blood p-tau181 analysis.

Plasma p-tau181's better diagnostic performance than the most well-known risk factors for amyloid deposition – age and *APOE* ε4 – both singly and jointly, indicate that the robust

performance of this diagnostic test does not require prior knowledge of an individual's age and *APOE* genotype. The higher performance than other plasma biomarkers indicates that our new assay significantly extends the clinical diagnostic potential of blood biomarkers for Alzheimer's disease.

To conclude, our high-performance blood p-tau181 assay may represent the first simple, practical and scalable test for the diagnosis of Alzheimer's disease. This technology has immediate applications for diagnosis and recruitment for disease-modifying trials. This assay has the potential to be incorporated in clinical practice as a rapid screening test to rule out Alzheimer's disease pathophysiology and guide therapy and clinical management of dementia patients.

CONTRIBUTORS

TKK, TAP, NJA, HK, OH, PR-N, and KB conceived the study. TKK developed and validated the blood p-tau181 assay with support from NJA, JLR, GB, KH, HZ, and KB. TKK, TAP, SJ, ALB, NJA, and OH performed statistical analysis. TAP, SJ, ALB, MC, MS, MSK, JT, NM, SP, EK, OH, and PR-N designed and implemented MRI and PET acquisition protocols, as well as performed image processing and quality control. GM, J-PS, NM, SP, SG, ES, HZ, OH, PR-N, and KB recruited participants, and collected clinical data. TKK, TAP, NJA, SJ, ALB, MS, KH, SP, SG, ES, HZ, OH, PR-N, and KB interpreted the data. TKK, TAP, NJA, SJ, JLR, HZ, OH, PR-N, and KB drafted the initial manuscript. All authors contributed to revision and editing of the manuscript.

DECLARATION OF INTERESTS

H.Z. has served at scientific advisory boards for Wave, Samumed, CogRx and Roche Diagnostics and has given open lectures for Alzecure. KB has served as a consultant or at

advisory boards for Axon, Biogen, CogRx, Lilly, MagQu, Novartis and Roche Diagnostics. HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. OH has acquired research support (for the institution) from Roche, Pfizer, GE Healthcare, Biogen, AVID Radiopharmaceuticals and Euroimmun. In the past two years, he has received consultancy/speaker fees (paid to the institution) from Biogen and Roche. The other authors declare no competing interest.

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631

TABLES

Table 1. Characteristics of participants in the discovery and the primary-care cohorts.

Characteristics	Discovery cohort		Primary-care clinical cohort			
	CU elderly	AD	Young adults	CU elderly	MCI	AD
Number (*n*)	18	19	11	72	12	10
Age, y, mean ± SD	63·8 ± 11·4	74·4 ± 5·4*	23·5 ± 2·0*	70·0 ± 9·1#	71·7 ± 10·5	62·7 ± 13·6*
Female, *n*. (%)	5/18 (27·8%)	10/20 (52·6%)	5/11 (45·5%)	49/72 (68·1%)	8/12 (66·7%)	4/10 (40·0%)
APOE *ε4*, *n*. (%)	-	-	2/11 (18·2%)	23/69 (33·3%)	5/12 (41·7%)	4/10 (40·0%)
Education, y, mean ± SD	-	-	17·8 ± 2·4	15·1 ± 3·6	14·1 ± 3·2	13·0 ± 3·3
CSF $A\beta_{1-42}$ pg/ml, mean ± SD	842·2 ± 175·9	388·9 ± 72·1*	-	-	-	-
CSF p-tau181 pg/ml, mean ± SD	35·4 ± 10·1	94·3 ± 28·6*	-	-	-	-

CSF total-tau pg/ml, mean ± SD	223·3 ± 68·7	669·5 ± 255·5*	-	-	-	-

In both cohorts, *CU elderly* refers to cognitively unimpaired elderly adults. *CU elderly* participants in the discovery cohort additionally tested negative for the CSF core biomarkers (Aβ, p-tau181, and total tau). The *Young adults* group consisted of cognitively unimpaired individuals with a mean age of 23.5 years.

Student's t-test (the discovery cohort) or analysis of variance followed by Tukey's post-hoc test (the primary-care cohort) revealed significant differences between groups for continuous variables except for gender and APOE ε4 where contingency chi-square tests were performed. Post-hoc analysis provided significant differences between groups compared with: CU elderly (*) or AD (#).

Note: CSF p-tau181, total tau and $A\beta_{1-42}$ were measured with the corresponding Innotest ELISA kits and the automated Lumipulse system in the discovery and primary-care clinical cohorts respectively.

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-β; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; p-tau181, tau phosphorylated at threonine-181; SD, standard deviation; y, years

Table 2. Characteristics of the TRIAD and BioFINDER-2 cohorts.

Characteristics	TRIAD cohort					BioFINDER-2 cohort						
	Young adults	CU elderly	MCI	AD	FTD	CU elderly	MCI	AD	bvFTD/PPA	PD/MSA	VaD	PSP/CBS
Number (*n*)	27	113	45	33	8	337	191	126	18	36	12	21
Age, y, mean ± SD	22·7 ± 1·9*#	69·2 ± 9·7	72·6 ± 6·8#	64·6 ± 9·2	59·3 ± 8·5*	63·1 ± 15·0#	70·6 ± 8·1*	74·0 ± 6·9*	67·4 ± 7·4	68·7 ± 11·0	74·8 ± 6·5*	69·0 ± 7·9
Female, *n*. (%)	17/27 (63%)	72/113 (63·7%)	23/45 (51·1%)	15/33 (45·5%)	7/8 (87·5%)	183/337 (54·3%)	85/191* (44·5%)	67/126 (53·2%)	13/18 (72·2%)	15/36 (41·7%)	4/12 (33·3%)	9/21 (42·9%)
APOE *ε4*, *n*. (%)	6/27 (22·2%)#	33/111 (29·7%)#	19/44 (43·2%)	17/32 (53·1%)*	0/8 (0%)#	147/335 (43·9%)#	98/186 (52·7%)#	87/123* (70·7%)	3/17* (17·6%)#	12/34 (35·3%)#	3/12 (25·0%)#	5/21 (23·8%)#
Education, y, mean ± SD	16·7 ± 1·5	15·3 ± 4·0	14·0 ± 3·7	15·2 ± 3·8	14·8 ± 3·9	12·7 ± 3·4	12·4 ± 4·1	12·2 ± 4·4	12·0 ± 3·1	13·2 ± 4·0	11·3 ± 2·8	12·5 ± 3·3
MMSE score, mean ± SD	29·8 ± 0·5#	29·1 ± 1·1#	27·3 ± 1·8*#	18·4 ± 5·7	22·9 ± 9·7*#	29·0 ± 1·2#	27·0 ± 2·0*#	20·1 ± 4·5*	24·1 ± 4·0	28·2 ± 2·1	23·1 ± 3·5	26·1 ± 3·5
CSF $A\beta_{1-42}$ pg/ml, mean ± SD	789·8 ± 262·7	1023·7 ± 451·3#	824·1 ± 381·5*	414·3 ± 142·2*	742·8 ± 146·3	948·7 ± 255·6#	740·1 ± 281·8*#	485·3 ± 133·6*	946·6 ± 193·5	907·1 ± 233·9	1011·8 ± 255·7	777·0 ± 242·4
CSF p-tau181 (Lumipulse) pg/ml, mean ±SD	20·8 ± 7·5#	40·5±19·3#	71·4 ± 57·0*	96·6 ± 51·4*	25·8 ± 9·4#	45·0 ± 18·2#	55·5 ± 25·8*#	86·9 ± 35·7*	38·9 ± 12·8	40·1 ± 17·1	36·7 ± 13·4	31·0 ± 13·0

CSF total-tau (Lumipulse) pg/ml, mean ± SD	198·6 ± 49·7#	331·6 ± 132·5$^{\#}$	475·1 ± 301·4*	651·9 ± 338·9*	255·2 ± 78·4$^{\#}$	312·7 ± 159·0$^{\#}$	448·5 ± 260·9$^{*\#}$	800·7 ± 378·9*	346·6 ± 137·8	277·3 ± 122·2	287·9 ± 128·2	234·3 ± 104·6
Aβ-PET SUVR, mean ± SD	1·2 ± 0·1$^{*\#}$	1·5 ± 0·3$^{\#}$	2·0 ± 0·6$^{*\#}$	2·4 ± 0·5*	1·2 ± 0·1$^{\#}$	0·5 ± 0·2$^{\#}$	0·7 ± 0·3$^{*\#}$	1·0 ± 0·1*	-	-	-	-
Tau-PET SUVR (Braak I-II ROI), mean ± SD	0·8 ± 0·4$^{*\#}$	1·0 ± 0·2$^{\#}$	1·3 ± 0·5$^{*\#}$	1·9 ± 0·6*	0·8 ± 0·12$^{*\#}$	1·2 ± 0·2$^{\#}$	1·4 ± 0·4$^{*\#}$	2·0 ± 0·4*	1·2 ± 0·6	1·1 ± 0·1	1·2 ± 0·2	1·1 ± 0·2
Tau-PET SUVR (Braak III-IV ROI), mean ± SD	1·02 ± 1·1$^{\#}$	1·05 ± 0·1$^{\#}$	1·4 ± 0·5$^{*\#}$	3·1 ± 1·2*	1·0 ± 0·1$^{\#}$	1·2 ± 0·2$^{\#}$	1·3 ± 0·4$^{*\#}$	2·1 ± 0·7*	1·2 ± 0·2	1·1 ± 0·1	1·1 ± 0·1	1·2 ± 0·1
Tau-PET SUVR (Braak V-VI ROI), mean ± SD	1·1 ± 0·16$^{\#}$	1·1 ± 0·1$^{\#}$	1·2 ± 0·3$^{*\#}$	2·9 ± 2·0*	1·0 ± 0·2$^{\#}$	1·1 ± 0·1$^{\#}$	1·1 ± 0·2$^{\#}$	1·5 ± 0·4*	1·0 ± 0·1	1·1 ± 0·1	1·0 ± 0·1	1·0± 0·1
Plasma p-tau181 (Simoa), pg/ml, mean ± SD	7·9 ± 2·6$^{\#}$	10·0 ± 3·3$^{\#}$	14·8 ± 6·7$^{*\#}$	24·9 ± 7·8*	6.9 ± 2·1$^{\#}$	9·4 ± 6·0$^{\#}$	12·5 ± 8·6$^{*\#}$	19·2* ± 9·4	11·2 ± 7·4$^{\#}$	11·9 ± 9·3$^{\#}$	9·9 ± 6·0$^{\#}$	9·9 ± 3·8$^{\#}$

In both cohorts, *CU elderly* refers to cognitively unimpaired elderly adults (mean ages approximately 63 years in BioFINDER-2 and 69 years in TRIAD) who were also CSF biomarker-negative. *Young adults* refer to cognitively unimpaired young adults (mean age approximately 23 years old) who also showed a CSF biomarker-negative profile.

Analysis of variance followed by Tukey's *post hoc* test assessed differences between groups for continuous variables except for gender and *APOE ε4* where a contingency chi-square was performed. Post-hoc analysis provided significant differences between groups from: CU elderly (*) or AD ($^{\#}$). All FTD/PPA, PD/MSA, VaD, PSP/CBS patients were Aβ-negative. Additional stratification using Aβ status may be found in Tables S2 (appendix p 19) and S3 (appendix p 20). Data were unavailable for BioFINDER-2 participants for the following variables: APOE *ε4* n=13, education n=6, MMSE n=3, CSF $A\beta_{42}$ and t-tau n=1, CSF p-tau n=3, Aβ-PET n=332, Tau-PET n=95.

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-β; bvFTD, behavioral variant frontotemporal dementia; CBS, corticobasal syndrome; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MSA, multiple systems atrophy; PD, Parkinson's disease; PET, positron emission tomography; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; p-tau181, tau phosphorylated at threonine-181; ROI, region of interest; SD, standard deviation; VaD, vascular dementia; y, years

FIGURES

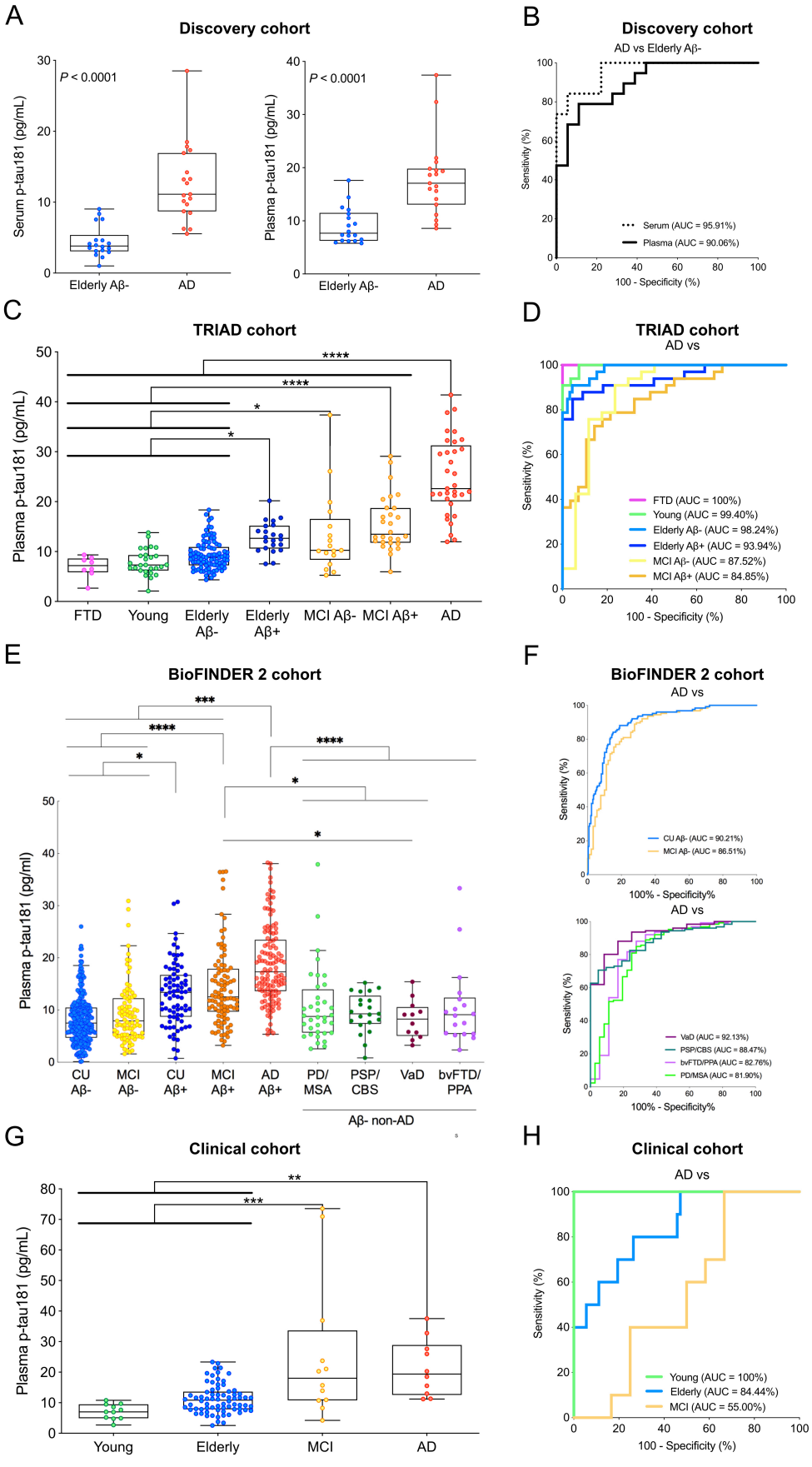
A
Discovery cohort
P < 0.0001
Serum p-tau181 (pg/mL)
Plasma p-tau181 (pg/mL)
Elderly Aβ-
AD
B
Discovery cohort
AD vs Elderly Aβ-
Sensitivity (%)
100 - Specificity (%)
Serum (AUC = 95.91%)
Plasma (AUC = 90.06%)
C
TRIAD cohort
Plasma p-tau181 (pg/mL)
FTD
Young
Elderly Aβ-
Elderly Aβ+
MCI Aβ-
MCI Aβ+
AD
D
TRIAD cohort
AD vs
Sensitivity (%)
100 - Specificity (%)
FTD (AUC = 100%)
Young (AUC = 99.40%)
Elderly Aβ- (AUC = 98.24%)
Elderly Aβ+ (AUC = 93.94%)
MCI Aβ- (AUC = 87.52%)
MCI Aβ+ (AUC = 84.85%)
E
BioFINDER 2 cohort
Plasma p-tau181 (pg/ml)
CU Aβ-
MCI Aβ-
CU Aβ+
MCI Aβ+
AD Aβ+
PD/ MSA
PSP/ CBS
VaD
bvFTD/ PPA
Aβ- non-AD
F
BioFINDER 2 cohort
AD vs
Sensitivity (%)
100% - Specificity%
CU Aβ- (AUC = 90.21%)
MCI Aβ- (AUC = 86.51%)
AD vs
VaD (AUC = 92.13%)
PSP/CBS (AUC = 88.47%)
bvFTD/PPA (AUC = 82.76%)
PD/MSA (AUC = 81.90%)
G
Clinical cohort
Plasma p-tau181 (pg/mL)
Young
Elderly
MCI
AD
H
Clinical cohort
AD vs
Sensitivity (%)
100 - Specificity (%)
Young (AUC = 100%)
Elderly (AUC = 84.44%)
MCI (AUC = 55.00%)

Figure 1. Plasma p-tau181 concentration in the four cohorts.

The box-and-whisker plots (left side) show blood p-tau181 concentrations across groups. *P* values indicate the results of analysis of variance models with post hoc multiple comparisons at $P < 0.05$. For each plot, the horizontal bar shows the median, and the upper and lower boundaries show the 25th and 75th percentiles, respectively. The figure also displays ROC curves in the four cohorts studied (right side). Each AUC value indicates overall biomarker performance, with 50% indicating no difference from chance and 100% indicating a biomarker with sensitivity and specificity of 100%. (**A-B**) In the discovery cohort (*n* = 37), serum and plasma p-tau181 concentrations accurately discriminated Alzheimer's disease from CU elderly Aβ-negative cognitively normal controls (mean age 64 years). In the TRIAD (**C-D**) and BioFINDER-2 (**E-F**) cohorts (*n* = 226 and 763, respectively), plasma p-tau181 showed a gradual increase along the Alzheimer's disease continuum; from cognitively normal young adults of mean age 23 years to Aβ-negative CU elderly and MCI, Aβ-positive CU elderly and MCI and Alzheimer's disease dementia patients. For illustrative purposes only, four cognitively impaired individuals with high plasma p-tau181 concentrations (50-90 pg/ml) were not shown in (**E**) but were fully included in the statistical analyses. Aβ positivity in the discovery cohort was based on CSF $A\beta_{1-42}$ (INNOTEST) <530 ng/L profile[19]. In the TRIAD and BioFINDER-2 validation cohorts, Aβ positivity was determined by Aβ PET uptake. Thresholds for Aβ positivity were independently determined using Aβ PET uptake; based on visual rating and a consensus of two neurologists blinded to the diagnosis[29] for TRIAD, and mixture modelling techniques for BioFINDER-2[30] (further details in the appendix pp 6-7). (**G-H**) In the primary-care clinical cohort (*n* = 105), clinically-diagnosed Alzheimer's disease dementia cases had higher plasma p-tau181 than CU elderly but not than the MCI group which may likely include MCI patients having Alzheimer's disease dementia, who were not excluded in this cohort without the evaluation of a dementia specialist. Abbreviations: AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; CBS, corticobasal syndrome; CU, cognitively unimpaired; FTD, frontotemporal dementia; MCI, mild cognitive impairment; MSA, multiple systems atrophy; PD, Parkinson's disease; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; and VaD, vascular dementia. CU and CU elderly both refer to cognitively unimpaired elderly participants.

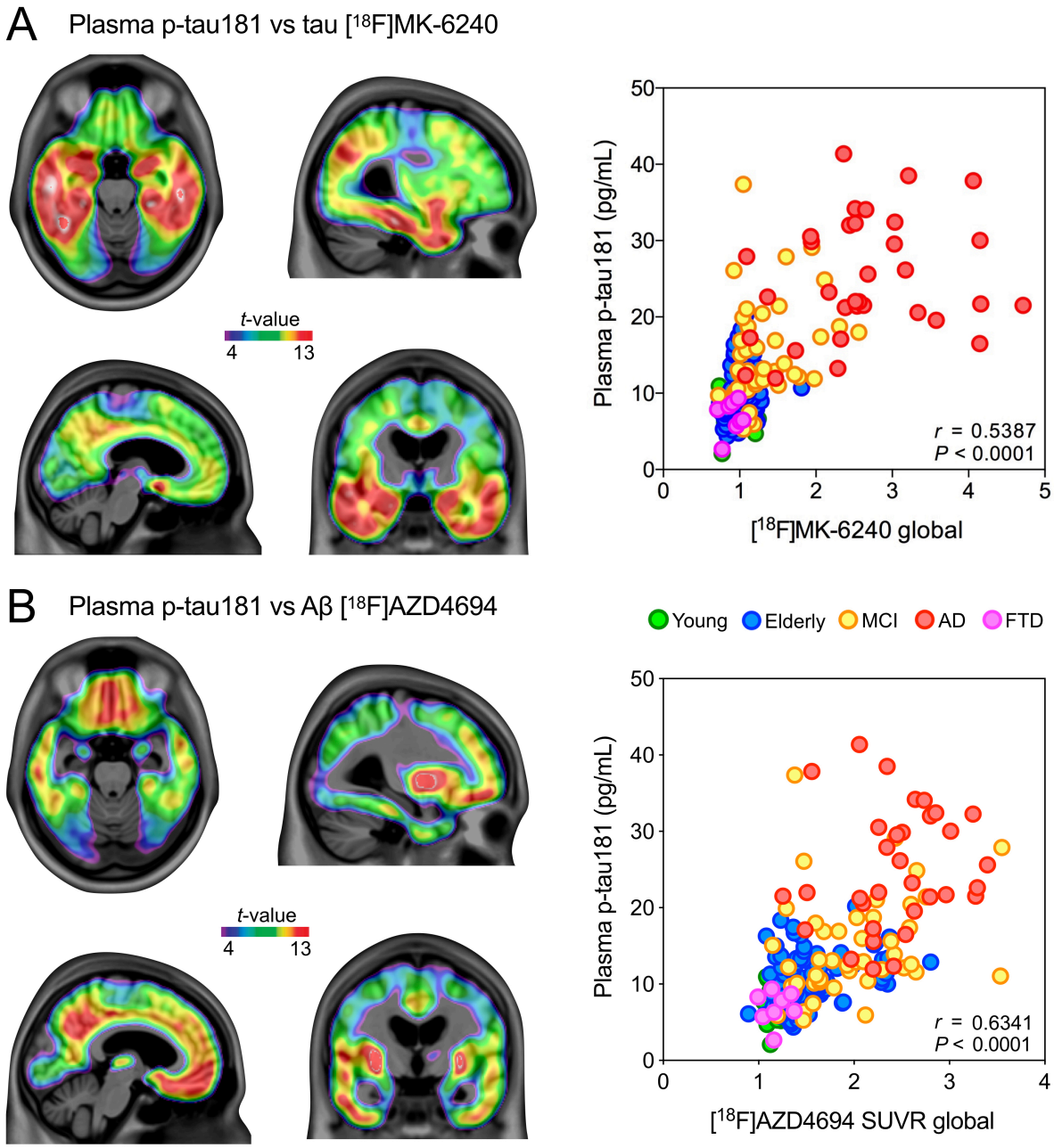

Figure 2. Plasma p-tau181 concentration according to PET tau and Aβ load.

The images on the left hand side of panels A and B show the results of voxel-wise regressions (false discovery rate corrected for multiple comparisons at $P < 0.05$) overlaid on a structural MRI template, whereas the scatter plots on the right hand side show the results of Spearman correlations between plasma p-tau181 and tau PET and Aβ PET ligands uptake ($n = 226$). PET [^{18}F]MK-6240 standardized uptake value ratio (SUVR) and [^{18}F]AZD4694 SUVR global values were estimated from Braak I-VI regions composite and typical brain regions used to assess global PET Aβ, as described in the Supplementary Methods (appendix pp 6-7), respectively. The panels show that plasma p-tau181 correlates well with global estimates of (**A**) [^{18}F]MK-6240 tau PET and (**B**) [^{18}F]AZD4694 Aβ PET. Plasma p-tau181 correlated better with tau PET and Aβ PET in Aβ-positive cases than in Aβ-negative individuals. For tau PET, r=0.6280, $P < 0.0001$ for Aβ-positive cases, and r=0.1636, $P = 0.0492$ for Aβ-negative cases. For Aβ PET, r=0.4454, $P < 0.0001$ for Aβ-positive individuals, and r=0.2890, $P = 0.0004$ for Aβ-negative cases.

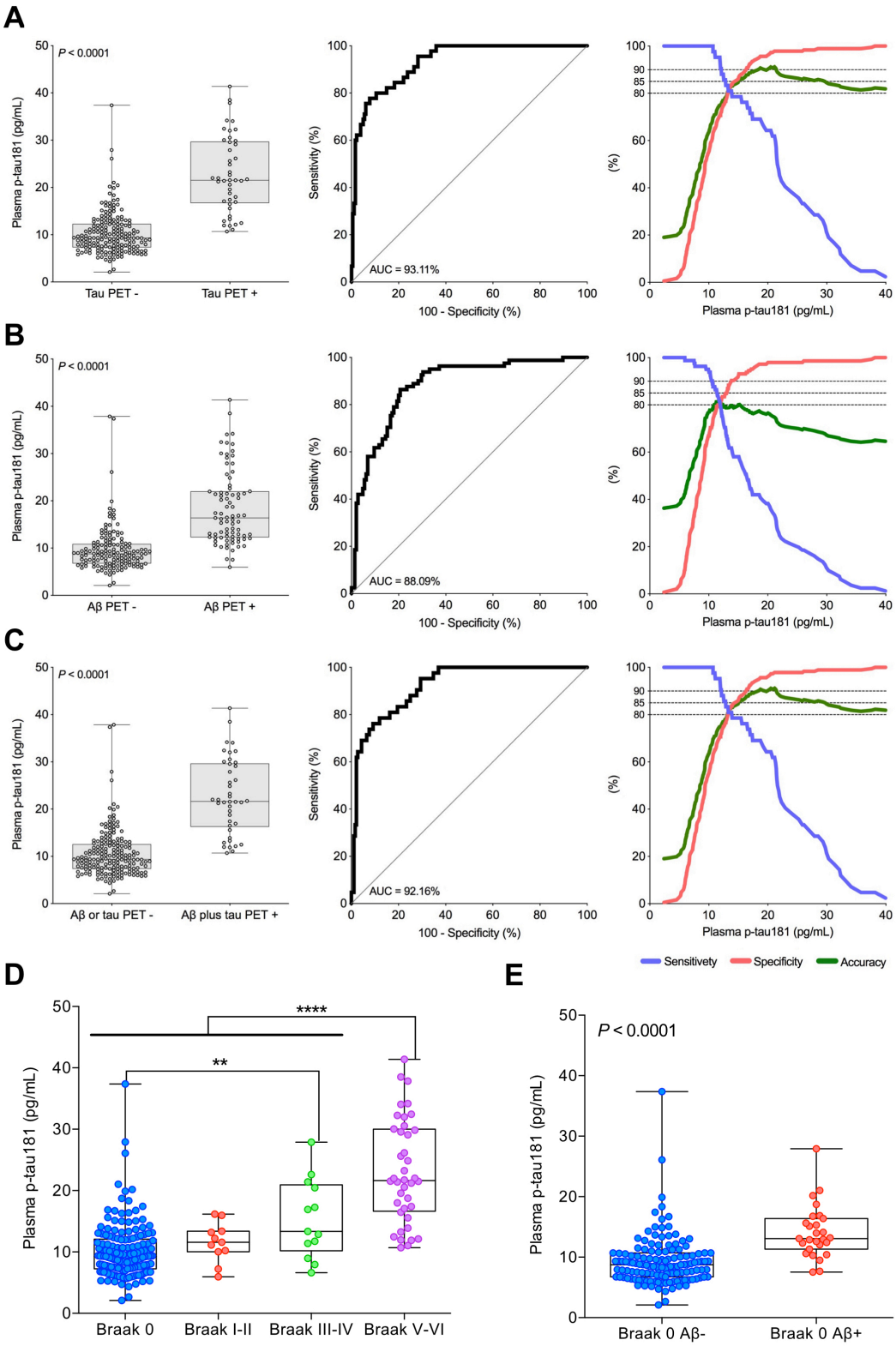

Figure 3. Plasma p-tau181 concentration according to PET tau and Aβ positivity.

The figure shows the results of two-tailed *t*-test (left side), AUC ROC curves (middle), and sensitivity, specificity, and accuracy for biomarker positivity (right side). Sensitivity is the ability of the test to correctly determine positive cases, while specificity is the ability of the test to determine the negative cases correctly. Accuracy is the ability of the test to correctly identify positive and negative cases. (**A**) Plasma p-tau181 accurately differentiated tau PET positive (*n* = 181) (composite [^{18}F]MK-6240 Braak I-VI, showed in Figure 2B) from tau PET negative (*n* = 45) individuals. (**B**) Plasma p-tau181 accurately differentiated Aβ PET positive (*n* = 145) ([^{18}F]AZD4694 composite, showed in Figure 2B) from Aβ PET negative (*n* = 81) individuals. (**C**) Plasma p-tau181 accurately identified individuals who were positive for both tau PET and Aβ PET (*n* = 184) from individuals negative for at least one of these biomarkers (*n* = 42). (**D**) Plasma p-tau181 concentrations increased with disease severity, as measured by tau PET Braak. Grouping into different Braak stages was according to *in vivo* tau PET uptake in brain regions known for the accumulation of tau neurofibrillary tangles in Alzheimer's disease; transentorhinal cortex (stage I-II), spreading to limbic (III-IV), and isocortical (V-VI) regions[21]. (**E**) Among tau PET-negative participants (Braak stage 0), plasma p-tau181 distinguished Aβ-positive (*n* = 139) from Aβ-negative (*n* = 29) cases.

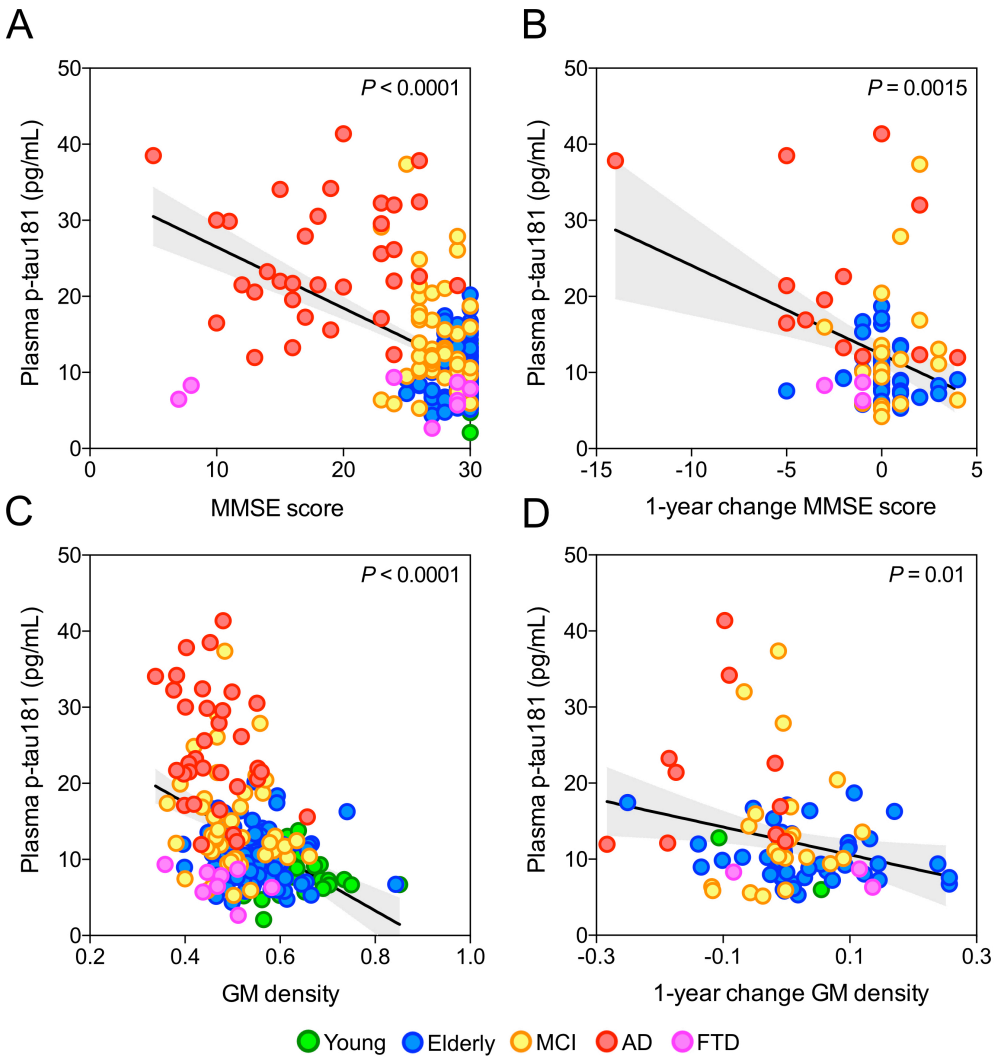

Figure 4. Association between plasma p-tau181 concentration and one-year longitudinal neurodegeneration and cognitive decline.

The scatter plots show the results of linear regressions between plasma p-tau181 with Mini Mental State Examination (MMSE) score and gray matter (GM) density in the hippocampus accounting for age, gender, *APOE* ε4 genotype and years of formal education in all individuals of the TRIAD cohort (n = 226, left side) as well as the subset who had one-year follow-up assessments (n = 88, right side). Plasma p-tau181 concentration was associated with (**A**) baseline (β = -0.34, R^2 = 0.31, P < 0.0001) and (**B**) one-year worsening (β = -0.11, R^2 = 0.164, P = 0.0015) in MMSE scores. Furthermore, plasma p-tau181 was associated with (**C**) baseline (β = -0.0035, R^2 = 0.38, P < 0.0001) and (**D**) one-year reduction in hippocampus GM density (β = -0.0037, R^2 = 0.1, P = 0.01). For longitudinal changes in MMSE score and hippocampus atrophy, lower scores represent cognitive decline and decrease in hippocampal volume, respectively.